SYNTHESE D'ACTIVTE SCIENTIFIQUE

Pour l'obtention de

l'Habilitation à Diriger des Recherches

Ecole Doctorale des Sciences de la Vie et de la Santé

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Maître de Conférence des Universités - Praticien Hospitalier

Discipline universitaire :
Anesthésiologie et Réanimation Chirurgicale

Activité hospitalière :
Responsable d'Unité Fonctionnelle,
Réanimation Chirurgicale Polyvalente,
Nouvel Hôpital Civil
Hôpitaux Universitaires de Strasbourg

Activité universitaire :
Maître de Conférence à la Faculté de Médecine de Strasbourg
Référent pédagogique du Pôle Anesthésie, Réanimation Chirurgicale, SAMU-SMUR des Hôpitaux Universitaires de Strasbourg

Activité scientifique :
Membre de l'équipe d'accueil 3072 : « Mitochondries, Stress Oxydant et Protection Musculaire »
Faculté de Médecine de Strasbourg
Fédération de Médecine Translationnelle de Strasbourg (FMTS)
Université de Strasbourg

Activité d'intérêt général :
Membre du CNU d'anesthésiologie, réanimation et médecine d'urgence
Membre de la CME des Hôpitaux Universitaires de Strasbourg

Synopsis

Mes études de médecine ont débuté à Strasbourg en 1990. J'ai choisi la spécialité **d'anesthésie-réanimation** et j'ai effectué mon internat au CHU de Rouen sous la direction du Professeur Bertrand Dureuil (1996-2000). Dès cette période, les effets délétères liés aux phénomènes **d'agression systémique et d'ischémie reperfusion** ont particulièrement attiré mon attention. Cette thématique a d'abord été développée lors d'un clinicat (CHU de Rouen) dans l'unité INSERM U 644 dirigée par le Professeur Thuillez sur un modèle d'ischémie-reperfusion intestinale. Ces travaux ont été enrichis dans le domaine de la recherche clinique sur les états critiques **lors de la mobilité (2003-2004) au *CRISMA center*** (*Clinical Research Investigation and System Modeling of Acute illness*) **du département de réanimation de l'Université de Pittsburgh**, Pennsylvanie, USA (Pr Derek Angus). Je suis revenu en Alsace en 2004, d'abord en tant que praticien hospitalier dans l'unité d'anesthésie de chirurgie et transplantation hépatique, puis en tant que **MCU-PH** (2007) dans le service de réanimation chirurgicale de l'Hôpital de Hautepierre (Pr Thierry Pottecher).

Je travaille aux côtés des **Professeurs Annick Steib et Michel Mertes** dans le service d'Anesthésie-Réanimation du Nouvel Hôpital Civil à Strasbourg. Plus précisément, je suis **responsable de la réanimation chirurgicale polyvalente**. Cette unité de 17 lits prend en charge plus de 1000 patients par an, en particulier les patients **transplantés pulmonaires**. Strasbourg est actuellement le premier centre de transplantation pulmonaire en France hors APHP. Enfin, notre hôpital bénéficie de **l'implantation d'un Institut Hospitalo-Universitaire** centré sur la chirurgie guidée par l'image. Mon objectif est de développer un ***« Intestinal Stroke Center »***, c'est-à-dire une filière de soin régionale pour que les patients présentant une ischémie intestinale puissent bénéficier des innovations techniques en matière de diagnostic et de traitement.

Sur le plan académique, je suis le **référent pédagogique du Pôle d'Anesthésie-Réanimation SAMU-SMUR des Hôpitaux Universitaires de Strasbourg**. Mon activité d'enseignement est associée à des publications centrées sur la pédagogie et notamment sur la simulation. J'ai dirigé 10 thèses de médecine, 10 mémoires pour le diplôme d'enseignement spécialisé en anesthésie-réanimation et co-dirigé deux masters II. Je participe également aux enseignements de l'école d'infirmières anesthésistes de Strasbourg ainsi qu'à diverses formations professionnelles. Je suis régulièrement invité comme orateur dans des congrès régionaux, nationaux et internationaux. Enfin, je suis membre de la **Commission Médicale d'Etablissement** et du **Conseil Scientifique** des Hôpitaux Universitaires de Strasbourg depuis 2011. Je suis également membre, depuis 2011, du **Conseil National Universitaire** de la sous-section Anesthésiologie - Réanimation ; Médecine d'Urgence (sous-section 48-01).

Ma **recherche** est centrée sur **l'étude des états d'agression systémique critique liés aux phénomènes d'ischémie-reperfusion et aux états de choc**. Cette thématique est en effet un sujet de préoccupation quotidien tant en anesthésie qu'en réanimation. **Les objectifs** de la recherche clinique et expérimentale sont triples : (1) **caractériser** les états d'agression systémique critiques en particulier développer des outils diagnostiques et de monitorage pertinents ; (2) **étudier les mécanismes** physiopathologiques et les voies de transduction impliquées et (3) **tester des nouveaux traitements** et de nouvelles prises en charge. Cette thématique de recherche a fait l'objet de 11 publications originales dans des revues internationales

Plusieurs perspectives peuvent être tracées pour les années à venir :

- **Recherche clinique : l'objectif est de créer** un **« *Intestinal Stroke Center* »,** c'est-à-dire une filière de soin spécifique pour les patients présentant une ischémie mésentérique aigue. Les patients bénéficieront des techniques diagnostiques et thérapeutiques innovantes développées dans notre équipe d'accueil et dans l'institut hospitalo-universitaire (IHU) de Strasbourg.

- **Recherche expérimentale:** le but est de poursuivre **l'étude du métabolisme cellulaire et mitochondrial des états d'agression systémique critique** et développement des **collaborations locales** (étude du métabolisme cellulaire myocardique par SPET, étude de l'implication du *Platelet Activating Factor)* **et internationales** (étude de la fusion-fission mitochondriale, Dr Jamal Bouitbir, *Clin. Pharmacology and Toxicology, Univ. Hospital Basel, Switzerland*).

I. Titres et activité Universitaires

A. Titres Universitaires

- Doctorat en Médecine, Faculté de Médecine de Rouen (2000)
- Diplôme d'Etudes Spécialisées en Anesthésiologie et Réanimation Chirurgicale, Faculté de Médecine de Rouen (2000)
- Maîtrise de Science Biologique et Médicale, Faculté de médecine de Rouen (2000)
- Diplôme d'Étude Approfondie en Physiologie et Biologie de la Circulation et de la Respiration – option Circulation-, Université Paris VII (2001)
- Doctorat de l'Université de Strasbourg en Physiologie et biologie des organismes, populations, interaction (2011)

B. Diplômes Universitaires

- Diplôme Universitaire de Défaillance Circulatoire Aiguë, Faculté de Médecine Paris-Sud (2002)
- Diplôme Universitaire de Ventilation Artificielle, Faculté de Médecine de Paris XII (2003)
- *Business English for International Course, University of Pittsburgh, Pittsburgh, Pennsylvania*, USA (2004)
- Diplôme Interuniversitaire de Pédagogie Médicale, Faculté de Médecine de Strasbourg (2009)

C. Fonctions Hospitalo-Universitaires

- Étudiant hospitalier, Hôpitaux Universitaires de Strasbourg (1992-1996)
- Interne des Hôpitaux, CHU de Rouen (1996-2000)
- Médecin attaché, CHU de Rouen (2000-2001)
- Chef de Clinique – Assistant, CHU de Rouen (2001-2003)
- *Research fellowship* au *CRISMA laboratory* (Pr. Derek Angus), *Critical Care Department, University of Pittsburgh, Pennsylvania*, USA (2003-2004)
- Praticien Hospitalier, Hôpitaux Universitaires de Strasbourg (2004-2007)
- Maître de Conférence des Universités - Praticien Hospitalier, Hôpitaux Universitaires de Strasbourg (depuis 2007)

D. Mobilité

- Clinicat au CHU de Rouen (2001-2003)
- *Research fellowship* au *CRISMA laboratory* (Pr. Derek Angus), *Critical Care Department, University of Pittsburgh, Pennsylvania*, USA (2003-2004)

E. Société savante

- Membre de la Société Française d'Anesthésie et de Réanimation (SFAR)
  - Membre du Comité Réanimation de la SFAR de 2006 à 2012
  - Expert pour les recommandations formalisées sur la « Stratégie du remplissage péri-opératoire », SFAR (2012).
  - Coordinateur du groupe d'experts pour les recommandations sur la « trachéotomie en réanimation », RFE SFAR-SRLF (en cours)
- Membre de l'ARCOTHOVA (Association des Anesthésistes Réanimateurs du Cœur, du Thorax et des Vaisseaux) (depuis 2012)

F. Bourses et prix

- Bourse de la Fondation pour la Recherche Médicale, 96 KF (2001)
- Bourse du Cercle des Anesthésistes Réanimateurs d'Alsace, 1,5 K€ (2003)
- Bourse de l'Institut de Recherche Contre les Cancers de l'Appareil Digestif, 15 K€ (2003).
- Bourse conjointe des Sociétés Française d'Anesthésie Réanimation et de Réanimation de Langue Française, 13 K€ (2003).
- ***Educational Scholarship Award, Society of Critical Care Medicine* (2005).**

II. Activités Hospitalières

1996 - 2000	Interne des Hôpitaux de Rouen, département d'anesthésie-Réanimation du Pr Bertrand Dureuil
2000 - 2001	Médecin Anesthésiste-Réanimateur au CHU de Rouen (vacations)
2001 - 2003	Chef de Clinique – Assistant, département d'Anesthésie 6 Réanimation, CHU de Rouen, Pr Bertrand Dureuil
2004 - 2007	Praticien contractuel puis Praticien Hospitalier, CHU de Strasbourg (Pr Thierry Pottecher)
2007-2012	Maitre de Conférence des Universités – Praticien Hospitalier, CHU de Strasbourg (Pr Thierry Pottecher)
Depuis 2012	Maitre de Conférence des Universités – Praticien Hospitalier, CHU de Strasbourg (Pr Annick Steib et Paul Michel Mertes)

Médecin Anesthésiste-Réanimateur, **responsable de l'Unité de Réanimation chirurgicale Polyvalente**, Nouvel Hôpital Civil, Hôpitaux Universitaires de Strasbourg (depuis 2012).

Membre de la **Commission Médicale d'Établissement** des Hôpitaux Universitaires de Strasbourg (depuis 2011)

- Membre du conseil scientifique (depuis 2011)
  Membre de la commission du médicament et des dispositifs médicaux (2011-2015)

III. Enseignement

A. Certification SIDES, enseignementométrie et types d'enseignement

Certification SIDES (niveau 2) depuis 2014.

Enseignementométrie

Score SIAPS : 300

Au total : 138 heures d'activité liée à l'enseignement en 2015 comprenant 41h heures de cours, 10h de surveillance d'examen et 87 heures de participation à des jurys. Les heures d'enseignement concernent des enseignements à la faculté de médecine en deuxième cycle (6.5h), en troisième cycle (10h), à l'école d'IADE (13h) et dans diverses autres formations (11h).

Ces temps ne prennent en compte ni le temps d'enseignement au lit du malade, ni les temps de formation lors des staffs quotidiens, ni les temps de préparation, de déplacement, de correction de copies, d'organisation ou de gestion des enseignements.

B. Types d'enseignements

- Enseignements premier et second cycle de la faculté de médecine de Strasbourg
- Enseignements du troisième cycle de la faculté de médecine de Strasbourg : DES d'anesthésie-réanimation, DES de chirurgie générale
- Enseignements à l'école d'Infirmier Anesthésiste DE des Hôpitaux Universitaires de Strasbourg
- Enseignements DIU Oncologie Thoracique
- Enseignements pour l'adaptation à l'emploi (IDE en réanimation)*
- Enseignements aux Cours Européens d'Anesthésie-Réanimation*
- Enseignements Post-Universitaires nationaux et internationaux (SFAR, MAPAR, JEPU, ARCOTHOVA, CAPSO, CEEA, ISICEM)* (cf. détails des conférences sur invitation p. 46)

C. Organisation

Enseignement

- Référent pédagogie du Pôle d'anesthésie-réanimation SAMU-SMUR depuis 2010
- Responsable du module optionnel d'anesthésie-réanimation chirurgicale de la faculté de médecine de Strasbourg

Organisation de congrès

- 2e Journée de Biotechnologie de la SFAR : « Biologie délocalisée », en partenariat avec l'Association Française des Ingénieurs Biomédicaux (2009)
- 12 intervenants, 6 sponsors, 75 participants
- « Les gestes qui sauvent », Journée de formation grand public organisée par le Cercle des Anesthésistes Réanimateurs d'Alsace, en partenariat avec le SAMU 67, le SDIS 67 et l'ensemble des associations de secourisme (2009)
- 350 participants
- 3e Journée de Biotechnologie de la SFAR : « Monitorage hémodynamique », en partenariat avec l'Association Française des Ingénieurs Biomédicaux (2010)
- 12 intervenants, 6 sponsors, 125 participants
- Atelier de réanimation de la SFAR à Strasbourg : « Ventilation mécanique »
- 4 intervenants, 30 participants

D. Encadrement

Thèses de Doctorat en Médecine:

1. Évaluation du D-lactate dans le diagnostic de souffrance colique lors de la chirurgie aortique. Guillaume Meyer, 2003, Rouen.

2. Effet de la position du patient sur les vélocités cérébrales au Doppler transcrânien après hémorragie sous-arachnoïdienne. Stéphanie Maison, 2008, Strasbourg.

3. Prise en charge des traumatismes fermés du foie au CHU de Strasbourg. Gaspard Miguel, 2009, Strasbourg.

4. Effets de l'anesthésie sur la fonction rénale après transplantation foie-rein chez les patients hyperoxaluriques. Delphine Vallaud, 2009, Strasbourg.
Travail publié sous forme d'abstract

5. Utilisation du PVI (Pleth Variability Index) pour détecter des variations de volémie efficace lors de la chirurgie hépatique. Bruno Saumade, 2010, Strasbourg.

6. Évaluation des connaissances du grand public en réanimation cardio-pulmonaire : vers quel public orienter en priorité la formation ? Carole Schroeder, 2011, Strasbourg.

7. Pré-oxygénation et ventilation mécanique peropératoire: enquête régionale de pratique. François Fischer, 2011, Strasbourg.

8. Comparaison de l'index de variabilité pléthysmographique et de la pression veineuse centrale lors de la greffe rénale. Lina Jazaerli, 2013, Strasbourg.

9. Manoeuvres de recrutement alvéolaire: comparaison du décubitus dorsal et du décubitus ventral lors de la chirurgie majeure du rachis. Vianney Kieffer, 2015, Strasbourg.

10. Dysfonction primaire de greffon après transplantation pulmonaire à Starsbourg : à propos d'une série de 153 cas. Guillaume Hafner, 2016, Strasbourg

Mémoires de DES :

1. Validation de la mesure de la lactatémie capillaire par microméthode chez les patients en état de choc. Stéphanie Maison, 2008, Strasbourg.
2. Interactions médicamenteuses délétères entre un inhibiteur de pompe à proton et une benzodiazépine. Hervé Kuntzman, 2009, Strasbourg.
3. Comparaison des indices dynamiques de volémie et d'un indice volumétrique pour l'évaluation de la réserve de la précharge en réanimation avec le système PiCCO®. Nawfel Ben Hamouda, 2010, Strasbourg.
4. Concordance entre les deux méthodes de mesure de l'Index Cardiaque par le PiCCO: thermodilution transpulmonaire et pulse contour. Saad Bouzoubaa, 2010, Strasbourg.
5. Différence artério capillaire en lactate dans les états de choc septiques et hémorragiques. Vincent Garcia, 2011, Reims.
6. Reproductibilité de la mesure du débit cardiaque: comparaison entre la thermodilution transpulmonaire et l'échocardiographie transthoracique. Adrien Sprunck, 2011, Reims.
7. Évaluation des bénéfices d'une formation par la simulation parmi les étudiants strasbourgeois en DCEM4. Clément Bongarzone, 2013, Reims.
8. Variation de la différence artério-veineuse en CO2 avec la ventilation alvéolaire. Aude Ruimy, 2014, Nancy.
9. Étude expérimentale: fonctions mitochondriales lors du choc anaphylactique. Walid Oulehri, 2015, Nancy.
10. Les ischémies mésentériques en réanimation : étude de la cohorte strasbourgeoise. Louis Profumo, 2015, Nancy.

Master II (co-direction):

1. Capacités oxydatives de différents organes dans un modèle expérimental de choc anaphylactique. Walid Oulehri, 2014, Strasbourg.
   Papier soumis
2. Protection mitochondriale par le bleu de méthylène lors du choc anaphylactique. Charles Tacquard, en cours.

IV. Recherche

Ma **recherche** est centrée sur **l'étude des états d'agression systémique critique (EASC) liés aux phénomènes d'ischémie-reperfusion et aux états de choc**. Cette thématique est en effet un sujet de préoccupation quotidien tant en anesthésie qu'en réanimation. **Les objectifs** de la recherche clinique et expérimentale sont triples : (1) **caractériser** les états d'agression systémique critiques en particulier développer des outils diagnostiques et de monitorage pertinents ; (2) **étudier les mécanismes** physiopathologiques et les voies de transduction impliquées et (3) **tester des nouveaux traitements** et de nouvelles prises en charge.

Les EASC sont des états caractérisés par une **lésion cellulaire initiale** (agression) suffisamment importante pour mettre en jeu le **système immunitaire inné** et avoir ainsi des **conséquences systémiques** (réponse inflammatoire systémique) pouvant provoquer une ou plusieurs **dysfonctions d'organe et la mort de l'organisme**.

La réponse d'un organisme à une agression comme une lésion tissulaire, une infection ou une anoxie (ischémie-reperfusion) comprend la libération de cytokines inflammatoires et de catécholamines endogènes. La réponse à l'agression est associée à un **état hyper-métabolique** comprenant notamment **une accélération de la glycolyse** [1]. Parallèlement, les troubles hémodynamiques macroscopiques (hypovolémie, vasodilatation, défaillance myocardique) et microcirculatoires peuvent conduire à **une dysoxie cellulaire**. Au total, l'agression cellulaire initiale produit une **crise énergétique majeure** souvent caractérisée par une **hyperlactatémie** [2].

Les **mitochondries** sont essentielles au **métabolisme** cellulaire. Elles produisent en effet la majeure partie de l'énergie (ATP) lors de la phosphorylation oxydative. Elles jouent également un rôle prépondérant dans la régulation de l'ensemble de la vie cellulaire par le biais de la formation des espèces radicalaires de l'oxygène. Enfin, certains auteurs suggèrent que les mitochondries altérées lors de l'agression cellulaire initiale pourraient être la source du signal d'activation du système immunitaire inné (*mitochondrial Damage Associated Molecular Patterns*) [3,4].

A. Etat d'agressions systémique critique (EASC)

1. Situations cliniques

En anesthésie-Réanimation, les EASC sont extrêmement fréquents. Toute lésion cellulaire importante en volume ou en intensité peut entrainer un tel état. Les traumatismes, infections, phénomènes d'ischémie-reperfusion (infarctus du myocarde, accident vasculaire cérébral, ischémie de membres) et, plus généralement, toutes les pathologies associées à un syndrome inflammatoire systémique (par exemple une pancréatite aigüe nécrosante) peuvent générer des EASC.
Le médecin anesthésiste-réanimateur est particulièrement impliqué dans la gestion de ce type de situations (ischémie-reperfusion lors de la chirurgie vasculaire, syndrome inflammatoire systémique post circulation extracorporelle, transplantations d'organes, états de choc) et une action thérapeutique pourrait en limiter les effets délétères.
Ma thématique de recherche clinique et expérimentale est centrée sur deux situations particulièrement fréquentes en anesthésie-réanimation : l'ischémie-reperfusion et les états de choc. Ainsi, j'ai encadré 10 mémoires de DES et 10 thèses de médecine dont la plupart sont des travaux cliniques centrés sur le monitorage et l'optimisation hémodynamique (cf. encadrement de la recherche p. 10-11).

2. Modèles expérimentaux

a. Modèle non létal : ischémie-reperfusion intestinale (IRI)

Ischémie-reperfusion : description d'un paradoxe
L'ischémie peut être définie par la réduction ou l'interruption du débit sanguin dans un territoire vasculaire donné. La baisse du flux sanguin est telle qu'apparaît une inadéquation entre d'une part les apports en oxygène et en substrats énergétiques et, d'autre part, les besoins de la cellule pour son fonctionnement et sa survie. L'ischémie menace rapidement la viabilité des cellules. En quelques heures, les cellules meurent et le tissu nécrose. L'ischémie est un phénomène courant en pathologie humaine : l'infarctus du myocarde est secondaire à une ischémie myocardique, les accidents vasculaires cérébraux sont la conséquence d'une ischémie cérébrale, etc. L'ischémie associée à l'ensemble des maladies cardio-vasculaires est la première cause de mortalité et de morbidité dans les pays occidentaux. L'ischémie est ainsi un phénomène directement lié à la maladie et à la mort.
La reperfusion correspond au retour du flux sanguin, de l'oxygène et des substrats énergétiques dans le territoire vasculaire préalablement ischémié. Si la reperfusion

est suffisamment précoce, certaines peuvent être sauvées et redevenir fonctionnelles. La reperfusion précoce est donc nécessaire pour limiter les conséquences délétères de l'ischémie.
Pourtant, la reperfusion s'accompagne d'une extension des lésions cellulaires. L'extension de la zone de nécrose lors de la reperfusion pourrait compter pour près de 50 % du volume de nécrose final [5]. Lors de la reperfusion, non seulement les lésions cellulaires locales continuent de s'étendre, mais de nombreux facteurs « toxiques » sont diffusés à travers l'ensemble de l'organisme. La reperfusion peut ainsi être responsable de dysfonctions d'organes situés à distance, non impliqués dans l'ischémie initiale [6].
Au final, les conséquences délétères de la reperfusion peuvent dépasser le risque lié à l'ischémie et mettre directement en jeu le pronostic vital des patients. Ce paradoxe est le sujet de très nombreuses recherches depuis plus de 50 ans, depuis les premières descriptions du syndrome de défaillance multiviscérale.

IR intestinale
L'intestin est un organe particulièrement sensible à l'ischémie et à la reperfusion. L'IRI a souvent été décrite comme un élément essentiel à l'emballement du système immunitaire après une agression cellulaire. L'IRI est encore souvent décrite comme étant le moteur du syndrome inflammatoire systémique lors des EASC (concept du *« gut motor »*) [7]. Ce **concept** intègre la fragilité de la barrière intestinale, séparant d'un côté plusieurs milliards de bactéries (1000 milliards de bactéries de la flore intestinale) et d'un autre côté le système immunitaire le plus complexe et le plus développé de notre organisme (*GALT, gut associated lymphoïd tissue*) [8].
Nous avons utilisé deux modèles d'IRI chez le rat Wistar: un modèle d'agression sévère par occlusion de l'aorte supra-cœliaque (40 minutes d'occlusion et 60 minutes de reperfusion) et un modèle d'agression moins sévère par occlusion de l'artère mésentérique supérieure (60 minutes d'occlusion et 60 minutes de reperfusion).

b. Modèle d'agression systémique létal : le choc anaphylactique

L'anaphylaxie est une réaction allergique systémique suraiguë potentiellement mortelle. Le CA est l'expression clinique la plus critique (mortelle) des réactions d'hypersensibilité immédiates, c'est-à-dire survenant dans l'heure suivant l'exposition à l'allergène.
Notre modèle de CA utilise des rats Brown-Norway sensibilisés à l'ovalbumine. Ce modèle reproduit les caractéristiques d'un **CA sévère** : la baisse de la pression artérielle est quasi instantanée (chute de 60% en 3 minutes) et, en l'absence de traitement, le modèle est systématiquement létal en moins de 20 minutes. **Plusieurs aspects du CA ont déjà pu être précisés grâce à ce modèle** [9-11]. **Néanmoins, à notre connaissance, le rôle et le fonctionnement des mitochondries lors du CA n'ont jamais été publiés**.

B. Exposé analytique des travaux

Nous **travaux de recherche expérimentaux et cliniques** nous ont permis:

- de mieux caractériser le rôle du lactate dans le diagnostic de plusieurs EASC et en particulier de préciser la **place du D-lactate** dans le diagnostic des IRI
- d'appréhender le **métabolisme cellulaire et mitochondrial, la réponse inflammatoire systémique et** les **répercussions à distance** de la lésion initiale
- de proposer des **traitements** limitant les effets délétères de l'IRI et du choc anaphylactique.

Plan des études (les tirés à part sont en annexes p.50)

1. Diagnostic des EASC : intérêt des L- et D-lactatémies

1.1. (L-)lactate

1.1.1. Lactate artériel

Etude N° 1

Etude N°2

1.1.2. Lactate capillaire

Etude N°3

Etude N°4

1.2. D-lactate

Etude N°5

Etude N°6

Etude N°7

Synthèse : D-lactate et IRI

2. Physiopathologie

Etude N°1

Etude N°8

Etude N°9

Etude N°10

3. Traitement

Etude N°9

Etude N°10

Etude N°11

1. Diagnostic des EASC : intérêt des L- et D-lactatémies

L- et D-lactate sont les deux isomères du lactate ($C_3H_6O_3$), optiquement différents par la position de leur radical alpha-hydroxyle. Le L-lactate est la forme habituellement mesuré en clinique humaine, notamment en Anesthésie-Réanimation. Par convention, l'appellation « lactate » fait référence au L-lactate.

1.1. (L-) lactate

1.1.1. Lactate artériel

L'hyperlactatémie (artérielle) est souvent associée à l'ischémie mésentérique, si bien que pour certains, une lactatémie normale élimine le diagnostic d'ischémie mésentérique. Les données de la littérature ne confirment pas cette vision simple des choses.

Etude N° 1

Collange O, Charles AL, Lavaux T, Noll E, Bouitbir J, Zoll J, et al., Compartmentalization of Inflammatory Response Following Gut Ischemia Reperfusion.

Eur J Vasc Endovasc Surg, 2015. 49(1): p. 60-5.

L'intestin n'était pas la principale source de lactate lors de l'IRI. Ainsi après une heure d'ischémie et une de reperfusion la lactatémie veineuse mésentérique (3,7±1,8 mmol/L) n'est pas plus élevée que la lactatémie artérielle (4,0±0,3 mmol/L, p>0.05). A notre connaissance, seulement quatre travaux publiés étudient le métabolisme du lactate lors de l'IR-I. Ce nombre modeste est à mettre en perspective avec les 60 000 publications référencées sur PubMed concernant le lactate. Ljungdahl et coll. observent une augmentation importante de la lactatémie veineuse mésentérique (7,07 ± 1,23 *vs.* 2,58 ± 0,39 mmol/L, p<0,05) après seulement 30 minutes d'ischémie [12]. Utilisant un modèle de réduction de 80 % du flux de l'artère mésentérique supérieure, Jakob et coll. montrent que l'augmentation de la lactatémie veineuse mésentérique (> 4 mmol/L) est immédiate et perdure tout au long de l'ischémie intestinale (4h) [13]. Kurimoto et coll. obtiennent le même résultat : la lactatémie veineuse mésentérique est augmentée tout au long de l'ischémie intestinale (> 4 mmol/L, pendant 6h) [14]. Enfin, Bäckström et coll. montrent très clairement que l'hyperlactatémie veineuse mésentérique (> 8 mmol/L) est directement liée à l'hypoxie intestinale. En effet, le rapport lactate/pyruvate est fortement augmenté (> 50) [15]. **Cependant, sur ces quatre études, deux seulement mesurent la lactatémie veineuse mésentérique lors de la reperfusion intestinale**. Ljungdahl et coll. montrent que la différence mésentérico-systémique baisse rapidement (en 30 minutes) lors de la reperfusion, si bien que la lactatémie veineuse n'est plus significativement différente de la lactatémie systémique [12]. De façon plus démonstrative, Bäckström et coll. observent non seulement une baisse de la lactatémie veineuse, mais aussi une normalisation du rapport lactate/pyruvate [15], indiquant que l'hyperlactatémie systémique n'est plus liée à un processus dysoxique, mais plutôt à une augmentation de la glycolyse aérobie [16]. Les résultats obtenus

avec notre modèle d'occlusion de l'artère mésentérique supérieure chez le rat confirment ceux de Ljungdahl [12] et de Bäckström [15] en indiquant qu'à une heure de reperfusion, l'intestin ne produit pas de lactate, **suggérant ainsi que l'intestin n'est plus la source de l'hyperlactatémie systémique**.

Etude N°2

Leone M, Bechis C, Baumstarck K, Ouattara A, Collange O, Augustin P, et al.

Outcome of acute mesenteric ischemia in the intensive care unit: a retrospective, multicenter study of 780 cases.

Intensive Care Med, 2015. 41(4): p. 667-76.

Dans cette étude multicentrique colligeant 780 cas d'ischémie mésentérique aiguë (IMA) recensés dans 46 services de réanimation français, nous avons pu montrer que la L-lactatémie n'était pas nécessaire au diagnostic d'IMA. En effet, 23% des patients présentant une IMA avaient une L-lactatémie normale.

Nous menons actuellement un travail ancillaire en comparant la lactatémie de patient présentant une IMA à celle de patients ne présentant pas d'IMA. Les données préliminaires semblent indiquer qu'à score IGSII identique, les patients présentant une IMA ont une lactatémie plus élevée.

1.1.2. Lactate capillaire

Des analyseurs « portables » utilisent une méthode électrochimique pour mesurer le (L-)lactate [17]. Ils permettent de mesurer la lactatémie capillaire, de façon similaire à la mesure de la glycémie capillaire (hémoglucotest). La comparaison de ces analyseurs portables avec les méthodes de laboratoire habituelles a montré des biais limités [18]. La nature du sang prélevé semble aussi jouer un rôle marginal. En effet, la plupart des études montrent que la lactatémie veineuse ou capillaire est légèrement supérieure à la lactatémie artérielle, mais la concordance entre les différents sites de prélèvement est jugée satisfaisante [18]. Il faut néanmoins rester attentif, car cette concordance n'a pas toujours été testée dans des situations pathologiques, en particulier quand le territoire servant au prélèvement est associé à une vasoconstriction ou à une ischémie.

Etude N° 3

Collange O, Charton A, Greib N, Joshi GP, Schaeffer R, Diemunsch PA, Correlation between arterial and capillary lactate measurements in a porcine hemorrhagic shock model.

J Trauma, 2010. 68(1): p. 32-4.

Lors d'un EASC induit par état de choc hémorragique chez le cochon, la mesure de la L-lactatémie capillaire par microméthode était très bien corrélée à la L-lactatémie artérielle (biais = -0.11±0.2 mmol/L, r^2 = 0.99). Ainsi, cet outil diagnostique simple et portable pourrait être utilisé quand l'accès à un prélèvement artériel ou un laboratoire de biochimie est difficile : prise en charge pré-hospitalière, service d'urgence, bloc

opératoire, réveil. Par ailleurs, le caractère instantané de la mesure en fait un outil particulièrement intéressant dans les situations instables.

Etude N° 4

Noll E, Bouitbir J, Collange O, Zoll J, Charles AL, Thaveau F, et al., Local but not systemic capillary lactate is a reperfusion biomarker in experimental acute limb ischaemia. Eur J Vasc Endovasc Surg, 2012. 43(3): p. 339-40.

Néanmoins, la lactatémie capillaire mesuré au niveau d'un membre ischémié était significativement plus élevée (14,7±1,5 mmol/L) que la lactatémie systémique (6,0 mmol/L ; p=0.015). Il convient ainsi d'interpréter avec prudence la lactatémie capillaire lorsque la circulation périphérique est compromise. Par ailleurs, la lactatémie capillaire et la différence artério-capillaire en lactate (DACL) pourraient être des marqueurs de la vascularisation périphérique.

1.2. D-lactate

Le D-lactate est la forme dextrogyre du lactate habituellement dosé en réanimation. Les deux isomères (L- et D-lactate) diffèrent par la position de leur radical alpha-hydroxyle. Chaque isomère résulte de la réduction du pyruvate par une lactate déshydrogénase (LDH) optiquement spécifique. Ainsi, la formation de L-lactate à partir d'une molécule de pyruvate nécessite une L-LDH, alors qu'une D-LDH est nécessaire pour former du D-lactate (le pyruvate est optiquement neutre). Les mammifères ne possèdent pas de D-LDH et ne peuvent donc pas produire de D-lactate à partir du pyruvate. Néanmoins, le D-lactate peut être formé dans les organismes supérieurs à des concentrations micromolaires à partir du méthylglyoxal par le système des glyoxalases [19]. Dans le sens inverse, le D-lactate peut être oxydé en pyruvate par une D-2-hydroxy-acide-deshydrogénase (D-2-HDH). Étant équipées de D-LDH, les bactéries produisent du D-lactate lors de la fermentation lactique. La présence de quantité mesurable (millimolaire) de D-lactate dans l'organisme humain traduit ainsi un apport exogène. Un dosage positif de D-lactate dans des liquides biologiques normalement stériles (liquide articulaire, liquide pleural ou liquide céphalorachidien) signe leur contamination bactérienne [20,21]. La présence de D-lactate dans le sang peut traduire soit un apport de D-lactate lors d'une dialyse péritonéale [22] soit une translocation du D-lactate produit par les bactéries dans la lumière digestive. Des cas d'acidose lactique ont en effet été rapportés chez des patients porteurs d'un grêle court faisant un écart de régime [23]. Chez ce type de patient, les glucides rapides ne sont pas absorbés par l'intestin grêle. Leur arrivée dans le colon provoque une production massive d'acides organiques (acides gras volatils) et la baisse du pH intraluminal. Dans cet environnement, les lactobacilles se développent facilement et produisent par fermentation lactique du D- et du L-lactate. Cela contribue à acidifier encore la lumière colique et amplifier le phénomène. Une partie du D-lactate et du L-lactate produit dans la lumière intestinale passe dans la circulation veineuse mésentérique. Le L-lactate est rapidement métabolisé en pyruvate par les cellules hépatiques via une L-LDH (néoglucogenèse), alors que le

D-lactate ne peut être que faiblement métabolisé par la D-2-HDH. Quand le taux d'absorption dépasse celui de son métabolisme et de son excrétion, le D-lactate devient mesurable dans le sang et, à terme, l'acidose D-lactique peut être symptomatique.

Le D-lactate a été proposé comme marqueur de l'ischémie mésentérique notamment lors de l'ischémie colique post-chirurgie aortique. Il y a plus de 20 ans Murray et al. montraient dans une étude expérimentale que l'ischémie mésentérique était associée à une augmentation significative et précoce (après 5 minutes d'ischémie) de la D-lactatémie [24]. Les mêmes auteurs ont rapporté dans une série de 31 patients opérés pour un syndrome abdominal aigu que les patients présentant une ischémie colique avaient une D-lactatémie plus élevée [25]. Poeze et al. ont montré que les patients opérés d'un anévrisme de l'aorte abdominale rompu présentant une D-lactatémie augmentée (> 0,20 mmol/L) à l'admission en réanimation avait un risque plus élevé de développer une ischémie colique (sensibilité 82%, spécificité 77%) [26].

Dans le prolongement de ces études, nous avons voulu préciser la place de la D-lactatémie dans le diagnostic de l'IRI.

Etude N°5

Collange O, Veber B, Tamion F, Lavoine A, Plissonnier D,Dureuil B,
Interest of D-lactate as a colic hypoperfusion marker during aortic abdominal aneurysm surgery.
Ann Fr Anesth Reanim, 2006. 25(9): p. 940-6.

La D-lactatémie mesurée chez 10 sujets sains était nulle. Au contraire, la D-lactatémie mesurée chez les patients de réanimation étaient mesurable (140 ± 180 µmol/L) et pouvait être considérée comme légèrement (< 0.,2 mmol/L) augmentée par rapport aux sujets sains. L'analyse rétrospective de notre cohorte de patients suggérait que la D-lactatémie pouvait être plus élevée chez les patients présentant une souffrance intestinale lors de la chirurgie aortique.

Etude N°6

Collange O, Tamion F, Chanel S, Hue G, Richard V, Thuillez C, et al.,
D-lactate is not a reliable marker of gut ischemia-reperfusion in a rat model of supraceliac aortic clamping.
Crit Care Med, 2006. 34(5): p. 1415-9.

Utilisant un modèle de clampage de l'aorte supra-cœliaque chez le rat Wistar (40 minutes de clampage, une heure de reperfusion), nous avons caractérisé l'atteinte intestinale sur le plan tissulaire et sub-cellulaire. Notre modèle associait une diminution de 30% de l'épaisseur de la muqueuse intestinale, une diminution de 45% de la taille des villosités et une ballonisation des mitochondries de cellules intestinales. Au niveau systémique, la reperfusion était accompagnée d'une diminution significative de la pression artérielle moyenne et d'une augmentation franche de la L-lactatémie (7,1±1,6 mmol/L vs. 1,6±0,4 mmol/L, p=0,001) et du TNF-α (213 ± 129 vs. 47 ± 32 pg/mL, p < 0,05). Par contre, la D-lactatémie restait nulle à

15 minutes et à une heure de reperfusion. Ainsi, le D-lactate n'était pas un marqueur fiable de l'IRI dans notre modèle d'IRI.

Etude N°7

Collange O, Tamion F, Meyer N, Quillard M, Kindo M, Hue G, et al.,
Early detection of gut ischemia-reperfusion injury during aortic abdominal aneurysmectomy: a pilot, observational study.
J Cardiothorac Vasc Anesth, 2013. 27(4): p. 690-5.

Lors de cette étude prospective incluant 18 patients opérés à froid d'un anévrysme de l'aorte sous rénale, la perfusion intestinale était monitorée en continue par tonométrie. L'ischémie intestinale était définie par une différence entre le CO_2 intestinal et le CO_2 artériel (ΔCO_2) de plus de 2,6 kPa. La D-lactatémie n'était pas augmentée chez les patients présentant une ischémie intestinale peropératoire (n=6), ni pendant l'intervention ni dans les 24 heures suivantes. Enfin, la D-lactatémie mesurée sur le sang veineux mésentérique prélevé à la fin du clampage aortique n'était pas augmentée.
Cette étude montre que la D-lactatémie n'est pas un marqueur fiable de l'IRI lors de la chirurgie élective de l'aorte abdominale sous rénale.

Synthèse : D-lactate et IRI

Nos travaux expérimentaux et cliniques n'ont pas confirmé les résultats observés par Murray, Poeze et Gunel [24-27]. Gunel et Murray utilisaient des modèles animaux différents du nôtre (respectivement des lapins et des rats Fischer) et aucune de ces études ne caractérisait l'atteinte intestinale. En particulier, le caractère perforatif de la lésion intestinale n'était pas précisé. En cas de lésions perforatives, la péritonite stercorale secondaire vient compliquer et aggraver la lésion intestinale initiale. Ces études ne précisaient pas non plus l'état hémodynamique des sujets présentant une augmentation de la D-lactatémie.
Une étude clinique récente confirme nos résultats : la D-lactatémie n'est pas augmentée (elle est non significativement plus basse) chez les patients présentant une ischémie mésentérique avérée (0,41 mmol/L vs 0,56 mml/L chez les patients sans IMA) [28].
Ces résultats pour le moins contrastés mettent en lumière les approximations du raisonnement physiopathologique reliant le D-lactate à l'ischémie intestinale.
En effet, la transposition de la physiopathologie des acidoses D-lactiques observées chez les patients porteurs de grêle court à celle des ischémies intestinales semble rétrospectivement hasardeuse. Dans le syndrome du grêle court, l'acidose D-lactique n'est pas secondaire à une souffrance colique, mais à l'arrivée massive de sucres rapides dans un colon sain. Actuellement, aucune étude ne montre que l'ischémie mésentérique est associée à une augmentation de production du D-lactate dans la lumière intestinale. Par ailleurs, l'ischémie mésentérique est une pathologie complexe associant généralement une hypovolémie, un syndrome inflammatoire systémique, voire un état de choc septique en cas de perforation digestive et de

péritonite. Il est difficile d'affirmer dans ce type de situation multicompliquée qu'un marqueur biologique est spécifique de la souffrance intestinale.
Le D-lactate pourrait être un bio-marqueur d'infection sévère ou d'un état de choc plutôt que celui que d'une ischémie intestinale. En ce sens, d'autres études ont mis en évidence une augmentation de la D-lactatémie lors des états infectieux. La D-lactatémie est augmentée chez les patients en état de choc septique [29] et expérimentalement lors du syndrome du compartiment abdominal [30]. La D-lactatémie pourrait être le reflet de l'activation d'une voie métabolique alternative à la glycolyse, le système des glyoxalases, traduisant un état de glycolyse accéléré [19]. Si cette hypothèse était vérifiée, le D-lactate pourrait être un marqueur de la dérégulation métabolique systémique, phénomène non spécifique de l'ischémie intestinale.

2. Physiopathologie : réponse inflammatoire et atteinte à distance de l'agression initiale

Etude N° 1

Collange O, Charles AL, Lavaux T, Noll E, Bouitbir J, Zoll J, et al., Compartmentalization of Inflammatory Response Following Gut Ischemia Reperfusion.
Eur J Vasc Endovasc Surg, 2015. 49(1): p. 60-5.

Paradoxalement, lors de l'IRI, l'intestin n'est pas la principale source de cytokines. Par ailleurs, la réponse inflammatoire précoce après IRI est essentiellement anti-inflammatoire et concerne surtout l'IL-10 et l'IL6. Enfin, la réponse inflammatoire est compartimentalisée « organe par organe ». En particulier, c'est surtout le poumon qui surexprime l'IL-10 à la phase initiale de la reperfusion intestinale (20 fois plus que l'intestin et le foie).

Etude N°8

Collange O, Tamion F, Rives N, Thuillez C, Richard V, Dureuil B, et al., Pulmonary apoptosis after supraceliac aorta clamping in a rat model.
J Surg Res, 2005. 129(2): p. 190-5.

L'IRI est précocement associée à une atteinte pulmonaire. Après une heure de reperfusion, l'histologie standard ne montre pas de lésion pulmonaire et l'hématose est préservée. Nous avons néanmoins observé une augmentation de 50% des cellules apoptotiques au niveau pulmonaire. Notre hypothèse est que l'atteinte pulmonaire commence par une apoptose avant de devenir symptomatique. Cette hypothèse suggère que les mitochondries des cellules pulmonaires sont altérées de façon précoce lors de l'IRI.

Etude N°9

Collange O, Charles AL, Noll E, Bouitbir J, Zoll J, Piquard F, et al., Isoflurane anesthesia preserves liver and lung mitochondrial oxidative capacity after gut ischemia-reperfusion. Anesth Analg, 2011. 113(6): p. 1438-41.

L'hypothèse formulée précédemment a été vérifiée lors de cette étude expérimentale. L'IRI est précocement associée une diminution des capacités oxydatives maximales des mitochondries hépatiques (-50% : de 51±11 à 24±45 µmol/O_2/min/g, $p<0.05$) et pulmonaires (-30% : de 23±4 à 16±9 µmol O_2/min/g, $p<0.05$).

Etude N°10

Collange O, Charles AL, Bouitbir J, Chenard MP, Zoll J, Diemunsch P, et al., *Methylene blue protects liver oxidative capacity after gut ischaemia-reperfusion in the rat.* Eur J Vasc Endovasc Surg, 2013. 45(2): p. 168-75.

Cette étude confirme la précocité de l'atteinte mitochondriale à distance de l'agression initiale. Après une heure de reperfusion intestinale, les intestinales sont visibles en histologie standard (hématoxyline éosine) et en méthode TUNEL (fragmentation de l'ADN, signe d'apoptose). Par contre, aucune lésion histologique n'est décelable au niveau hépatique. Néanmoins, les capacités oxydatives des mitochondries hépatiques sont significativement diminuées (-30% : de 50±3 à 35±3 µmolO_2/min/g, $p< 0.01$) parallèlement à une augmentation significative des transaminases hépatiques (ASAT et ALAT).

3. Traitement : protection des capacités oxydatives à distance de l'agression initiale

Lors de l'IRI, l'isoflurane (**étude N°9**) protège les capacités oxydatives des mitochondries hépatiques et pulmonaires lors d'une IRI. Plus précisément, la diminution des capacités oxydatives maximales des mitochondries hépatiques (-50%) et pulmonaires (-30%) était observée chez les rats endormis par kétamine mais pas chez les rats endormis par isoflurane.

Le bleu de méthylène (**étude N°10**) protège également les capacités oxydatives maximales des mitochondries hépatiques après une IRI.

Etude N° 11

Zheng F, Barthel G, Collange O, Montemont C, Thornton SN, Longrois D, et al., Methylene blue and epinephrine: a synergetic association for anaphylactic shock treatment.

Crit Care Med, 2013. 41(1): p. 195-204.

Dans cette étude expérimentale utilisant un modèle de choc anaphylactique, le bleu de méthylène permettait de prolonger la survie des animaux, mais était insuffisant pour corriger à lui seul les perturbations hémodynamiques. Néanmoins, les effets thérapeutiques de l'adrénaline et du bleu de méthylène étaient synergiques. Comparés à l'adrénaline seule, les rats traités par adrénaline plus bleu de méthylène avaient une meilleure pression artérielle moyenne, une meilleure redistribution du flux sanguin cérébral et une meilleure oxygénation cérébrale. Le bleu de méthylène augmentait de façon spécifique le rapport lactate/pyruvate et le glutamate au niveau cérébral et musculaire. Ces observations suggéraient une interaction entre le bleu de méthylène et le métabolisme énergétique cellulaire.

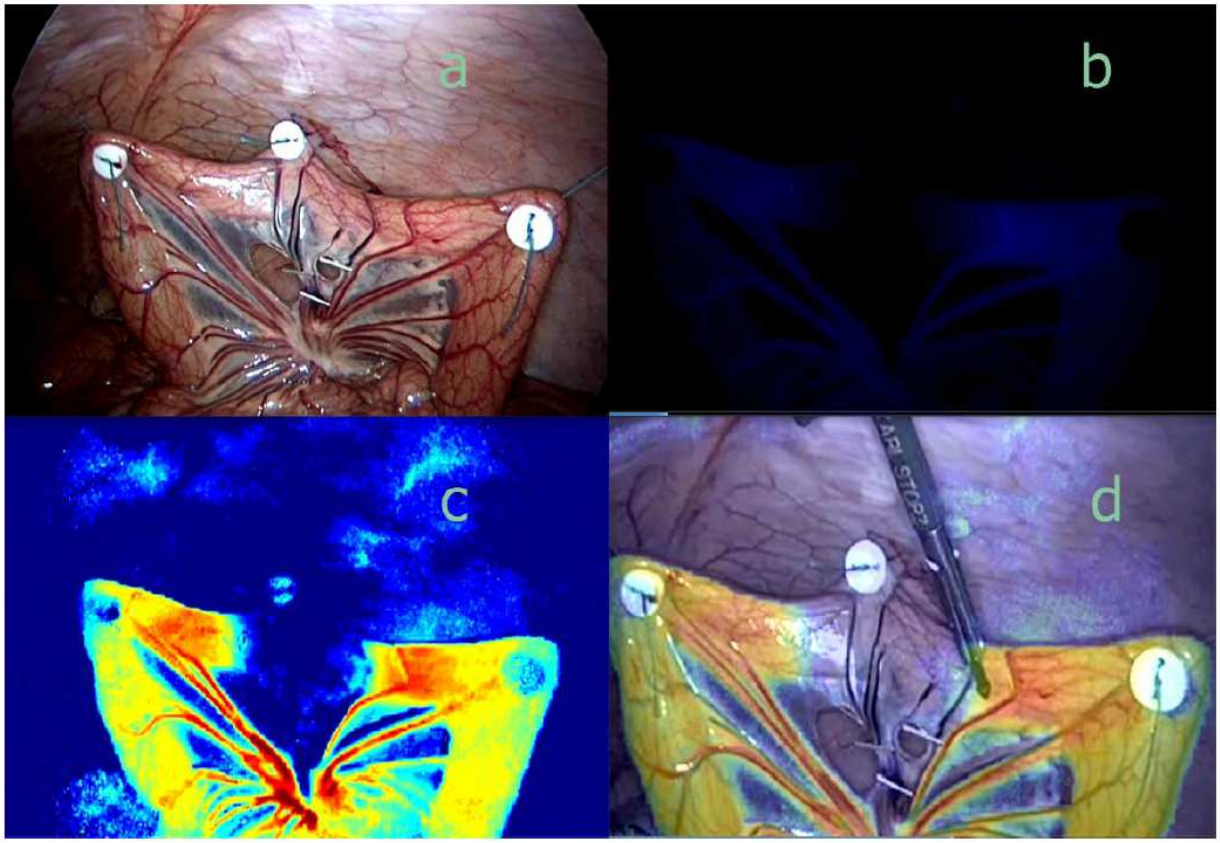
C. Perspectives

Le but est de poursuivre mon travail avec Annick Steib et Paul Michel Mertes, dans l'EA 3072 et aux Hôpitaux Universitaires de Strasbourg et de développer recherche clinique et expérimentale sur les états d'agression systémique critique.

1. Recherche clinique : création d'un « Intestinal Stroke Center ».

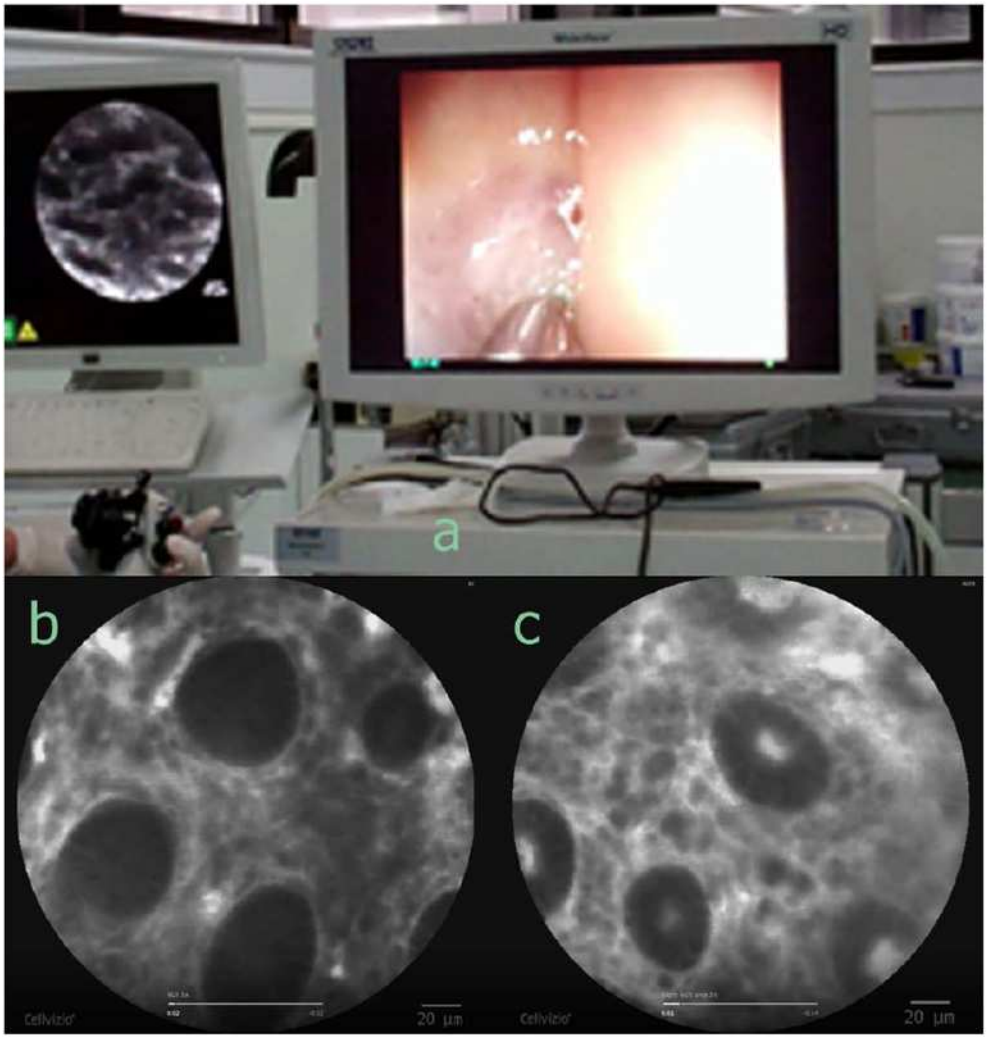
L'objectif est de créer une filière de soin multidisciplinaire pour les patients présentant une ischémie intestinale aigüe. La prise en charge des patients doit être précoce et doit intégrer les dernières innovations diagnostiques et thérapeutiques de notre Institut Hospitalo-Universitaire, en particulier, les techniques diagnostiques développées ces dernières années par le Dr Michele Diana à l'Institut de Recherche contre le Cancer de l'appareil Digestif (IRCAD) : la vidéographie par fluorescence et réalité augmentée (Figure 1) et la microscopie confocale (Figure 2) [31]. Ces techniques pourraient être utilement couplées avec la mesure de la tonométrie intestinale et de la lactatémie capillaire *in situ*. Sur le plan thérapeutique, l'équipe de chirurgie vasculaire de notre CHU développe des techniques de revascularisation endovasculaire innovantes, notamment au sein de Groupe Européen de Recherche sur les Prothèses Appliquées à la Chirurgie Vasculaire (GEPROVAS).
En termes de flux de patients, nous estimons que 60 patients par an pourraient bénéficier de cette filière de soin. En effet, sur les 43 centres contribuant à l'étude publiée en 2015 dans *Intensive Care Medicine* [32], notre service a fourni près du dixième des effectifs totaux (73 patients sur 5 ans). Ainsi, actuellement, près de 15 patients par an sont hospitalisés dans notre service de réanimation (chirurgicale, NHC) pour une ischémie intestinale. Nous estimons que le double de patients est hospitalisé au NHC, soit sans critère de gravité et ne nécessitant pas la réanimation (n=10), soit au contraire en présentant un tableau d'ischémie intestinale dépassée (n=5). Enfin, nous estimons que la création d'un centre d'expertise permettrait de prendre en charge de façon précoce des patients actuellement hospitalisés dans une autre structure de notre CHU (hôpital de Hautepierre), dans d'autres structures de notre GHT et enfin, dans d'autres structures de notre région. Au total, nous estimons que notre filière de soin pourrait concerner 60 patients par an.

Figure 1 : Vidéographie par fluorescence avec réalité augmentée [31]



Evaluation de la perfusion intestinale par vidéographie par fluorescence et Réalité Augmentée utilisant la camera à infrarouge D-Light P développée par Karl Storz (Tuttlingen, Germany) et un logiciel (VR Perfusion) développé à l'IRCAD. La figure montre la validation de la technique sur un modèle d'ischémie intestinale : a) une zone ischémique était créée sur une anse intestinale à l'aide de clips métalliques ; b) après injection de Vert d'Indocyanine la camera D-Light P est positionnée sur modalité infrarouge pour détecter la fluorescence émise par le Vert d'Indocyanine ; c) le logiciel reconstruit l'image sur la base du temps requis pour attendre le pic d'intensité que représente la perfusion du tissu ; d) le modèle virtuel est superposé à l'image réelle pour obtenir de la réalité augmentée et tracer avec précision la région ischémique.

Figure 2 : Microscopie confocale [31]



a) La sonde laser du Cellvizio® est conçue pour être introduite dans les endoscopes classiques et permet d'obtenir en temps réel des images microscopiques de la muqueuse intestinale. Nous avons utilisé le Cellvizio® pour déterminer le niveau de perfusion intestinale, sur la base des modifications précoces de la muqueuse intestinale subséquentes à l'ischémie : en b) visualisation des glandes dans un segment bien perfusé en c) aspect « en cible » des glandes due à l'accumulation de dépôts de fibrine suite à l'ischémie.

2. Recherche expérimentale : choc anaphylactique

En important et adaptant le modèle de CA au sein de notre équipe d'accueil (EA 3072), nous avons pu mesurer les capacités oxydatives des mitochondries musculaires (muscle squelettique et muscle cardiaque) lors du CA. Les résultats montrent que le **CA diminue les capacités oxydatives des mitochondries cardiaques** (-36%, p = 0.04) et **augmente celles des mitochondries musculaires squelettiques** (+73%, p = 0.04). Ces premiers résultats sont encourageants. Ils sont d'une part concordants avec les données de la littérature montrant que le CA est associé à une dysfonction myocardique précoce [33]. D'autre part, ces données précisent que l'effondrement de la PtiO2 musculaire observée lors du CA [11] est probablement dû à un sur-fonctionnement mitochondrial inapproprié. Cet effet semble propre au CA dans la mesure où la plupart des EASC (notamment les autres types de chocs comme le choc septique ou le choc hypovolémique) sont généralement associés à une PtiO2 musculaire normale ou augmentée [34] et à une baisse lente (quelques heures à quelques jours) des capacités oxydatives mitochondriales (concept d'hibernation cellulaire) [35].

Ces données ont fait l'objet du mémoire de master II de Walid Oulehri (actuellement chef de clinique dans notre service) [36] et sont soumises à publication.
Nous réalisons actuellement une deuxième étude caractérisant l'action de plusieurs traitements pouvant améliorer la survie des rats lors du CA. Nous testons ainsi le bleu de méthylène (protection mitochondriale) et un inhibiteur de *platelet activating factor*. Cette étude est en cours et fait l'objet du mémoire de **master II** de Charles Tacquard (interne en anesthésie-réanimation, année recherche 2015-2016).

Notre but est de progresser sur les 3 axes que nous nous sommes fixés :

- **Caractériser le choc anaphylactique** et en particulier le métabolisme cellulaire et mitochondrial myocardique et musculaire. Nous avons développé deux collaborations :
  - Avec l'équipe du Dr David Brasse nous souhaitons explorer le métabolisme des cellules myocardiques lors du choc anaphylactique (CNRS UMR 7178, AMISSA *« A Multimodality Imaging System for Small Animal* », Institut Pluridisciplinaire Hubert Curien, Strasbourg, France).
  - Avec l'équipe du Dr Jamal Bouitbir, nous souhaitons mettre en évidence les activités de fusion et de fission mitochondriale lors du choc anaphylactique (*Clin. Pharmacology and Toxicology, Univ. Hospital Basel, Switzerland*)
- **Etudier les mécanismes et les voies de transduction** opérants lors du choc anaphylactique et en particulier la voie du *Platelet Activating Factor.* Nous avons développé une collaboration avec l'équipe INSERM-UMR S-949 dirigée par le Dr Christian Gachet (« Biologie et pharmacologie des plaquettes sanguines », Strasbourg, France).

- **Tester de nouvelles approches thérapeutiques** comme les inhibiteurs du *Platelet Activating Factor*.

Enfin, nous continuons à développer et perfectionner les outils de monitorage hémodynamique permettant de mieux caractériser les EASC. Nous disposons actuellement d'un **« ban hémodynamique »** permettant le monitorage de la pression artérielle sanglante, de la fréquence cardiaque et de l'analyse de l'ECG, du débit cardiaque par thermodilution, de la mesure de dP/dt du ventricule gauche par sonde Millar, des mesures de la fraction de raccourcissement et du diamètre ventriculaire gauche (mesures échographiques), de la mesure de la pression partielle tissulaire en oxygène (PtiO2), de la mesure tissulaire des métabolites intermédiaires par microdialyse (lactate, pyruvate, glutamate) (collaboration avec le groupe « Choc » - Contrat Avenir Inserm-U961 de l'Université de Nancy). En sus de l'étude du choc anaphylactique, le ban hémodynamique permet d'étudier divers états d'agression systémique comme le choc septique (Dr Julien Pottecher) ou les ischémies aigues de membres (Dr Anne Lejay, MCU-PH en Chirurgie Vasculaire). **Nous avons formé ainsi un axe *« choc et ischémie-reperfusion »* pour la prochaine contractualisation de notre équipe d'accueil**.

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VI. Publications et communications

A. Bibliométrie

40 publications référencées dans PubMed**, score SIGAPS = 444, H-index = 10**

+ 2 publication <u>acceptées :</u>

- Collange O, Wolff V, Cebula H, Pradignac A, Meyer A, Kindo M, Diemunsch P, Proust F, Mertes P, Kremer S. *Spontaneous intracranial hypotension: An etiology for consciousness disorder and coma. Anesthesia & Analgesia.(IF = 3.4, SIGAPS B, 1er auteur, + 24 points SIGAPS)*
- Collange O, Jazaerli L, Lejay A, Biermann C, Caillard S, Moulin B, Chakfé N, Severac F, Schaeffer M, Mertes PM, Steib A. *Intraoperative Pleth Variability Index is linked to delayed graft function after kidney transplantation. Transplantion proceedings*.

+ 1 publication en revision favorable:

Collange O, Garcia V, Kindo M, Meyer N, Maison S, Lavaux T, Mertes PM, Joshi GJ, DIemunsch P. *Comparison of capillary and arterial lactate levels in patients with shock. Anesthesia Critical care and Pain Medicine*.

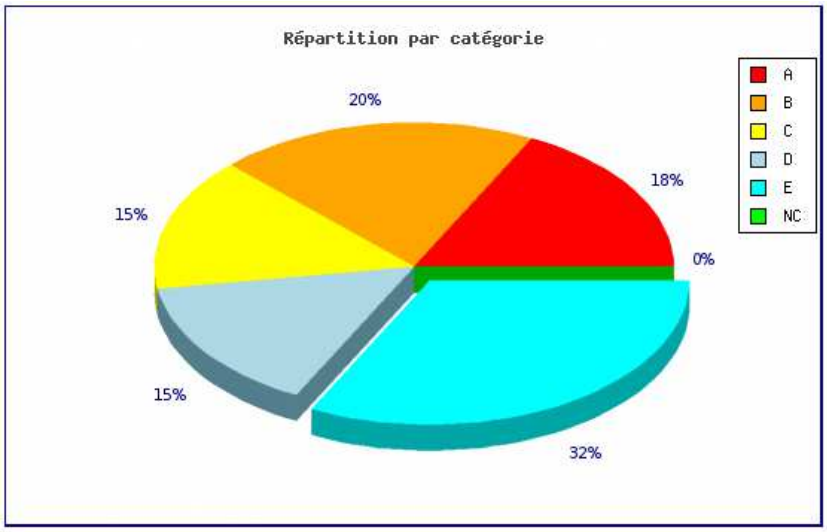
H index

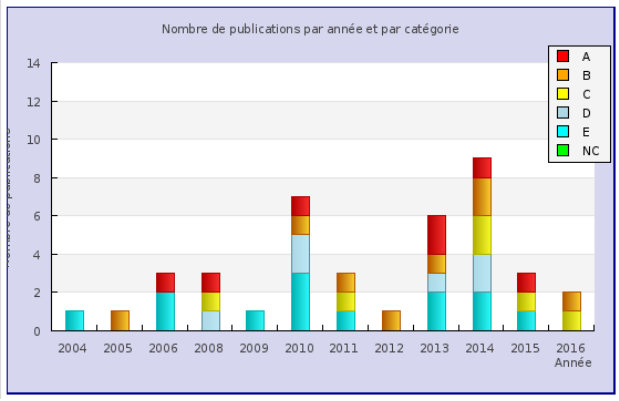
Citations	Toutes	Depuis 2011
Citations	543	446
indice h	10	8
indice i10	10	8

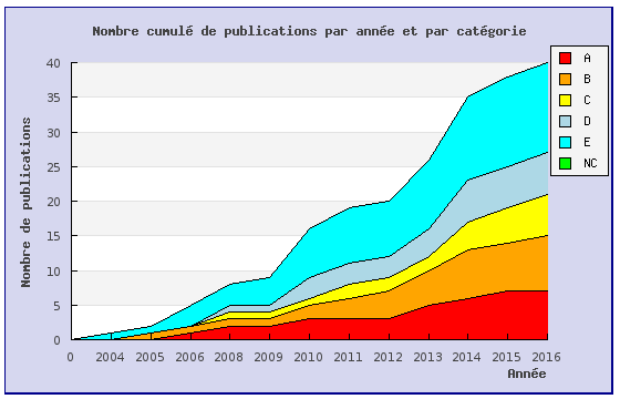
Score SIGAPS : 444

Répartition par catégorie et par année

Période : 2004 - 2016								
Année	**Total**	**A**	**B**	**C**	**D**	**E**	**NC**	**Score**
2004	1	0	0	0	0	1	0	4
2005	1	0	1	0	0	0	0	24
2006	3	1	0	0	0	2	0	42
2007	0	0	0	0	0	0	0	0
2008	3	1	0	1	1	0	0	36
2009	1	0	0	0	0	1	0	6
2010	7	1	1	0	2	3	0	82
2011	3	0	1	1	0	1	0	42
2012	1	0	1	0	0	0	0	12
2013	6	2	1	0	1	2	0	82
2014	9	1	2	2	2	2	0	62
2015	3	1	0	1	0	1	0	24
2016	2	0	1	1	0	0	0	28
Total	**40**	**7**	**8**	**6**	**6**	**13**	**0**	**444**







Nombre de publications par Discipline

Disciplines

Code	Discipline	Nb	%
BA	ANESTHESIOLOGY	20	50.00
YA	SURGERY	7	17.50
DS	CRITICAL CARE MEDICINE	7	17.50
ZD	PERIPHERAL VASCULAR DISEASE	6	15.00
DQ	CARDIAC & CARDIOVASCULAR SYSTEMS	4	10.00
WE	RESPIRATORY SYSTEM	3	7.50
MA	HEMATOLOGY	2	5.00
FF	EMERGENCY MEDICINE	2	5.00
RT	CLINICAL NEUROLOGY	1	2.50
VY	RADIOLOGY, NUCLEAR MEDICINE & MEDICAL IMA	1	2.50

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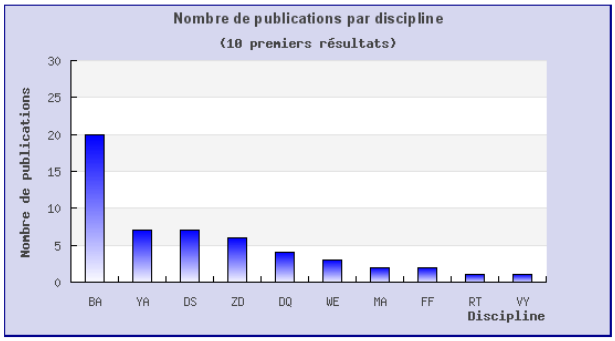

Mesh Terms

MeSH Term	Nb de publications	Nb de Major Topic
Reperfusion Injury	10	7
Lung Diseases	6	4
Shock, Septic	4	4
Lactic Acid	6	4
Gram-Negative Bacterial Infections	4	4
Mesentery	3	3
Aortic Aneurysm, Abdominal	3	3
Ischemia	4	3
Intensive Care Units	4	3
Myocardial Infarction	3	3

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B. Publications originales

42 publications : 40 actuellement référencées dans PubMed, 2 acceptées pur publication
Les 7 publications en 1er ou dernier auteur dans des revues à IF > 3 sont **marquées d'un astérisque ***.

1. **Collange O**, Wolff V, Cebula H, Meyer A, Kindo M, Diemunsch P, Proust F, Mertes PM, Kremer S. *Spontaneous intracranial hypotension: A new etiology for consciousness disorder and coma.* Accepté dans ***Anesthesia & Analgesia*.** (IF=3,42)*

2. **Collange O**, Jazaerli L, Lejay A, Biermann C, Caillard S, Moulin B, Chakfe N, Severac F, Schaeffer M, MertesPM, Steib A. *Intraoperative Pleth Variability Index is linked to delayed graft function after kidney transplantation.* Accepté dans *Transplantation Proceddings*. (IF=0,87)

3. Messas N, Willemain R, Bilger A, Rondeau-Lutz M, Kuhnert C, Mertes PM, Morel O, **Collange O**, *Takotsubo syndrome triggered by acute intermittent porphyria attack: An unusual stressor for catecholamine-induced cardiomyopathy.* **Int J Cardiol, 2016.** 207: p. 28-30.(IF=4,03)*

4. Haas E, Fischer F, Levy F, Degirmenci S-E, Grunebaum L, Kindo M, **Collange O**, Mertes P-M,Steib A, *Identifying optimal heparin management during cardiopulmonary bypass in obese patients: A prospective observational comparative study.* Eur J Anaesthesiol, 2016. (IF=2,94)

5. Zheng F, **Collange O**, Davidson J, Barthel G, Oulehri W, Thornton SN, Longrois D, Levy B, Audibert G, Malinovsky JM,Mertes PM, *Epinephrine, compared with arginine vasopressin, is associated with similar haemodynamic effects but significantly improved brain oxygenation in the early phase of anaphylactic shock in rats: An experimental study.* Eur J Anaesthesiol, 2015. 32(8): p. 563-70.(IF=2.94)

6. Noll E, Fischer F, **Collange O**, Ohlman P,Steib A, *Neuraxial catheter and antithrombotic therapy for myocardial infarction: When can we remove it?* Anaesth Crit Care Pain Med, 2015 (IF=0.84)

7. Leone M, Bechis C, Baumstarck K, Ouattara A, **Collange O**, Augustin P, Annane D, Arbelot C, Asehnoune K, Baldesi O, Bourcier S, Delapierre L, Demory D, Hengy B, Ichai C, Kipnis E, Brasdefer E, Lasocki S, Legrand M, Mimoz O, Rimmele T, Aliane J, Bertrand PM, Bruder N, Klasen F, Friou E, Levy B, Martinez O, Peytel E, Piton A, Richter E, Toufik K, Vogler MC, Wallet

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9. Tacquard C, **Collange O**, Olland A, Degot T,Steib A, *Post-intubation tracheal rupture: poor healing of the tracheal wall.* Can J Anaesth, 2014. 61(4): p. 357-61.(IF=2.53)

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C. Travaux de recherche en cours de publication

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D. Publications didactiques

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6. **Collange O**, Boet S, Freys G. *Problèmes ventilatoires du sujet obèse*. in Mise au Point en Anesthésie Réanimation. 2009. Paris: MAPAR éditions.
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E. Résumés scientifiques

Résumés scientifiques présentés lors congrès internationaux

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4. Jazaerli L, Heshmati N, **Collange O**, Biermann C, Lejay A, Muller C, Sauleau E, Mertes M,Steib A. *Intraoperative Pleth Variability Index Predicts Delayed Graft Function*. in American Society of anesthesiologists,. 2013. San Francisco, USA.
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Résumés scientifiques présentés lors de congrès nationaux

1. Tacquard C, **Collange O**, Gomis P, Petitpain N, Malinovsky JM, Steib A,Mertes M. *Réactions d'hypersensibilité immédiate per anesthésiques en France entre 2011 et 2012 : résultats de la 10ème enquête du GERAP*. in *Conférence Francophone d'Allergologie*. 2016.
2. Tacquard C, **Collange O**, Oulehri W, Charles AL, Zoll J, Geny B,Mertes M. *Effects of epinephrine and ABT-491 on left ventricle mitochondrial oxidative capacity during anaphylactic shock in sensitized-rats, a preliminary study*. in *Congrès de Physiologie et de Biologie Intégrative*. 2015. Strabourg.
3. Pottecher J, **Collange O**. *Septic shock resuscitation: time for a new paradigm*. in *Congrès de Physiologie et de Biologie Intégrative*. 2015. Strasbourg.
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16. Jeannot M, Mahoudeau G, Boet S, **Collange O**, Forestier C, Stojeba N, Ilie A,Pottecher T, *Intérêt d'un vidéolaryngoscope pour l'apprentissage de l'intubation orotrachéale par des étudiants en médecine novices.*, in *5e Forum International Francophone de Pédagogie des Sciences de la Santé* 2010: Québec, Canada.
17. Flicoteaux H, Castelain V, **Collange O**, Kummerlen C, Lebas B, Martinez C, Dhar A, Herbrecht J, Hasselmann, Pottecher T,Schneider F, *Suivi prospectif d'une cohorte de patients admis en réanimation pour coma et influence sur le nombre de prélèvements d'organes réalisé*, in *38e Congrès de la Société de Réanimation de Langue Française*. 2010: Paris. p. S42.
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F. Conférences sur invitation

Conférences internationales

1. Workshop: Airway Management. 29th International Symposium on Intensive care and Emergency Medicine, 2009, Bruxelles, Belgique.
2. Ventilation mécanique peropératoire. Société d'Anesthésie de Charleroi, 2015, Bruxelles, Belgique.
3. Cardiogenic Shock. Committee for European Education in Anesthesiology, 2010, Kosice, Slovaquie
4. Lactate in the ICU. Committee for European Education in Anesthesiology, 2010, Kosice, Slovaquie
5. Anesthesia for laparoscopic surgery, IRCAD-European Institute of TeleSurgery. 2005 et 2006, Strasbourg.

Conférences nationales

1. Lactate en Anesthésie – Réanimation, Congrès national d'Anesthésie et de réanimation, 2016, Paris.
2. Traumatismes thoraciques, Congrès du Collège de Réanimation et d'Urgence des Hôpitaux Extra-Universitaires de France (CREUF), 2016, Mulhouse.
3. Traumatisme trachéal. ARCOTHOVA, 2016, Nantes.
4. Canules double courant sous ECMO, ventilation spontanée et mobilisation. Consensus, Actulalité et Perspectives en Suppléance d'Organes, 2015, Bordeaux.
5. Le lactate capillaire comme marqueur de l'hypoperfusion périphérique. Congrès national d'Anesthésie et de Réanimation, 2014, Paris.
7. Choc inflammatoire: cytopathie ou mitochondriopathie? Congrès national d'Anesthésie et de Réanimation, 2014, Paris.
8. Anesthésie et mitochondries. Congrès national d'Anesthésie et de Réanimation, 2014, Paris.
9. Transplantation pulmonaire: comment conduire l'évaluation préopératoire? ARCOTHOVA, 2014, Tours.
10. Le monitorage hémodynamique lors de la greffe pulmonaire. ARCOTHOVA 2013, Marseille.
11. Bio-impédancemétrie, NICO et les autres. 3e Journée de Biotechnologie de la SFAR, 2010, Paris.
12. Lactates. 2e Journée de Biotechnologie de la SFAR, 2009, Paris.
13. Problèmes ventilatoires du sujet obèse. Mise au Point en Anesthésie Réanimation, 2009, Paris.
14. Ventilation periopératoire du patient obèse: en pression ou en volume? Journées d'Enseignement Post Universitaire d'Anesthésie-Réanimation, 2008, Paris.
15. Controverse : Intubation prolongée vs. trachéotomie précoce. Congrès national d'Anesthésie et de Réanimation, 2005, Paris.

Conférences Régionales

1. Anesthésie pour patient hémodynamiquement instable. Fondation Européenne d'Enseignement en Anesthésiologie, 2016, Strasbourg.

2. Concepts d'optimisation hémodynamique. Enseignement post-universitaire, 2015, Strasbourg.

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ANNEXES : Tirés à part des études

Etude N°1

Collange O, Charles AL, Lavaux T, Noll E, Bouitbir J, Zoll J, Chakfe N, Mertes PM, Geny B,
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Compartmentalization of Inflammatory Response Following Gut Ischemia Reperfusion ☆

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WHAT THIS PAPER ADDS

Gut ischemia reperfusion is thought to trigger the multi-organ failure that is associated with aortic and mesenteric vascular surgery. Each organ (gut, liver, and lung) was found to have a specific cytokine expression profile, suggesting that the inflammatory response is compartmentalized. Therapies focusing on remote organs that are specifically involved in the inflammatory response, for example lung protective ventilation strategies, might limit the deleterious effects of the inflammatory cascade.

Objective/background: Gut ischemia reperfusion (IR) is thought to trigger systemic inflammation, multiple organ failure, and death. The aim of this study was to investigate inflammatory responses in blood and in two target organs after gut IR.

Methods: This was a controlled animal study. Adult male Wistar rats were randomized into two groups of eight rats: control group and gut IR group (60 minutes of superior mesenteric artery occlusion followed by 60 minutes of reperfusion). Lactate and four cytokines (tumor necrosis factor-α, interleukin [IL]-1β, IL-6, and IL-10) were measured in mesenteric and systemic blood. The relative gene expression of these cytokines was determined by real time polymerase chain reaction in the gut, liver, and lung.

Results: Gut IR significantly increased lactate levels in mesenteric (0.9 ± 0.4 vs. 3.7 ± 1.8 mmol/L; $p < .001$) and in systemic blood (1.3 ± 0.2 vs. 4.0 ± 0.3 mmol/L; $p < .001$). Gut IR also increased the levels of four cytokines in mesenteric and systemic blood. IL-6 and IL-10 were the main circulating cytokines; there were no significant differences between mesenteric and systemic cytokine levels. IL-10 was upregulated mainly in the lung, suggesting that this organ could play a major role during gut reperfusion.

Conclusion: The predominance of IL-10 over other cytokines in plasma and the dissimilar organ responses, especially of the lung, might be a basis for the design of therapies, for example lung protective ventilation strategies, to limit the deleterious effects of the inflammatory cascade. A multi-organ protective approach might involve gut directed therapies, protective ventilation, hemodynamic optimization, and hydric balance.

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INTRODUCTION

Acute mesenteric ischemia is of major concern following impairment of the mesenteric circulation. It is associated with significant mortality and morbidity rates that have remained largely unchanged over the last few decades.[1–3] Gut reperfusion leads to the production of numerous mediators, such as lactate, cytokines, reactive oxygen metabolites, pro-inflammatory cytokines, and nitric monoxide, and also activates many enzymes.[4] Interactions between polymorphonuclear neutrophils and endothelial cells appear to play a key role in inducing damage in distant organs (the "gut origin of inflammation" hypothesis).[5,6] This secondary, inflammatory and distant organ injury is the leading cause of death in critically ill patients.[7–9] However, data on the site and kinetics of cytokine production and on the balance between pro- and anti-inflammatory responses are scarce.[10] Washout of toxic substances from the gut during reperfusion might explain multi-organ failure, and

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there is some evidence that the gut can become a cytokine generating organ after ischemia reperfusion (IR). The gut may also be influenced by inflammation and be a target of stress caused by severe insults such as sepsis, trauma, shock, and infection. The mechanism of the initiation, propagation and effects of inflammation on remote organs after gut IR has still not been fully elucidated.

Elucidating the relationship between gut IR, inflammatory response, and organ failure could have practical implications and help define new therapeutic strategies. Gut directed therapies such as early enteral feeding and, to a lesser extent, gut decontamination or probiotic therapies may help limit the systemic impact of gut IR.[11] However, a better understanding of the physiopathology of the inflammatory response after gut IR is needed in order to be able to devise novel multi-organ protective therapies. The aim of this study was to determine systemic, mesenteric, and organ inflammatory response in an animal model of gut IR.

MATERIALS AND METHODS

Ethical statement

Procedures were conducted in accordance with international guidelines for the care and use of laboratory animals.[12] The study was approved by the animal care committee of the University of Strasbourg, Strasbourg, France (CREMEAS authorization no. AL/03/11/06/09).

Animals

Adult male Wistar rats weighing between 330 and 380 g (Depré, Saint Doulchard, France) were used. They were housed under climate controlled conditions (22 ± 2 °C) on a 12:12 hour photoperiod, and were provided food and water *ad libitum*.

Experimental procedure

Sixteen rats were randomized into two groups of eight (control group, gut IR group) using sealed envelopes. Anesthesia was induced by an intraperitoneal (IP) injection of 20 mg/100 g of ketamine (Imalgène 1000, Merial, France) and maintained by IP injection of 3 mg/100 g of ketamine every 30 minutes under spontaneous ventilation. The rats were placed in a supine position on heating pads to maintain body temperature at 37 °C (rectal temperature probe). After midline abdominal incision, the superior mesenteric artery was isolated at its origin and occluded with an atraumatic clamp for 60 minutes followed by reperfusion for 60 minutes, as previously described.[13,14] Gut ischemia was confirmed by a complete stop of pulsation to the mesenteric arcade and by intestinal color change, and gut reperfusion was confirmed by the reappearance of pulsation and color. Control animals underwent sham surgery without superior mesenteric artery occlusion. Anesthetized animals were euthanized by exsanguination.

Blood and tissue sampling

All study parameters were determined at the end of reperfusion (gut IR group) or an equivalent lapse of time (control group). Mesenteric and systemic blood (2 mL) were sampled simultaneously from the superior mesenteric vein and the abdominal aorta, respectively. Immediately after blood withdrawal, fragments of the last ileal loop (2 cm wide ± 7 g), segment VI of the liver (± 1.7 g) and the lower lobe of the right lung (± 1.2 g) were harvested and separately frozen at −80 °C for subsequent determination of cytokine gene expression.

Blood lactate

Lactate was considered as a quantitative marker of hypoxia and a lactate level <2 mmol/L was considered as normal. Lactate was measured by a micromethod on a Lactate Pro device (LT1710; Arkray, KGK, Japan).

Determination of plasma cytokine levels

Plasma tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 levels were used as markers of inflammatory response to the IR insult. TNF-α and IL-1β are considered to be pro-inflammatory cytokines, and IL-10 is considered to be an anti-inflammatory cytokine. IL-6 may be a pro- or anti-inflammatory cytokine. The TNF-α/IL-10 and the IL-1β/IL-10 ratios were used to measure the balance between pro- and anti-inflammatory cytokines.[15]

Serum obtained by centrifugation (3500 *g* for 15 minutes) was stored at −80 °C for later measurement of cytokine levels using Luminex technology (Austin, TX, USA) and kits from Millipore (Milliplex Map Immunoassay, Molsheim, France) according to the manufacturers' instructions. Briefly, a standard curve for each of the target proteins was prepared by serial dilution in assay buffer. Serum samples were diluted 1:4. Standards and samples were incubated in a 96-well filter-bottom plate with target-specific microbeads for 2 hours at room temperature with shaking. Following the incubation, microbeads were washed three times using the wash buffer provided in the kit and a vacuum manifold. Biotinylated antibodies were added and incubated for 1 hour, and detected using a streptavidin−phycoerythrin conjugate. Following extensive washing, samples were analyzed on a Luminex plate reader. All samples were analyzed in duplicate and the concentrations calculated by comparison to the appropriate standard curve.

Relative cytokine gene expression in gut, liver, and lung

Total RNA was isolated from each tissue using RNeasy columns (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. The complementary DNA (cDNA) synthesis reaction was performed with 1 µg of total mRNA and 100 U of SuperScript II reverse transcriptase (InVitroGen, Paris, France) with random hexamers in a final volume of 20 µL, according to the manufacturer's instructions. The quality of the total RNA was assessed by the intensity of 28S and 18S bands after denaturing agarose electrophoresis. The RNA concentration was determined by

ultraviolet spectrophotometry. One round of cDNA synthesis was performed in a thermal cycler (65 °C for 5 minutes before adding reverse transcriptase mix, 42 °C for 50 minutes and 65 °C for 5 minutes). Real time polymerase chain reaction (RT-PCR) was performed according to the manufacturer's instructions (Qiagen, Courtaboeuf, France). QuantiTect Primers (Qiagen) were used to assay TNF-α, IL-1β, IL-6, and IL-10. Relative gene expression was quantified using the 2-ΔΔCT method and glyceraldehyde 3-phosphate dehydrogenase as gene reporter.[16]

Statistical methods

Results were expressed as means ± SD. Variable values for the gut IR and control groups, as measured on systemic and mesenteric blood, were compared using repeated measures analysis of variance (ANOVA) with one between-subjects factor and one within-subjects factor. Lung, liver, and gut RT-PCR values were compared using repeated measures ANOVA with one within-subjects factor after logarithmic transformation of the data (data initially on a multiplicative scale). Post hoc tests of mean lung, liver, and gut values were performed using the Bonferroni correction.

p-Values $< .05$ were considered statistically significant. Statistical analysis was performed using GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA, USA) and SPSS 12.0 for Windows (IBM, Armonk, NY, USA).

RESULTS

Lactate levels

Lactate levels in the gut IR group were significantly higher than those in the control group, whether in mesenteric or systemic blood (Table 1). Mesenteric and systemic lactate levels did not differ significantly within the control or within the gut IR group.

Plasma cytokine levels and pro-/anti-inflammatory balance

The levels of all four cytokines were significantly higher in the gut IR group than in the control group, whether in systemic or mesenteric blood (Table 1). There was no significant difference between mesenteric and systemic cytokine levels within each group.

Calculation of the TNF-α/IL-10 ratio was not always possible because of zero TNF-α values in both groups. When calculation was possible, the ratio indicated that IL-10 always predominated over TNF-α whether in mesenteric or systemic blood, and whether in the control or gut IR group. IL-10 also predominated over IL-1β in both mesenteric and systemic blood in both groups (Table 1). IL-10 predominated more markedly over IL-1βin the gut IR group than in the control group (Table 1).

Table 1. Lactate and cytokine levels in mesenteric and systemic blood.

	Origin	Controls	After gut IR	*p*
Lactate (mmol/L)	Mesenteric	0.9 ± 0.4	3.7 ± 1.8	<.01
	Systemic	1.3 ± 0.4	4.0 ± 1.0	<.01
TNF-α (ng/L)	Mesenteric	8 ± 13	32 ± 18	.01
	Systemic	10 ± 10	27 ± 17	.04
IL-1β (ng/L)	Mesenteric	11 ± 4	19 ± 7	.01
	Systemic	11 ± 7	36 ± 28	.02
IL-6 (ng/L)	Mesenteric	201 ± 189	759 ± 613	<.01
	Systemic	171 ± 200	395 ± 176	.03
IL-10 (ng/L)	Mesenteric	540 ± 856	3519 ± 1837	<.01
	Systemic	597 ± 999	3115 ± 1782	<.01
IL-1β/IL-10	Mesenteric	0.09 ± 0.04	0.006 ± 0.001	.05
	Systemic	0.26 ± 0.15	0.018 ± 0.008	.09

Note. Values are expressed as means ± SD for absolute values and as means ± SEM for ratios. IR = ischemia reperfusion; TNF = tumor necrosis factor; IL = interleukin.

Relative gene expression of cytokines in gut, liver, and lung

After gut IR, cytokine mRNA (all four cytokines) increased in almost all organs. IL-10 expression tended to decrease in the liver (−0.13 ± 0.18, arbitrary unit of relative gene expression). Upregulation differed significantly between the gut, liver, and lung ($p < .01$) (Fig. 1). TNF-α was upregulated to a lesser extent in the lung (1.15 ± 0.95, 2.33 ± 0.24 and 0.17 ± 0.14, respectively; $p = .05$), whereas IL-10 was upregulated significantly more in the lung than other organs (0.1 ± 0.4, −0.13 ± 0.18, and 2.36 ± 0.26, respectively; $p < .01$). IL-1β was upregulated more in the liver and lung than gut (0.94 ± 0.42, 2.2 ± 0.25, and 2.6 ± 0.27, respectively; $p = .02$). IL-6 was upregulated to fairly similar extents in all three organs (2.07 ± 0.45, 3.34 ± 0.48, and 2.65 ± 0.24, respectively; $p = .01$).

DISCUSSION

After 1 hour of gut ischemia and 1 hour of reperfusion in the rat model, the plasma levels of all four cytokines increased, with IL-6 and IL-10 being the main circulating cytokines. Gene expression of all four cytokines increased in the three organs under study (gut, liver, and lung) but to

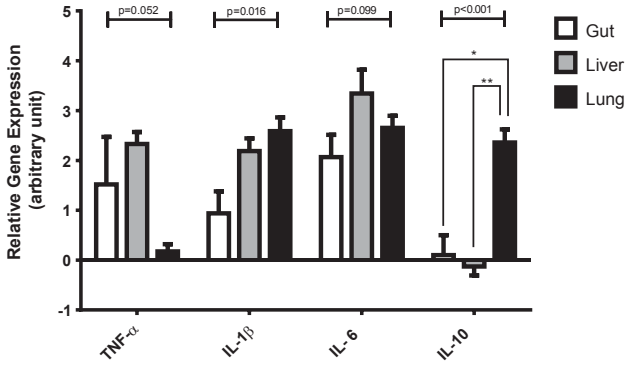

Figure 1. Cytokine gene expression (real time polymerase chain reaction) in gut, liver, and lung after gut ischemic reperfusion (IR). Profile plots showing 95% Bonferroni intervals for the difference in mRNA expression of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 between organ pairs after gut IR. IL-10 gene expression was significantly higher in the lung than gut or liver (*, **$p < .01$). *Note.* Results expressed in relative gene expression arbitrary unit.

different extents according to organ. IL-10 upregulation was highest in the lung compared with gut and liver.

There was almost no difference between mesenteric and systemic lactate levels, indicating that the gut is probably not the main source of lactate after IR in rats. Sources such as muscle or circulating white blood cells may thus explain inflammation related hyperlactatemia.[17,18] In the rat model, after 1 hour of reperfusion, the increase in plasma lactate was probably due to systemic inflammation rather than to gut hypoxia.

The relatively low level of TNF-α supports the findings of Grotz et al.[19] Using a similar rat model with superior mesenteric artery occlusion, these authors recorded a significant increase in mucosal and serosal TNF-α levels after 75 minutes of occlusion but not after 45 minutes of occlusion and 30 minutes of reperfusion. In the sham group of Grotz et al.,[19] the SD exceeded the mean value (66.9 $\pm$ 75.3 pg/mL), suggesting low, even null, TNF-α values, as in the present study. Likewise, the authors found no difference between mesenteric and systemic levels of TNF-α and IL-6.[19] One hour of reperfusion in the present study may have been too short to observe a TNF-α peak, although a significant TNF-α peak was observed 1 hour after mild gut IR during elective infrarenal aortic aneurysm surgery (183 $\pm$ 53 ng/L).[20]

The low IL-1β/IL-10 ratio recorded in the control group in the present study indicated that, under normal conditions (laparotomy under ketamine anesthesia), the cytokine balance is in favor of the anti-inflammatory cytokine IL-10. After IR, this ratio decreased in both mesenteric and systemic blood in further favor of IL-10 (55-fold more than the level of IL-1β in systemic blood), refuting the accepted view that the initial inflammatory response after gut IR is predominantly pro-inflammatory but supporting experimental findings showing that IL-10 is the predominant circulating cytokine after gut IR. The inflammatory response after gut IR is complex. According to Cryer,[21] the exaggerated pro-inflammatory response probably coexists with an exaggerated counter-inflammatory response. According to Moldawer et al.,[15] the situation is more complicated than a pro- followed by an anti-inflammatory response.

Whether the gut is a source of cytokines after gut IR is a matter of controversy and has undergone little study. Cabie et al. observed that mesenteric TNF-α levels were higher than systemic levels in patients undergoing aortic surgery after bowel manipulation and after aortic cross clamp removal[22]; however, aortic cross clamping is not a true model of gut IR. The results of Cabie et al. are thus not directly comparable with those of the present study.[22] Prospective studies using routine sigmoidoscopy or pHi-guided sigmoidoscopy have reported a 5—9% incidence of colon ischemia after elective surgery and a 15—60% incidence after surgery for ruptured abdominal aortic aneurysm.[23] However, on abdominal aortic occlusion, ischemia is probably greater in the limbs than in the gut. Limb IR is known to increase cytokine levels significantly.[24]

Cytokine gene expression in the present study was organ specific and did not reflect plasma cytokine levels, supporting the concept of inflammatory compartmentalization.[25] Evidence for compartmentalization of cytokine gene expression during inflammation comes from several studies: (i) during human septic shock, injured tissue expressed pro-inflammatory cytokines, whereas mainly anti-inflammatory cytokines were present in plasma[26]; (ii) the expression of 15 sepsis related genes in rats was upregulated in the liver and downregulated in the spleen[27]; (iii) TNF-α production on lipopolysaccharide injection in mice was organ specific[28]; (iv) in a human model, 45 minutes of duodenal ischemia enhanced tissue mRNA expression of IL-6, IL-8, and TNF-α, and increased the levels of IL-6 and IL-8 in systemic blood. The IL-6 blood levels in the study of Grootjans et al. were similar to those found in the present study ($\pm$500 ng/L).[29]

In the present study, gut IR activated the lung immune system after only 1 hour of reperfusion. Mechanical ventilation also activates the lung immune system, leading to lung inflammation and a systemic inflammatory response (biotrauma), which can contribute to multiple organ failure.[30] It is a known cause of ventilator induced lung injury.[31] The accumulation of two sources of inflammatory activation might be highly detrimental to the lung and point to a need for protective ventilation. Lung protective ventilation usually refers to the use of low tidal volumes and positive end expiratory pressure, and may also include the use of recruitment maneuvers (periodic hyperinflation of the lungs). This ventilation strategy has been shown to reduce mortality among patients with the acute respiratory distress syndrome,[32] and to improve clinical outcomes in intermediate and high risk patients undergoing major abdominal (non-vascular) surgery.[33] Although further clarification of the relationship between cytokine kinetics and lung dysfunction is needed, the results of the present study support the design of a clinical study comparing protective and standard ventilation during vascular surgery, in particular when surgery involves gut IR.

The main limitations of the present study are lack of kinetic data and the small sample size. Cytokine gene expression and production vary with time, and the cytokine peak, whether in tissue or blood, may have been missed. Cytokine levels were measured after 1 hour of reperfusion because 1 hour of ischemia and 1 hour of reperfusion is a commonly used gut IR model, and because this corresponded to the inflammatory peak in an earlier clinical study.[21] An animal model of 45 minutes of supraceliac aortic occlusion and 1 hour of reperfusion led to a greater increase in lactate.[34] Unfortunately, the design of the present study, which included lung and liver harvesting, did not allow multiple measurements to be made on the same animal.

CONCLUSIONS

In summary, gut IR significantly increased lactate levels. IL-6 and IL-10 were the main circulating cytokines after gut IR. Cytokine gene upregulation differed in the gut, liver, and lung (i.e., was organ specific), suggesting compartmentalization of

the inflammatory response to gut IR. The predominance of IL-10 over other cytokines in plasma and the dissimilar organ responses, especially of the lung, might be a basis for the design of therapies, for example lung protective ventilation strategies, to limit the deleterious effects of the inflammatory cascade. A multi-organ protective approach might involve gut directed therapies, protective ventilation, hemodynamic optimization, and hydric balance.

CONFLICT OF INTEREST

None.

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Etude N°2

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ORIGINAL

Outcome of acute mesenteric ischemia in the intensive care unit: a retrospective, multicenter study of 780 cases

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Take-home message: Acute mesenteric ischemia was associated with a 58 % death rate in ICU patients. Age and severity score at diagnosis were risk factors for mortality; plasma lactate concentration above 2.7 mmol/l was also an independent risk factor.

For the AtlanRea and AzuRea Collaborative Network Investigators; members are listed in the "Appendix".

Electronic supplementary material
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Abstract *Background:* In the intensive care unit (ICU), the outcomes of patients with acute mesenteric ischemia (AMI) are poorly documented. This study aimed to determine the risk factors for death in ICU patients with AMI. *Methods:* A retrospective, observational, non-interventional, multicenter study was conducted in 43 ICUs of 38 public institutions in France. From January 2008 to December 2013, all adult patients with a diagnosis of AMI during their hospitalization in ICU were included in a database. The diagnosis was confirmed by at least one of three procedures (computed tomography scan, gastrointestinal endoscopy, or upon surgery). To determine factors associated with ICU death, we established a logistic regression model. Recursive partitioning analysis was applied to construct a decision tree regarding risk factors and their interactions most critical to determining outcomes. *Results:* The death rate of the 780 included patients was 58 %. Being older, having a higher sequential organ failure assessment (SOFA) severity score at diagnosis, and a plasma lactate concentration over 2.7 mmol/l at diagnosis were independent risk factors of ICU mortality. In contrast, having a prior history of peripheral vascular disease or an initial surgical treatment were independent protective factors against ICU mortality. Using age and SOFA severity score, we established an ICU mortality score at diagnosis based on the cutoffs provided by recursive partitioning analysis. Probability of survival was statistically different ($p < 0.001$) between patients with a score from 0 to 2 and those with a score of 3 and 4. *Conclusion:* Acute mesenteric ischemia in ICU patients was associated with a 58 % ICU death rate. Age and SOFA severity score at diagnosis were risk factors for mortality. Plasma lactate concentration over 2.7 mmol/l was also an independent risk factor, but values in the normal range did not exclude the diagnosis of AMI.

Keywords Ischemia · Mesenteric · Occlusion · Lactate · Surgery

Introduction

Acute mesenteric ischemia (AMI) is subdivided into several forms according to the mechanism of inadequate blood flow. Arterial emboli are responsible for approximately 50 % of cases. Most arterial emboli are cardiac in origin, resulting in a dramatic onset of symptoms. Arterial thrombosis constitutes 25–30 % of all ischemic events. Arterial thrombosis develops in patients with severe atherosclerotic disease. Most patients can tolerate major visceral artery obstruction because of the slow progressive nature of atherosclerosis. Approximately 20 % of patients with AMI have nonocclusive mesenteric ischemia, probably related to low cardiac output associated with diffuse mesenteric vasoconstriction [1].

The outcomes of ICU patients with AMI are poorly reported. The sparse data available related to AMI concerns patients undergoing surgery. The operative mortality of patients with AMI ranges from 26 to 72 % [2]. In these surgical patients, the risk factors for death include preoperative do not resuscitate orders, open wounds, low albumin, contaminated versus clean-contaminated cases, and poor functional status [3]. Thus, AMI is associated with high mortality. Early diagnosis and extensive knowledge of risk factors for death are important issues, which by being addressed may contribute to decreased mortality [1–3]. However, previous studies did not clearly identify patients either diagnosed with AMI in the ICU or requiring ICU.

Improving the knowledge about the outcomes of ICU patients with AMI would allow one to provide accurate information to both patients and their relatives, planning appropriate management and designing future clinical trials. We hypothesized that AMI is associated with high mortality in ICU patients. Therefore, the aim of our study was to assess the mortality associated with AMI in ICU patients and the risk factors associated with ICU mortality. Finally, we developed a score aimed at predicting ICU mortality in patients with AMI.

Methods

Study design and population

We conducted a retrospective, observational, non-interventional, multicenter study in 43 ICUs from 38 public healthcare institutions. These 43 institutions belonged to different networks of ICUs. Of note, initially, 46 ICUs were contacted and three units declined to participate. All adult patients with a diagnosis of AMI were screened. Retrospectively from December 2013, each participating ICU included all consecutive patients with a diagnosis of AMI until reaching a maximum of 25 inclusions. The inclusion period ranged from January 2008 to December 2013. Patients were included if at least one of the three diagnostic procedures (computed tomography scan, gastrointestinal endoscopy, or surgery) supported the diagnosis of AMI.

Ethics and consent

As an observational, non-interventional, retrospective study, according to French legislation (articles L.1121-1 paragraph 1 and R1121-2, Public Health Code), neither informed consent nor approval of the ethics committee was required to use data from patient records for an epidemiologic study. All data were collected anonymously from medical records only. Data representing patient identifiers were not collected.

Data collection

Cases of AMI were identified using the French national healthcare system administrative coding for diagnosis (K550). The data were retrospectively collected from patient records, either electronic or paper. At ICU admission, we collected demographics, reasons for admission, simplified acute physiology score (SAPS) II, and presence of peripheral vascular disease or cancer.

From admission to AMI diagnosis, we collected the following data: time between ICU admission and AMI diagnosis, recent vascular surgery, use of vasopressors in the 24 h prior to AMI diagnosis, type of vasoactive drugs, maximal dose of vasoactive drug, any type of shock 10 days prior to AMI diagnosis, anticoagulation (type), atrial fibrillation, duration of mechanical ventilation prior to AMI diagnosis, and route of feeding (enteral or parenteral).

On the day of AMI diagnosis, we collected the diagnostic procedure(s) for AMI (requiring written diagnosis of AMI on the reports of computed tomography (CT) scan, gastrointestinal (GI) endoscopy, or surgery), date of diagnosis, type of ischemia (following aortic surgery/intravascular procedures or spontaneous), administration of antimicrobial treatment, sequential organ failure assessment score (SOFA) [4], and plasma lactate concentration. For each patient, we also identified four events reflecting organ failure: use of renal replacement therapy (renal failure), need for inotrope or central venous oxygen saturation below 70 % (heart failure), platelet count under 100 G/l (coagulation failure), and prothrombin time ratio under 50 % (liver failure).

From the day of AMI diagnosis to the ICU discharge (or ICU death), we reported the duration of antimicrobial treatment, duration of vasoactive drug support, maximum dosage of vasoactive drug, initial surgical procedure (i.e., in the first 24 h of AMI diagnosis), any additional diagnostic imaging procedures, anticoagulation (type), plasma lactate concentration measured 24 h after the AMI diagnosis, duration of mechanical ventilation following AMI diagnosis, route of feeding (enteral or parenteral), and ICU outcome (survivors or nonsurvivors).

Statistical analysis

Statistical analyses were performed using the SPSS 15.0 software package (SPSS Inc., Chicago, IL). For continuous and ordinal variables, data were expressed as mean with standard deviation or median with interquartile range. Two groups were defined according to ICU outcome, namely survivors or nonsurvivors. The comparison between the two groups was performed on continuous variables using Student *t* tests or Mann–Whitney tests and on qualitative variables using Chi square or Fisher's exact tests.

The discriminative performance of plasma lactate concentration at diagnosis, plasma lactate concentration at 24 h, and the change in plasma lactate concentrations between diagnosis and 24 h were assessed through receiver operating characteristics (ROC) curve analysis. The optimal cutoff values were defined by the value of the Youden index (sensitivity + specificity − 1). Kaplan–Meier survival analyses were performed to estimate the probability of survival following ICU admission. Comparisons of probabilities of survival were performed using log-rank test according to plasma lactate concentration levels ($\leq$3, >3 and $\leq$9, and >9 mmol/l) and number of organ failures (0–2 vs. 3–4).

To determine factors potentially associated with ICU death, a logistic regression model was established using nine variables: age, prior history of cancer, prior history of peripheral vascular disease, shock in the 10 days prior to AMI diagnosis, SOFA at AMI diagnosis, plasma lactate concentration at AMI diagnosis, antimicrobial administration, initial surgical treatment, and time between ICU admission and AMI diagnosis. The variables relevant to the model were selected by their clinical and/or statistical relevance (previously reported as risk factors and/or $p < 0.05$ from the univariate analysis). The final model expressed the odds ratios (OR) and 95 % confidence intervals (CI).

ICU mortality score

Recursive partitioning analysis (RPA) was applied to determine outcome groups of ICU survival from the major well-known risk factors using the RPART routine in R software (Package 'rpart', Recursive Partitioning and Regression Trees. 2014) [5]. RPA is a method of classification providing homogeneous groups of individuals based on the status of the outcome (ICU nonsurvivor or ICU survivor). This method creates a decision tree according to risk factors and their interactions that are most important in determining outcome [6]. The optimal cutoffs of the variables were provided by the RPA. The initial continuous variables were transformed into categorical variables from these cutoffs; points were arbitrarily allocated to each category to be as intuitive as possible. The total score ranged from 0 to 4 (lower to higher risk of ICU mortality). The discriminative performance of the score was determined from ROC analysis. The optimal cutoff value was defined by the Youden index. Kaplan–Meier survival analysis was performed to estimate the probability of survival following ICU admission, and comparison between individuals under and over the optimal cutoff value was assessed using the log-rank test.

Results

In 43 ICUs, 780 patients were included in the database. ICU death occurred in 454 (58 %) patients. The in-hospital death rate was 63 %. Each ICU included 19 ± 13 patients. Patient characteristics are reported in Table 1. At ICU admission, patients were 71 (61–79) years of age, with a median SAPS II of 59 (46–74). Concerning prior medical history, peripheral arterial disease, vascular surgery, and cancer were reported in 44, 32, and 21 % of patients, respectively. In the 24 h prior to AMI diagnosis, vasopressors were administered to 44 % of patients. Anticoagulants were used in 51 % of patients. Feeding was given via either enteral (49 %) or parenteral (12 %) routes.

The time between ICU admission and AMI diagnosis was 0 (0–1) day in survivors and 1 (0–3) day in nonsurvivors ($p = 0.1$). At the AMI diagnosis, atrial fibrillation was found in 25 % of patients. It was identified in 23 % of patients with "spontaneous" AMI and 33 % of those with AMI occurring after vascular surgery ($p = 0.04$). The median SOFA score was 10 (7–13). The mean plasma lactate concentration was 4.0 ± 3.3 mmol/l in survivors and 6.6 ± 5.1 mmol/l in nonsurvivors ($p < 0.001$). Mean plasma lactate was under 2 mmol/l in 23 % of patients overall and 16 % of nonsurvivors. The best cutoff value of plasma lactate concentration at diagnosis of AMI to discriminate survivors from nonsurvivors was 2.7 mmol/l (Fig. 1a). At this threshold, plasma lactate had a sensitivity of 77 %, a specificity of 50 %, and a Youden index of 0.27. At 24 h, the best cutoff value was 3.9 mmol/l with a sensitivity of 60 %, a specificity of 83 %, and a Youden index of 0.43. In contrast, the variations of plasma lactate concentrations between diagnosis and 24 h did not differ between survivors and nonsurvivors (Fig. 1b). As shown by Fig. 2a, survival differed between patients according to plasma lactate concentration measured at diagnosis of AMI. Of note, the plasma lactate concentration decreased in patients undergoing surgery and increased in those not undergoing surgery, although the difference did not reach statistical significance ($p = 0.48$).

Concerning diagnostic procedures for AMI, CT scan, surgery, and endoscopy were used in 58, 27, and 15 % of patients, respectively. Antibiotics, vasopressors, and anticoagulants were used in 79, 79, and 72 % of patients, respectively. The duration of antibiotic treatment was 6.5 ± 5.8 days. Following AMI diagnosis, heart failure, liver failure, thrombocytopenia, and renal replacement therapy were reported in 31, 46, 28, and 45 % of patients, respectively (Table 2). Figure 2b shows that the cumulative number of these events impacted survival. Time to death after diagnosis was 11 (9–13) days.

Several variables differed between ICU survivors and nonsurvivors. Using a logistic regression model comprising nine risk factors (age, prior history of cancer, prior history of peripheral vascular disease, shock in the 10 days prior to AMI diagnosis, SOFA at diagnosis, plasma lactate concentration at diagnosis, antimicrobial administration, initial surgical treatment, time from ICU admission to AMI diagnosis), we identified five independent variables associated with increased risk of ICU death (Table 3). Being older, having a higher SOFA score at AMI diagnosis, and plasma lactate concentration over 2.7 mmol/l (optimal cutoff value) at AMI diagnosis were independent risk factors of ICU mortality. In contrast, having a prior history of peripheral vascular disease and an initial surgical treatment were independent protective factors against ICU mortality. In Supplemental Tables 1 and 2 we identified the results of the univariate analysis in the 176 patients who did not undergo initial surgical treatment and the 604 patients undergoing initial surgical treatment.

Using age and SOFA score, we developed an ICU mortality score at diagnosis based on the cutoffs provided by the RPA method (Supplemental Table 3). Plasma lactate concentrations, history of peripheral vascular disease, and initial treatment were excluded from the score because they could not adequately discriminate patient outcome given the low number of patients per group (less than 20). Probability of survival was statistically different ($p < 0.001$) between individuals with a score from 0 to 2 and those with a score of 3 and 4 (Fig. 2c).

Table 1 Features of patients according to their outcomes in intensive care unit

	MD	Total ($n = 780$)	Survivors ($n = 326$)	Nonsurvivors ($n = 454$)	p
Demographics					
Gender					
Male (%)	0	58	58	58	0.961
Age (years)					
Mean ± SD	0	69 ± 14	66 ± 15	71 ± 12	0.001
History					
PVD (%)	2	44	44	43	0.904
Cancer (%)	1	21	15	25	0.001
Vascular surgery (%)	0	32	35	29	0.07
Reasons for ICU admission					
Medical (%)	0	30	18	38	0.001
Planned surgery (%)		13	14	13	
Urgent surgery (%)		54	66	46	
Trauma (%)		3	3	3	
SAPS II					
Median (IQR)	15	59 (46–74)	48 (37–62)	66 (53–80)	0.001
Diagnosis day					
Shock (<10 days) (%)	17	38	32.7	41.9	0.01
Diagnosis					
CT scan (%)	4	58	63	54	0.016
GI endoscopy (%)		15	16	15	
Surgery (%)		27	21	31	
Type of ischemia					
No surgery (%)	0	73	71	74	0.320
Aorta surgery (%)		27	28	26	
Cardiac surgery (%)		0.5	0.9	0.2	
Atrial fibrillation (%)	2	25	26	25	0.873
SOFA					
Median (IQR)	74	10 (7–13)	8 (4–11)	11 (9–14)	0.001
Lactate (mmol/l)					
Mean ± SD	76	5.6 ± 3.6	4.0 ± 3.3	6.6 ± 5.1	0.001
Platelet (G/l)					
Mean ± SD	15	185 ± 130	214 ± 140	165 ± 117	0.001
ICU stay					
Lactate 24 h (mmol/l)					
Mean ± SD	159	5.1 ± 5.1	2.8 ± 2.3	7.1 ± 5.7	0.001
Heart failure (%)					
Inotrope or $ScvO_2 < 70$ %	32	31	21	37	0.001
Liver failure (%)					
PT < 50 % or jaundice	10	46	30	58	0.001
Renal failure (%)					
RRT	2	45	33	55	0.001

CT computed tomography, *GI* gastrointestinal, *ICU* intensive care unit, *IQR* interquartile range, *MD* missing data, *p p* value between survivors and nonsurvivors, *PT* prothrombin time, *PVD* peripheral vascular disease, *RRT* renal replacement therapy, *SAPS* simplified acute physiology score, $ScvO_2$ central venous oxygen saturation, *SD* standard deviation, *SOFA* sequential organ failure assessment

Discussion

From the present database, 58 % of ICU patients with AMI did not survive their ICU stay. Variables, which were associated with death, included age, SOFA score at admission, and plasma lactate concentration above 2.7 mmol/l at diagnosis of AMI. Prior history of peripheral vascular disease and initial surgical treatment of AMI were protective. In contrast, the administration of antibiotics or anticoagulants was not associated with improved survival. To our knowledge, this is the first multicenter series of ICU patients with AMI that is available in the literature. In addition, we provide a score predictive of ICU mortality in these patients, which may assist in informing patients and family or be used as part of the medical decision-making process.

The ICU mortality rate of ICU patients with AMI that we found (58 %) can only be compared to rates reported in other settings. Previously reported operative mortality of AMI ranged from 26 to 72 % with a pooled mortality rate of 47 % (95 % CI 40–54 %) [2]. However, this series did not specifically include ICU patients. The authors stated that the mortality rate for missed mesenteric ischemia that did not undergo surgery was close to 100 % [2]. In our study, 14 % of patients who did not undergo

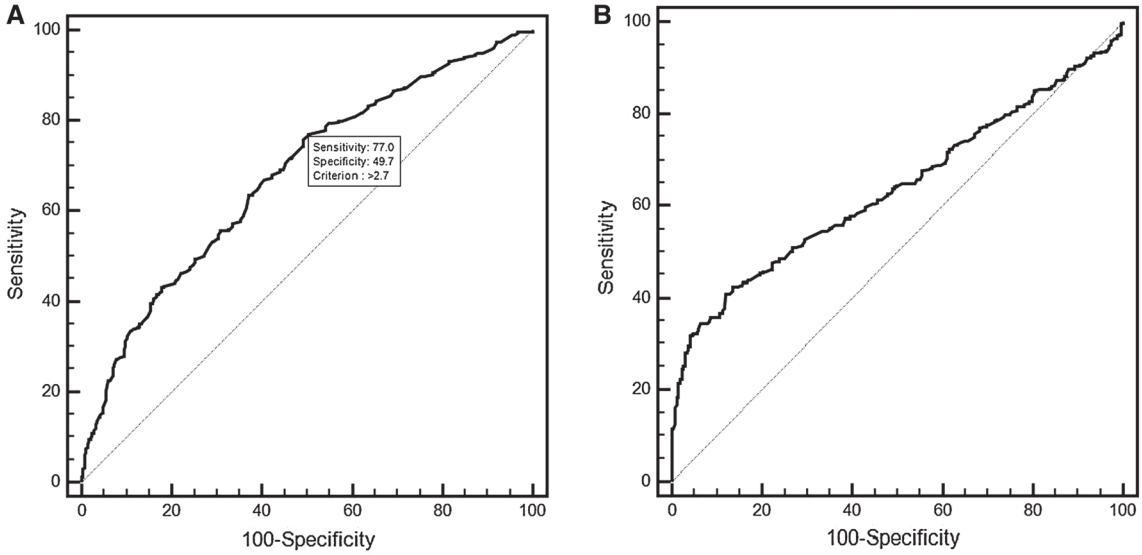

Fig. 1 Intensive care unit (ICU) mortality: receiver operating characteristics curves of **a** plasma lactate concentration at diagnosis and **b** change of plasma lactate concentration between diagnosis and 24 h

surgery survived during their ICU stay. This is probably related to nonocclusive forms of AMI. Similarly, the protection conferred by a prior history of peripheral vascular disease is also probably related to the type of AMI. In such patients, one can speculate that the disease was related to progressive thrombosis, allowing the development of important collaterals [1].

Several independent factors of mortality and protective factors have already been reported. As elsewhere [7–9], we confirmed that advanced age is an independent risk factor of mortality. Initial surgical management of AMI, i.e., performed within the first 24 h of AMI diagnosis, was associated with improved outcomes. Surgery for AMI was performed in 86 % of survivors and 71 % of nonsurvivors, resection representing 57 % of procedures. This finding is in line with previous studies showing that delay in surgery was associated with impaired outcomes [1–3]. In contrast, in the entire cohort, the time elapsed from ICU admission to AMI diagnosis was not associated with mortality rate.

Plasma lactate concentrations can successfully predict the outcomes of AMI in ICU patients. The best level for prediction of ICU death was 2.7 mmol/l. However, even if an increased plasma lactate concentration was an independent factor of mortality, the performance of plasma lactate appears disappointing. One should note that the plasma lactate concentrations remained under 2 mmol/l in 16 % of nonsurvivors. Therefore, normal plasma lactate concentrations cannot exclude the diagnosis of AMI and should not be taken into account in the diagnostic process. Nor did lactate variations over time have any added value, although we observed a trend in plasma concentration decrease from diagnosis to 24 h in patients undergoing surgery and increase in those who did not undergo surgery. Finally, as a result of the retrospective observational design of the study, we could not measure D-lactate, which may be useful [1, 10].

Although univariate analysis showed that the rates of individual organ failures were higher in the nonsurvivors than in the survivors, in line with previous reports of liver and renal failure associated with poor outcomes in AMI [2], multivariate analysis did not confirm any one organ failure as an independent factor. However, the cumulative number of organ failures strongly impacted the outcomes. The SOFA score was higher in the nonsurvivors than in the survivors, which has not been reported elsewhere since it is a score for predicting outcomes of ICU patients only [11]. One should note that the use of this score increases the risk of re-analyzing previously utilized data in various abstracted forms. We then established a composite score including both age and SOFA score for facilitating the prediction of outcomes at the bedside. Of note, in our study heart failure was defined as the inotrope requirement or a central venous oxygen saturation below 70 %. Since central venous oxygen saturation depends on cardiac output, oxygen consumption, hemoglobin level, and arterial oxygen saturation, our definition supposed that patients were adequately resuscitated regarding preload, hemoglobin level, and arterial oxygen saturation [12]. Heart failure, shock, and vasopressor can generate the conditions for developing nonocclusive mesenteric ischemia [1]. Finally, the rate of enteral nutrition was higher in survivors than in nonsurvivors, supporting once again the use of this route in the ICU patients [13].

Several variables commonly thought to influence outcome in the ICU did not differ between survivors and

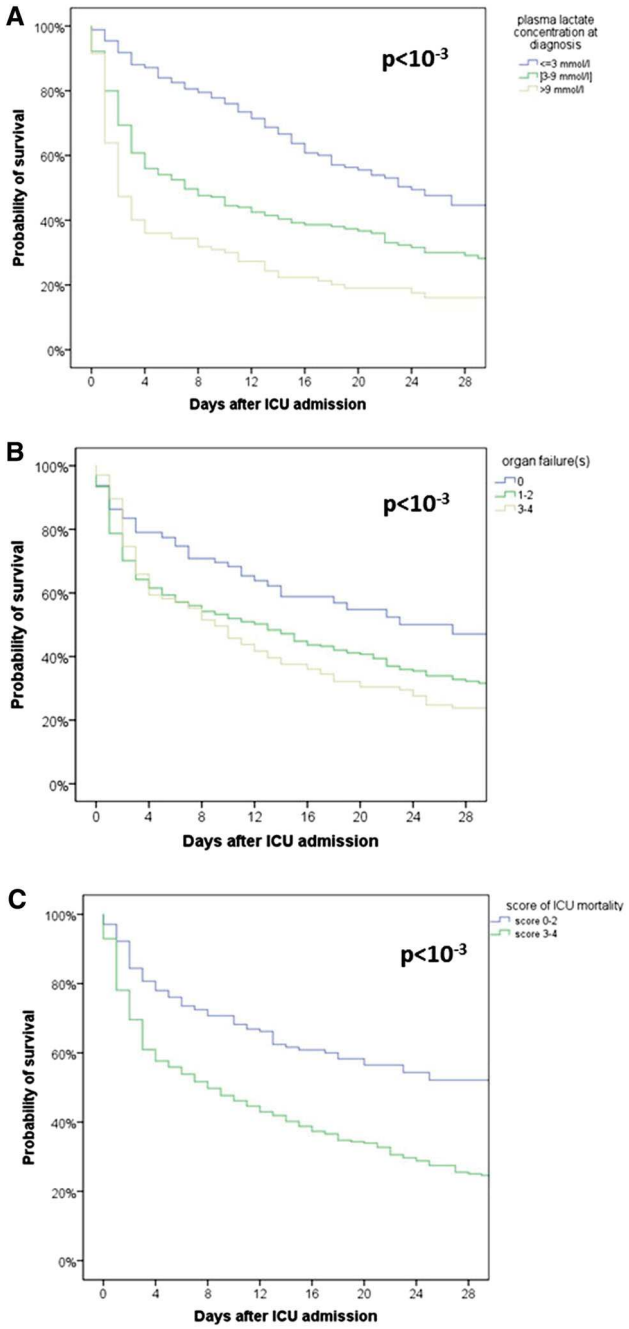

Fig. 2 Probabilities of intensive care unit (ICU) survival according to **a** the plasma lactate concentration at diagnosis, **b** the number of organ failures, and **c** the score of ICU mortality

nonsurvivors. Antibiotics were used in 79 % of patients with AMI, including 82 % of ICU survivors and 77 % of ICU nonsurvivors Interestingly, multivariate analysis did not show significant difference in terms of mortality. While such data cannot serve to decide whether or not antibiotics should be administered in patients with AMI, they raise questions as to their systematic use [14]. Use of vasopressors both before and after the diagnosis of AMI was more frequent in ICU nonsurvivors than in survivors. Vasopressor use in itself was not identified as an independent factor in the multivariate analysis, although it may play a role since it is taken into account in the SOFA score, which was an independent factor. Before the diagnosis of AMI, anticoagulants were used in 51 % of patients. This finding underlines that it is not reasonable to exclude this diagnosis in patients on anticoagulant therapy. In terms of mortality, no difference was observed between survivors and nonsurvivors. In contrast, after AMI diagnosis, anticoagulants were used in 91 % of survivors and 58 % of nonsurvivors. However, the use of anticoagulants was not an independent protective factor for mortality.

In our study, CT scan, surgery, and GI endoscopy were performed to confirm AMI in 58, 27, and 15 % of cases. CT scan findings were used to diagnose AMI in 63 % of survivors and 54 % of nonsurvivors. The CT scan findings of AMI depend on its origin, bowel perfusion status, and the degree of ischemia [15]. In our database, we did not collect CT scan features. The AMI was diagnosed during surgery in 21 % of survivors and 31 % of nonsurvivors. GI endoscopy led to diagnoses only of ischemic colitis. For unclear reasons, the outcome seemed impaired when AMI was diagnosed during surgery [16]. That might reflect a difference in the severity of patients, since this finding was not confirmed in the multivariate analysis.

We have to acknowledge several study limitations. As the data were retrospectively collected, we cannot determine the actual causes of death. Specifically, we did not collect data on the do not resuscitate orders, which were probably frequent in this population. The type of AMI was poorly reported, although this feature may be of interest in terms of outcomes as suggested by our data. We could not discriminate AMI due to arterial occlusions from AMI due to venous occlusions; however, this difference changes the management of patients. In addition, we did not collect data on several confounding factors such as history of hypertension, stroke, and coronary artery disease. Nor did we seek clinical or other features that had given rise to the suspected diagnosis of AMI. For instance, our data cannot discriminate if atrial fibrillation was an acute event or a chronic disease. Likewise, we did not collect on other relevant variables such as blood pressure or duration of hypotension although they are included to some degree in the SOFA score. Finally, we did not determine the delay between AMI diagnosis and surgery although surgical timing is probably one of the critical end-points in patients with AMI as it was reported elsewhere as an independent risk factor of death [1, 2, 16]. However, indirectly and only as a trend, we showed that plasma lactate concentration tended to increase in the patients who did not undergo surgery, whereas a decrease was observed in those undergoing surgery. Finally, our

Table 2 Treatments of patients with acute mesenteric ischemia according to their outcomes in intensive care unit

	MD	Total ($n = 780$)	Survivors ($n = 326$)	Nonsurvivors ($n = 454$)	p
Management at diagnosis					
Initial surgery (%)					
No	0	23	14	29	0.001
Yes		77	86	71	
Type of surgery					
Resection	0	442	229	213	
Bypass		22	17	5	
Thrombectomy		2	2	0	
CT scan (%)					
Yes	0	1.9	1.8	2.0	
Antibiotic at diagnosis (%)	0	79	82	77	0.147
Antibiotic duration (days)					
619 patients, mean ± SD		6.5 ± 5.8	8.7 ± 4.9	4.8 ± 6.0	0.001
Mechanical ventilation (days)					
702 patients, mean ± SD		8.8 ± 11.8	10.3 ± 12.9	7.9 ± 11.0	0.012
Before diagnosis					
Vasopressor (%)	0	44	29	55	0.001
Anticoagulants (%)	2	51	49	52	0.512
Mechanical ventilation (days)					
319 patients, mean ± SD		5.9 ± 8.7	5.9 ± 10.2	5.9 ± 8.1	0.97
Enteral nutrition (%)	7	49	54	46	0.021
Parenteral nutrition (%)	7	12	9	15	0.017
After diagnosis					
Vasopressor (%)	22	79	73	83	0.001
Anticoagulants (%)	6	72	91	58	0.001
Mechanical ventilation (days)					
671 patients, mean ± SD		6.6 ± 9.4	8.6 ± 9.7	5.1 ± 8.9	0.001
Enteral nutrition (%)	8	37	65	16	0.001
Parenteral nutrition (%)	7	45	67	29	0.001

CT computed tomography, *MD* missing data, *SD* standard deviation

Table 3 Risk factors of ICU mortality

	OR	95 % CI	p
Age at admission (class: 10 years)	1.27	1.11–1.46	<0.001
History of cancer (ref: no)	1.56	0.97–2.49	0.065
History of peripheral vascular disease (ref: no)	0.56	0.38–0.82	0.003
Shock in the preceding 10 days (ref: no)[a]	1.01	0.69–1.49	0.948
SOFA at diagnosis (class: 4 points)	2.08	1.73–2.50	<0.001
Lactate at diagnosis (ref: ≤2.7 mmol/l)[b]	2.36	1.52–3.66	<0.001
Antibiotic at diagnosis (ref: no)	0.71	0.44–1.14	0.108
Initial surgical treatment (ref: no)	0.27	0.16–0.43	<0.001
Time between ICU admission and diagnosis	1.01	0.99–1.04	0.297

OR odd ratio, *95 % CI* 95 % confidence interval, *p p* value of the logistic regression, *SOFA* sequential organ failure assessment

[a] Before intensive care unit (ICU) admission

[b] Optimal cutoff value of the receiver operating characteristics

data cannot serve to determine the incidence of AMI among ICU patients.

In conclusion, our retrospective analysis of 780 ICU patients with AMI shows that the ICU mortality of patients with AMI was high, around 58 %. Increasing age, SOFA score at diagnosis, and plasma lactate concentrations above 2.7 mmol/l at diagnosis were associated with increased ICU death rate, whereas a prior history of peripheral vascular disease and an initial surgical treatment were associated with positive outcomes. A composite score integrating both age and SOFA score was established in order to facilitate the prediction of outcomes in ICU.

Conflicts of interest The authors have no conflict of interest to disclose related to this topic.

Appendix: List of investigators

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Etude N° 3

Collange O, Charton A, Greib N, Joshi GP, Schaeffer R, Diemunsch PA,

Correlation between arterial and capillary lactate measurements in a porcine hemorrhagic shock model.

J Trauma, 2010. 68(1): p. 32-4.

Correlation Between Arterial and Capillary Lactate Measurements in a Porcine Hemorrhagic Shock Model

Olivier Collange, MD, Antoine Charton, MD, Nicolas Greib, MD, Girish P. Joshi, MBBS, MD, FFARCSI, Roland Schaeffer, MD, and Pierre A. Diemunsch, MD, PhD

Lactate is the end product of anaerobic glucose metabolism. In physiologic conditions, lactate is produced by striated muscle, skin, brain, erythrocytes, and intestine.[1] Lactate metabolism takes place in the liver, brain, heart, kidney, and placenta. Hyperlactatemia is usually interpreted as a marker of secondary anaerobic metabolism because of the cellular distress often associated with inadequate oxygen supply.[2] Therefore, hyperlactatemia is considered as a key marker of shock. Conventional measurement of lactate levels is based on the enzymo-colorimetric method, which is performed on arterial blood samples in the biochemistry laboratory or with advanced point-of-care equipment.[3]

The Lactate Pro device (LT1710; Arkray, KGK, Japan) is a capillary lactate measurement method, which is validated and used to assess fetal well-being during labor,[4,5] lactate production during extreme exercise,[6,7] or in critical care.[8] However, this micromethod device has not yet been evaluated specifically during hemorrhagic shock, which is a common clinical situation producing metabolic acidosis. We hypothesized that the lactate levels obtained from the Lactate Pro device would correlate with those from the arterial blood samples using the conventional method, during hemorrhagic shock. This study was designed to compare the lactate measurements obtained from arterial blood samples (i.e., the conventional method) with the capillary lactate measurements (using the Lactate Pro device), in a porcine hemorrhagic shock model.

METHODS

After institutional approval, five white pigs (27 ± 4 kg) received standardized general anesthesia. Premedication was performed with intramuscular ketamine (50 mg/kg) and azaperone (2 mg/kg), followed by intravenous induction of general anesthesia with thiopental (5 mg/kg). Tracheal intubation was facilitated with pancuronium (0.2 mg/kg). Anesthesia and neuromuscular blockade were maintained with isoflurane and nitrous oxide in 50% oxygen. Minute ventilation was adjusted to keep the end-tidal carbon dioxide concentrations between 35 mm Hg and 45 mm Hg. In addition to routine monitoring (electrocardiography, pulse oximetry, and capnography), a femoral arterial catheter was placed to obtain continuous arterial blood pressure, draw blood samples, and perform controlled bleeding.

Progressive blood loss was performed over 60 minutes to achieve a mean arterial pressure of 40 mm Hg, which was maintained for 2 hours. No additional intravenous fluids were administered during the study period. Lactate levels from arterial blood and capillary sampling were obtained at baseline every hour for 3 hours (T0, T1, T2, and T3). Arterial blood samples were drawn from the femoral arterial line in a Pro-Vent (Portex; Keene, NH) type syringe and was immediately analyzed on an ABL 800 type automate (Radiometer; Copenhagen, Denmark). Capillary blood samples were obtained from the snout region with a BD Genie single-use lancet (2.0 × 1.5 mm automatic retraction blade; Becton Dickinson; Franklin Lakes, NJ) as done for capillary glucose assessment.

The conventional arterial lactate measurement method used by us is based on the enzymatic reaction followed by colorimetric measurement.[3] In the presence of lactate oxydase and oxygen, lactate is oxidized into pyruvate and hydrogen peroxide. Hydrogen peroxide makes a specific chromogen turn to violet coloration, whose absorbance variation is measured at 545 nm and 694 nm and is conversely proportional to the lactate concentration in the arterial blood sample.

Contrary to that, the Lactate Pro capillary lactate measurement method is based on electrical current measurement. When the microdroplet of blood reaches the reaction layer of the test strip in the presence of lactate oxydase and oxygen, the lactate is oxidized into pyruvate and hydrogen peroxide. The hydrogen peroxide oxidizes ferricyanide into ferrocyanide. The ferrocyanide is then oxidized under the polarization tension of an anode and produces an electrical current. The measured electrical intensity is proportional to the initial lactate concentration in the capillary blood sample. The numeric result of this amperometric method is displayed on an LCD screen in mmol/L.

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The authors declare no conflict of interest.
Presented at the ASA Annual Meeting, October 13–17, 2007, San Francisco, CA.
Address for reprints: Pierre Diemunsch, MD, PhD, Department of Anesthesiology, Hautepierre University Hospital, 1, Avenue Molière, 67000 Strasbourg, France; email: pierre.diemunsch@chru-strasbourg.fr.

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The lactate levels were compared by linear regression and calculation of Pearson's correlation coefficient R^2. The agreement between the two techniques of measurement was analyzed as described by Bland and Altman.[9,10] Bias (mean of differences), precision (SD [SD] of differences), and the limits of agreement (bias ± $t_{0,95}$ SD) along with their 95% confidence intervals (CI)s were calculated. The influence of time over the differences of lactate levels was analyzed by Kruskal Wallis test.

RESULTS

All animals survived the duration of study. The mean amount of blood withdrawn to achieve a mean arterial pressure of 40 mm Hg was 949 mL ± 55.5 mL (mean ± SD). The evolution plot of capillary and arterial lactate levels in function of time displays two parallel curves (Fig. 1). The correlation coefficient R^2 of Pearson was 0.989 (Fig. 2). The bias was −0.115 mmol/L with a 95% CI of −0.115 mmol/L ± 0.197 mmol/L (−0.312; 0.082) and the precision was ± 0.420 mmol/L (Fig. 3). The limits of agreement are equal to bias ± 2.093 SD, where 2.093 is the value of the *t* distribution for a risk alpha of 0.05 with 19*df*. The upper and lower limits of agreement were 0.764 mmol/L and −0.994 mmol/L with their 95% CIs from 0.424 mmol/L to 1.104 mmol/L and from−1.334 mmol/L to −0.654 mmol/L, respectively. The bias was not significantly influenced by time (Kruskal Wallis test).

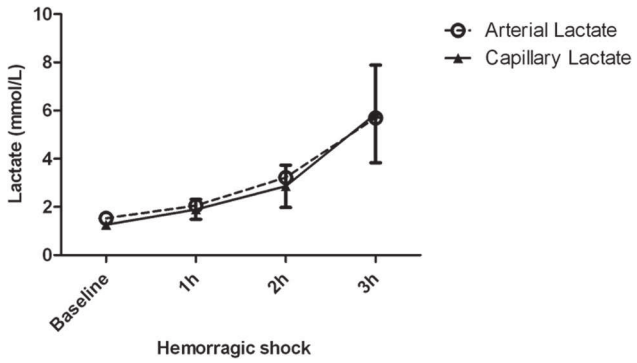

Figure 1. Arterial and capillary lactate levels evolution during hemorrhagic shock in pigs.

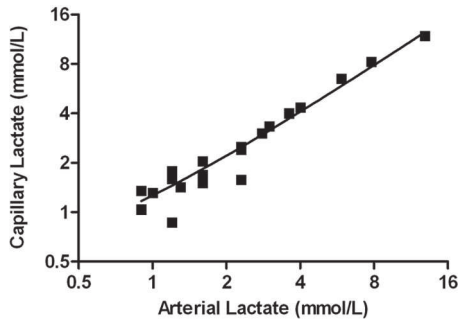

Figure 2. Linear regression (logarithmic scales).

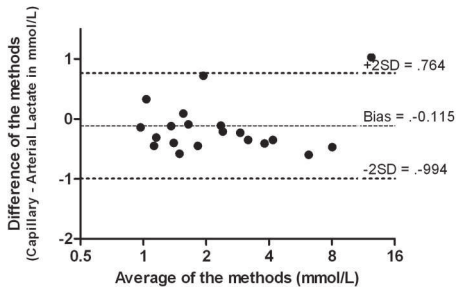

Figure 3. Bland-Altman plot (logarithmic scale).

DISCUSSION

In our experimental setting, an excellent correlation between arterial blood lactate levels (measured by conventional method) and capillary blood lactate levels (measured by the amperometric micromethod Lactate Pro) was observed over the course of hemorrhagic shock. Thus, capillary lactate monitoring may help to assess severity and guide therapy during hemorrhagic shock in the operation room, intensive care units, and prehospital situations. However, human studies in different clinical situations are necessary to assess the capillary lactate measurement as reliable as observed in the animal model.

The capillary blood lactate monitoring device is a simple, fast, and bedside technique. Similar to obtaining blood sugar levels using d-sticks, this micromethod needs 5 μL of capillary blood to provide a lactate level within 60 seconds. Moreover, at our institution, the cost of one capillary lactate measurement is about half the conventional measurement.

Some potential bias has to be taken in account before generalization to clinical situations. First, it is possible that lactate metabolism differs between pigs and human; however, no study supports this idea. Second, we explored lactate only during a controlled hemorrhagic shock. Some other conditions such as inflammatory shock, anaphylactic shock, or mesenteric ischemia could produce significant differences between capillary and arterial lactate. Third, it is possible that pig snout capillaries may have different perfusion than human fingertips, which may be influenced by peripheral vasoconstriction during hemorrhagic shock state. However, there is no data to suggest such a difference. In fact, our results confirm a clinical study comparing capillary and arterial lactate measurements in other types of shock.[8]

In summary, in a hemorrhagic shock setting, capillary lactate levels correlate with arterial blood lactate levels. These preliminary findings suggest that the micromethod could be used at the bedside to assess the severity of hemorrhagic shock and to guide its therapy. The capillary lactate measurement device is easy to use, cost effective, and could be used as point-of-care monitoring in the hospital as well as prehospital settings.

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Etude N° 4

Noll E, Bouitbir J, Collange O, Zoll J, Charles AL, Thaveau F, Diemunsch P,Geny B,

Local but not systemic capillary lactate is a reperfusion biomarker in experimental acute limb ischaemia.

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Short Report

Local but not Systemic Capillary Lactate is a Reperfusion Biomarker in Experimental Acute Limb Ischaemia☆

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ABSTRACT

Introduction: Systemic capillary lactate, an end product of cellular anaerobic metabolism, has not established credibility in monitoring limb reperfusion. We assessed, in mice, whether local capillary lactate, arising from the reperfused limb, might be a relevant biomarker of reperfusion.
Report: Systemic and local capillary lactate were sampled in the non-ischaemic and in the ischaemic limb. Only local lactate concentrations significantly increased after 2 h of ischaemia and decreased after reperfusion.
Discussion: Local, but not systemic, capillary lactate appeared as a potential reperfusion biomarker in this experimental acute limb ischaemia model.

Acute limb ischaemia is a common and critical vascular disease with a mortality rate of 15–20%. Post-revascularisation care requires careful limb vascular monitoring for early critical ischaemia recurrence. However, in emergencies, such clinical follow-up might be difficult. Peripheral pulse status is not strongly correlated with tissue perfusion and to clearly determine whether the revascularisation is successful remains a challenge.

Lactate, the end product of cellular anaerobic energetic metabolism, which is a key biomarker of tissue hypoxia, is of interest as an ischaemia biomarker. Systemic lactate has not convincingly been useful in monitoring limb reperfusion.[1] However, microdialysis demonstrated a short time course of lactate normalisation in muscular tissue after revascularisation.[2]

We tested the hypothesis that local capillary lactate arising from the ischaemic limb might be a better biomarker for early reperfusion than systemic lactate, and determined simultaneously systemic and local lactate kinetics in a one-limb ischaemia–reperfusion (IR) mouse model. Skeletal muscle mitochondrial function, impaired following IR, was also analysed.[3,4]

Report

Experimental design, blood and muscle sampling and analysis

Mice were separated into two groups: the sham group (SHAM, $n = 13$), which did not undergo, IR, and the ischaemic group (IR, $n = 12$), which had left limb ischaemia induced by a tourniquet applied for 2 h followed by a 2-h reperfusion. Then, gastrocnemius muscles were excised for mitochondrial respiration analysis. The left leg was considered as ischaemic (IR-ISCH) and the right contralateral leg was considered as non-ischaemic (IR-CTL).[3]

Local and systemic capillary lactates were measured simultaneously before ischaemia, 2 h after ischaemia (I) and 2 h after IR, using the Lactate Pro© device (LT170, Arkray, KGK, Japan).[5]

Mitochondrial maximal oxidative capacities were investigated using glutamate–malate in saponin-skinned gastrocnemius fibres.[3]

The investigation was carried out in accordance with Helsinki Accords for Humane treatment of Animals during Experimentation (institutional approval no. AL/02/21/12/09).

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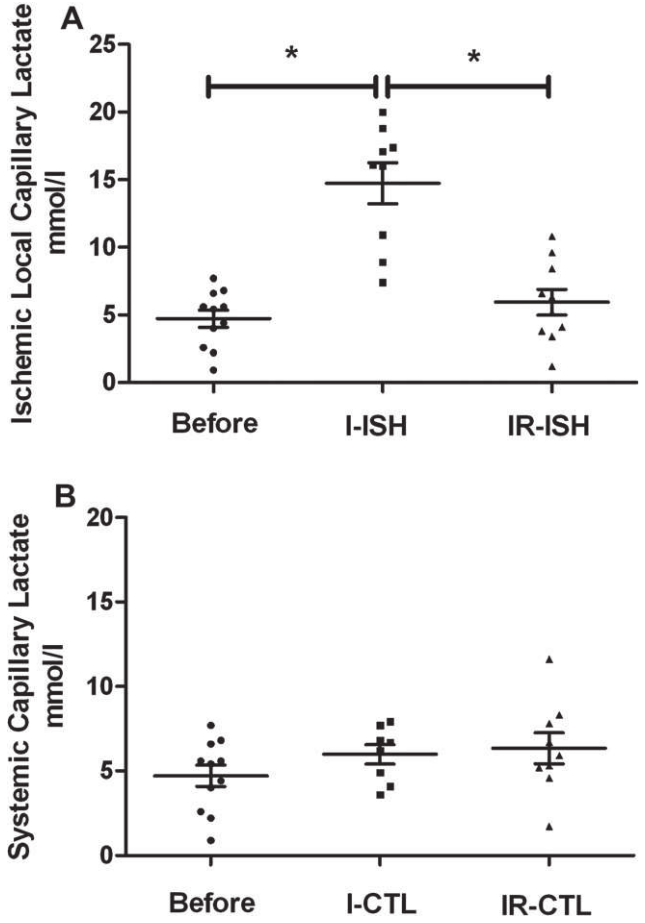

Figure 1. Capillary lactate values (A): local capillary lactate sampled at the ischaemic leg level (B): systemic capillary lactate sampled at the contralateral non-ischaemic leg level Data are presented in three experimental conditions: before ischaemia, after ischaemia and after ischaemia–reperfusion in the ischaemic leg (IR-ISCH) and in the non-ischaemic leg (IR-CTL). Horizontal line at the mean and vertical bar for the SEM. ($n = 11$) $^*p < 0.05$ (before vs. I-ISCH: $p = 0.00013$, I-ISCH vs. IR-ISH: $p = 0.00017$).

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). A one-way analysis of variance (ANOVA) was used, with the Newman–Keuls post-test analysis and *t*-test for requiring analysis. $p < 0.05$ was accepted as significant.

Results

Mitochondrial respiratory chain oxidative capacity (V_{max}) was similar in sham animals and in the non-ischaemic contralateral leg of the IR animals (8.58 ± 0.85 vs.9.39 ± 1.33 μmol min^{-1} g^{-1} dry weight, $p = 0.59$, in SHAM and IR-CTL, respectively).

V_{max} was significantly decreased in ischaemic muscle as compared with SHAM and IR-CTL (5.40 ± 0.95 vs. 8.58 ± 0.85 and 9.39 ± 1.33 μmol min^{-1} g^{-1} dry weight, $p = 0.04$, −30% ± 14% and $p = 0.03$, −32% ± 12%, respectively).

Ischaemia significantly increased local capillary lactate levels in the ischaemic leg, compared to baseline value (14.73 ± 1.50 vs.4.71 ± 0.64 mmol l^{-1}, in I-ISCH, $p = 0.00013$, Fig. 1).

The small increase in lactate value in the systemic leg failed to reach statistical significance (5.99 ± 0.57 mmol l^{-1} in I-CTL, $p = 0.62$).

Thus, lactate increase was significantly greater in the ischaemic than in the non-ischaemic leg (+419% ± 173, vs. +108% ± 93, for I-ISCH and I-CTL; $p = 0.031$).

After 2 h of reperfusion, local capillary lactate levels significantly decreased (5.94 ± 0.95 mmol l^{-1}, $p = 0.00017$). Systemic lactate value failed to change significantly (6.34 ± 0.91 mmol l^{-1}).

Thus, lactate variation was significantly greater in the ischaemic than in the non-ischaemic leg (−59% ± 8, vs. +13% ± 22, for IR-ISCH and IR-CTL respectively, $p = 0.015$).

Discussion

In accordance with data reported after IR in rodents, we observed a decreased muscle mitochondrial maximal oxidative capacity after this 2-h ischaemia–2-h reperfusion protocol in mice. Such results further support that mitochondria are early target of IR injury.[3]

Although systemic lactate is of prognostic interest, its slow and progressive changes preclude use in the early diagnosis of re-thrombosis.[1]

Local capillary lactate, sampled directly at the ischaemic leg level, demonstrated both a rapid increase during ischaemia and a rapid decrease during reperfusion. Such kinetics are in agreement with data obtained using microdialysis, and could allow an early diagnosis of successful reperfusion.[2]

In summary, this study demonstrates that local, but not systemic, capillary lactate levels might be reperfusion biomarkers after limb IR. Since capillary blood lactate monitoring is a rapid and repeatable bedside method,[5] clinical studies are warranted to test whether local lactate kinetics might be helpful in determining the success of revascularisation in vascular surgery in man.

Conflict of Interest/Funding

None.

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Etude N°5

Collange O, Veber B, Tamion F, Lavoine A, Plissonnier D, Dureuil B,

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Article original

Intérêt du D-lactate comme marqueur de l'hypoperfusion colique lors de la chirurgie des anévrismes de l'aorte abdominale

Interest of D-lactate as a colic hypoperfusion marker during aortic abdominal aneurysm surgery

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Résumé

Objectif. – Le D-lactate est la forme dextrogyre du lactate habituellement dosé en réanimation. Son origine bactérienne pourrait en faire un marqueur de translocation lors de l'ischémie digestive. Le but de l'étude a été de tester le D-lactate comme marqueur de l'hypoperfusion colique lors de la chirurgie aortique.

Type d'étude. – Étude prospective, observationnelle, ouverte.

Patients et mesures. – Patients opérés d'un anévrisme de l'aorte abdominale. Deux groupes de patients étaient constitués selon la pression résiduelle de l'artère mésentérique inférieure (PrAMI) mesurée lors de l'opération : groupe hypoperfusion colique peropératoire (HCop) : patients dont la PrAMI était inférieure à 40 mmHg. Groupe témoin : patients dont la PrAMI était supérieure ou égale à 40 mmHg. Des données générales telles que l'âge, le temps de clampage aortique et l'indice de gravité simplifié (IGS II) ont été recueillies. Le D-lactate était dosé après l'opération : lors de l'admission du patient en réanimation puis quotidiennement jusqu'à sa sortie. Le D-lactate$_{max}$, définissait le taux de D-lactate le plus élevé parmi l'ensemble des prélèvements réalisés pour un patient.

Résultats. – Vingt-neuf patients ont été inclus, 23 dans le groupe témoin, six dans le groupe HCop. Les groupes étaient comparables en termes de données générales. Le D-lactate$_{max}$ du groupe HCop était significativement plus élevé (médiane : 0,13 mmol/l ; min-max : 0,03–0,9 mmol/l) que celui du groupe témoin (0,03 ; 0–0,26 mmol/l, $p = 0{,}007$).

Conclusion. – Le D-lactate pourrait être un marqueur postopératoire de l'hypoperfusion colique survenant pendant la chirurgie des anévrismes de l'aorte abdominale.

Abstract

Objective. – D-lactate is the dextogyre form of the lactate usually measured in intensive care. Its bacterial origin should make it a marker of translocation during gut ischemia. The aim was to test D-lactate as a postoperative marker of colic hypoperfusion measured during aortic surgery.

Study design. – Prospective observational cohort study.

Patients and measurements. – Patients operated for abdominal aortic aneurysm. Two groups were stratified on inferior mesenteric arterial residual pressure (IMArP) measured during the surgery: Colic hypoperfusion during surgery (CHs) group: patients with an IMArP < 40 mmHg. Control Group: patients with an IMArP ≥ 40 mmHg. Baseline data such as age, duration of aortic clamping and severity score (IGS II) were collected. The D-lactate was measured in postoperative at admission time in ICU and then daily. D-lactate$_{max}$ defined the maximum value of D-lactate for one patient.

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Main results. – Twenty-nine patients were included, 23 in the control group and 6 in the CHs group. Groups were comparable at baseline. D-lactate$_{max}$ was significantly higher in the CHs group (median: 0.13 mmol/l; min-max: 0.03–0.9 mmol/l) than in the control group (0.03; 0–0.26 mmol/l, $p = 0.007$).

Conclusion. – D-lactate could be postoperative marker of colic hypoperfusion measured during surgery for abdominal aortic aneurysm.

Mots clés : D-lactate ; Hypoperfusion digestive ; Ischémie–reperfusion digestive ; Ischémie colique ; Artère mésentérique inférieure ; Chirurgie aortique ; Réanimation

Keywords: D-lactate; Gut hypoperfusion; Gut ischaemia reperfusion; Colic ischaemia; Inferior mesenteric artery; Aortic surgery; Critical care

1. Introduction

Le D-lactate a été proposé comme marqueur spécifique de l'ischémie digestive [1–4], notamment de l'ischémie colique après chirurgie aortique [4]. Il constitue la forme dextrogyre du lactate habituellement dosé en réanimation. Les deux isomères (L- et D-lactate) diffèrent par la position de leur radical alpha-hydroxyl. Chaque isomère résulte de la réduction du pyruvate par une lactate déshydrogénase (LDH) optiquement spécifique. Ainsi, la formation de L-lactate à partir d'une molécule de pyruvate optiquement neutre nécessite une L-LDH alors qu'une D-LDH est nécessaire pour former du D-lactate. Les mammifères ne possèdent qu'un type d'enzyme : la L-LDH. Lors de l'anaérobiose, les cellules humaines produisent spécifiquement des molécules de L-lactate. Étant équipées de D-LDH, les bactéries produisent du D-lactate lors de la fermentation lactique. La présence de D-lactate dans l'organisme humain traduit un apport exogène. Ainsi, un dosage positif de D-lactate dans des liquides biologiques normalement stériles (liquide articulaire, liquide pleural ou liquide céphalorachidien) signe leur contamination bactérienne [5–7]. La présence de D-lactate dans le sang peut traduire soit un apport de D-Lactate (intraveineux [8] ou lors d'une dialyse péritonéale [9]) soit une translocation du D-lactate produit par les bactéries dans la lumière digestive. Des cas d'acidose lactique ont en effet été rapportés chez des patients porteurs d'un grêle court [10–16]. Dans ces cas, la translocation de D-lactate était secondaire à l'augmentation de la fermentation (D-)lactique des bactéries coliques [17,18]. Le même mécanisme, associé à une augmentation de la perméabilité digestive, peut expliquer la translocation de D-lactate lors de la souffrance ischémique. Ainsi, il a été montré que le D-lactate pouvait être un marqueur prédictif de la survenue d'une ischémie colique après chirurgie pour anévrisme aortique rompu [4].

Néanmoins, nous ne disposons pas de données associant directement l'hypoperfusion colique à une augmentation de D-lactate sanguin. Le but de cette étude était de comparer une mesure hémodynamique de la perfusion colique réalisée pendant la chirurgie aortique aux taux de D-lactate postopératoires.

2. Matériel et méthodes

Cette étude était prospective, observationnelle et ouverte. Pendant une période de six mois (novembre 1999–avril 2000), les patients opérés de l'aorte abdominale et hospitalisés dans le service de réanimation chirurgicale du CHU de Rouen étaient inclus.

2.1. Population

Deux groupes de patients étaient constitués en fonction de la pression résiduelle de l'artère mésentérique inférieure (PrAMI), habituellement mesurée dans notre équipe lors de la chirurgie des anévrismes de l'aorte abdominale : groupe hypoperfusion colique peropératoire : patients dont la perfusion digestive était basse (pression résiduelle de l'artère mésentérique inférieure à 40 mmHg) ; groupe témoin : patients dont la perfusion colique était satisfaisante (pression résiduelle de l'artère mésentérique supérieure ou égale à 40 mmHg).

2.2. Mesures

2.2.1. Mesure de la perfusion digestive : pression résiduelle de l'artère mésentérique inférieure (PrAMI)

L'artère mésentérique inférieure (AMI) vascularise le rectum et la partie gauche du côlon. Lors de l'occlusion de l'AMI, le réseau des anastomoses artérielles est habituellement suffisant pour assurer la perfusion colique et rectale. La PrAMI rend compte de la qualité du réseau de suppléance lors du clampage aortique. Une PrAMI élevée traduit la bonne fonctionnalité du réseau. Une PrAMI basse révèle une hypoperfusion colorectale et traduit, en l'absence d'hypotension artérielle systémique et/ou d'état de choc, la mauvaise qualité du réseau de suppléance. Nous avons considéré qu'une PrAMI inférieure à 40 mmHg traduisait une hypoperfusion de la partie gauche du côlon et du rectum lors du clampage aortique [19,20]. Dans ce cas, l'AMI était réimplantée en fin d'intervention.

2.2.2. Données générales

Des données générales pré-, per- et postopératoires ont été recueillies. Les données préopératoires comprenaient :

- l'âge ;
- le sexe ;
- le score IGS II ;
- le caractère urgent de l'opération ;
- la présence d'un état de choc avant l'intervention.

Les données peropératoires étaient le niveau et la durée du clampage aortique, la durée de l'intervention, le nombre de culots globulaires transfusés et le volume de Ringer lactate per-

fusé durant l'intervention, le nombre de patient sous substance vasoactive au déclampage. En période postopératoire, le nombre de patient en état de choc, ayant développé une insuffisance rénale ou une infection systémique, était recueilli. L'état de choc était défini par le recours à un agent vasopresseur pendant plus de 24 heures (dopamine à plus de 5 µg/kg par minute, noradrénaline ou adrénaline). L'insuffisance rénale était définie par une créatinémie supérieure à 150 µmol/l. Le diagnostic d'infection systémique reposait sur un examen bactériologique positif (pulmonaire, urinaire, sanguin ou du site opératoire).

2.2.3. D-lactate$_{max}$: prélèvement et dosage

Le D-lactate$_{max}$ définissait le taux de D-lactate le plus élevé parmi l'ensemble des prélèvements réalisés pour un patient. Le D-lactate était prélevé en période postopératoire : lors de l'admission du patient en réanimation puis quotidiennement jusqu'au jour de sa sortie du service. Le prélèvement sanguin était effectué dans un tube contenant de l'acide perchlorique assurant la déprotéinisation de l'échantillon. Il était ensuite centrifugé à 3500 tours/min pendant 15 minutes et congelé à –80 °C jusqu'au dosage. Le dosage employait une méthode fondée sur la spectrochimie [21]. De manière à valider in vitro la qualité de la méthode de dosage et à déterminer son seuil de détection, des dosages-tests d'échantillons de D-lactate étalonnés ont été effectués conformément au protocole « Validation de techniques » préconisé par la Société française de biologie clinique. Par ailleurs, une validation in vivo a été réalisée pour vérifier la faisabilité de la procédure prélèvement–acheminement–dosage et pour tester la capacité de notre méthode de dosage à détecter le D-lactate chez des sujets de réanimation. Pour ce test in vivo, les taux de D-lactate de dix sujets sains étaient comparés à ceux de dix sujets de réanimation arbitrairement choisis parmi des patients de réanimation non opérés de l'aorte.

2.2.4. L-lactate$_{max}$

Des prélèvements de L-lactate étaient réalisés en même temps que les prélèvements de D-lactate. Le L-lactate$_{max}$ définissait le taux de L-lactate le plus élevé parmi l'ensemble des prélèvements réalisés pour un patient.

2.2.5. Ischémie colique postopératoire

Le diagnostic d'ischémie colique postopératoire était fait lors d'une coloscopie. Tout patient présentant des signes cliniques évoquant une ischémie colique (diarrhée, état de choc, syndrome fébrile ou inflammatoire non expliqué) avait une coloscopie.

2.3. Analyse statistique

Un test de Mann et Whitney et le calcul des coefficients de corrélations de Spearman étaient utilisés pour mettre en évidence une différence entre les groupes. Les résultats sont exprimés en médiane et valeurs extrêmes. Une valeur de $p < 0{,}05$ était considérée comme statistiquement significative.

4. Résultats

4.1. Validation de la méthode de dosage

4.1.1. Validation in vitro

La méthode de dosage a été validée dans les limites de linéarité et dans le domaine de mesure grâce à un étalon primaire de D-lactacte à 200 mg/l. Dix dilutions progressives ont permis de couvrir la totalité du domaine de mesure. Le seuil de détection a été déterminé à 0,14 mmol/l grâce à une série de dix mesures effectuées sur le blanc réaction.

4.1.2. Validation in vivo

Le taux de D-lactate de dix sujets sains, cinq femmes et cinq hommes âgés de 25 à 45 ans, était nul. Les dix sujets de réanimation (quatre femmes et six hommes) avaient un âge moyen de 58 ans et étaient hospitalisés pour différentes raisons (quatre péritonites, une pancréatite aiguë, une pneumopathie hypoxémiante, une désinsertion traumatique de l'artère mésentérique supérieure, un syndrome d'Ogilvie, une hémorragie méningée et un état de choc cardiogénique). Sept de ces sujets présentaient un état de choc lors du dosage. Le D-lactate$_{max}$ des sujets de réanimation était de $0{,}14 \pm 0{,}18$ mmol/l (médiane 0,08 ; min–max : 0–0,6 mmol/l), soit un taux significativement supérieur à celui des sujets sains ($p = 0{,}027$) [Fig. 1].

4.2. Données générales

Trente-cinq patients ont été opérés d'un anévrisme de l'aorte abdominale et hospitalisés dans le service de réanimation durant la période d'étude. Six n'ont pas pu être inclus dans l'étude. Sur les 29 patients inclus, 26 étaient opérés à froid d'un anévrisme sous-rénal ($n = 19$), d'un anévrisme nécessitant un clampage supracœliaque ($n = 6$) et un patient nécessitait un clampage sous-rénal associé à un clampage de l'artère mésentérique supérieure. Trois patients ont été opérés en urgence pour un anévrisme de l'aorte abdominal fissuré. Les caractéristiques des 29 patients inclus et leur répartition dans les groupes témoins ($n = 23$) et HCop ($n = 6$) sont précisées dans le Tableau 1. Les populations des deux groupes n'étaient pas différentes en terme d'âge, de sexe ou de score IGS II. La durée de clampage, la durée d'intervention, le nombre de concentrés globulaires transfusés n'étaient pas significativement différents. Le volume de Ringer Lactate perfusé semblait plus important dans le groupe témoin (2,4 vs 1,9 l) mais cette différence n'était pas significative ($p = 0{,}21$). En ce qui concerne la période postopératoire : il n'y avait pas de différence en termes de durée de séjour et le nombre de patients en état de choc, ayant une infection ou une insuffisance rénale était comparable dans les deux groupes.

4.3. D-lactate$_{max}$

Le D-lactate$_{max}$ était significativement plus élevé dans le groupe HCop (médiane : 0,13 mmol/l ; min–max : 0,03–

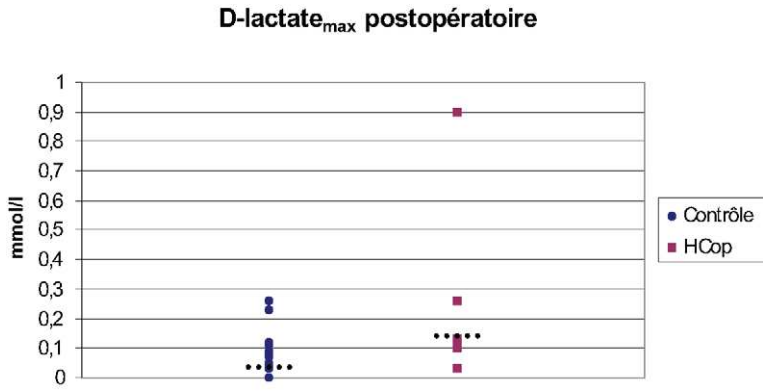

Fig. 1. D-lactate$_{max}$.
Groupe témoin : absence d'hypoperfusion digestive durant la chirurgie aortique (PrAMI ≥ 40 mmHg).
Groupe HCop : hypoperfusion colique durant la chirurgie aortique (PrAMI < 40 mmHg).
Les pointillés représentent la valeur médiane de chaque groupe (témoin : 0,03 mmol/l ; HCop : 0,13 mmol/l).
Le D-lactate$_{max}$ est significativement plus élevé dans le groupe hypoperfusion colique peropératoire (HCop) ; $p = 0{,}007$.

Tableau 1
Données pré-, per- et postopératoires

	Groupe Contrôle ($n = 23$)	**Groupe HCop** ($n = 6$)	*p*
Caractéristiques des deux populations			
Âge	67 (37–82)	69 (54–79)	ns
Sexe ratio (H/F)	23/0	6/0	ns
IGS II	22 (11–58)	22 (19–34)	ns
État Préopératoire			
Opération en urgence	2	2	ns
État de choc	2	0	ns
Données peropératoires			
Hauteur du clampage			
Cœliaque	4	2	ns
Sous-Rénal	19[a]	4	ns
Durée du clampage aortique (minute)	67 (18–120)	69 (54–79)	ns
Durée de l'intervention (minute)	198 (130–300)	195 (180–260)	ns
PrAMI (mmHg)	52 (42–66)	31 (22–38)	0,002
CG transfusé *n*	3 (0–10)	2 (1–7)	ns
Volume de RL perfusé	2,4 (2–3)	1,9 (1–3)	ns
Patients sous dopamine	17	4	ns
Patients sous noradrénaline	4	1	ns
Données postopératoires			
Durée du séjour en réanimation (jour)	4 (2–12)	5 (3–20)	ns
État de choc	2	2	ns
Infection	2	1	ns
Insuffisance rénale	3	2	ns
Ischémie colique	0	1	ns
L-lactate$_{max}$ (mmol/l)	1,4 (0,5–8)	1,4 (0,8–4,2)	ns
D-lactate$_{max}$ (mmol/l)	0,03 (0–0,26)	0,13 (0,03–0,90)	0,007

Les valeurs sont données en médianes et (valeurs extrêmes). ns = non significative ($p > 0{,}05$) ; groupe témoin : absence d'hypoperfusion digestive durant la chirurgie aortique (PrAMI ≥ 40 mmHg) ; groupe hypoperfusion digestive : hypoperfusion digestive durant la chirurgie aortique (PrAMI < 40 mmHg) ; IGS II : indice de gravité simplifié ; L-lactate$_{max}$: valeur de L-lactate la plus élevée pour chaque patient ; D-lactate$_{max}$: valeur de D-lactate la plus élevée pour chaque patient.

[a] Une intervention a nécessité la réimplantation de l'artère mésentérique supérieure.

0,9 mmol/l) que dans le groupe témoin (0,03 ; 0–0,026 ; $p = 0{,}007$) [Fig. 2].

4.4. L-lactate$_{max}$

Les taux de L-lactate$_{max}$ des groupes témoins (médiane : 1,4 mmol/l ; min–max : 0–8 mmol/l) et HCop n'étaient pas significativement différents (1,4 mmol/l ; 0,8–4,2 mmol/l ; $p = 0{,}6$).

4.5. Comparaison du D-lactate au L-lactate et à l'IGS II

Il n'y avait pas de corrélation entre les taux de D- et L-lactate prélevés au même moment ($p = 0{,}89$; coefficient de corrélation : 0,01 ; Fig. 3A) ni entre les taux de D- et L-lactate$_{max}$ ($p = 0{,}72$; coefficient de corrélation : –0,08 ; Fig. 3B). Les taux de D-lactate$_{max}$ n'étaient pas corrélés à l'IGS II ($p = 0{,}87$; coefficient de corrélation : 0,03 ; Fig. 3C), contrairement aux taux de L-lactate$_{max}$ qui étaient positivement corrélés à l'IGS II ($p = 0{,}01$; coefficient de corrélation 0,48 ; Fig. 3D).

4.6. Ischémie colique

Un seul patient a développé une ischémie colique postopératoire. Il s'agissait d'un homme de 54 ans opéré à froid d'un anévrisme de l'aorte sous-rénale. La PrAMI était de 25 mmHg et traduisait une hypoperfusion colique pendant la durée (38 min) du clampage aortique (groupe HCop). L'AMI a été réimplantée en fin d'intervention. À j1 postopératoire, l'apparition d'une diarrhée profuse a motivé la réalisation d'une coloscopie confirmant le diagnostic d'ischémie colique grade I. L'apparition d'un état de choc à j2 imposa la reprise chirurgicale. Celle-ci permit de faire le diagnostic de thrombose de l'AMI, la réalisation d'un pontage de l'AMI et d'une colectomie gauche. Le patient a été extubé le lendemain de la

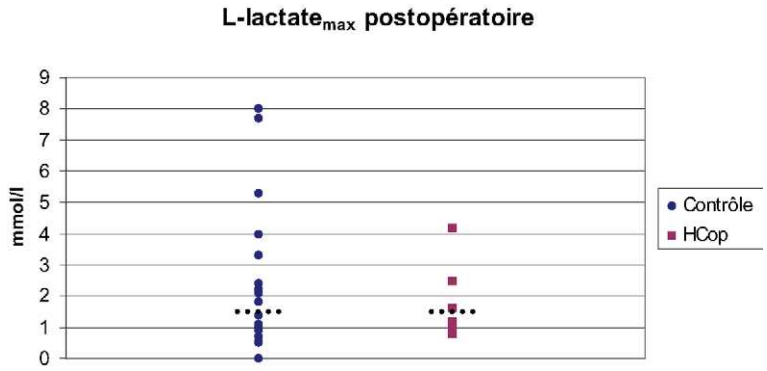

Fig. 2. L-lactate$_{max}$.
Groupe témoin : absence d'hypoperfusion digestive durant la chirurgie aortique (PrAMI ≥ 40 mmHg).
Groupe HCop : hypoperfusion colique durant la chirurgie aortique (PrAMI < 40 mmHg).
Les pointillés représentent la valeur médiane de chaque groupe (témoin : 1,4 mmol/l ; HCop : 1,4 mmol/l).

reprise chirurgicale (j3) et la dopamine a pu être sevrée à j4. Le patient est sorti du service de réanimation à j20. Le D-lactate$_{max}$ de ce patient était de 0,13 mmol/l. Il s'agissait du premier prélèvement réalisé à l'entrée dans le service. Tous les autres prélèvements étaient inférieurs à 0,04 mmol/l. En particulier, la reprise chirurgicale n'a entraîné aucune modification des taux de D-lactate. Le taux de L-lactate$_{max}$ était de 4,2 mmol/l et correspondait également au premier prélèvement réalisé à l'entrée dans le service. Les taux de L-lactates sont restés augmentés jusqu'à j4 (successivement 3,4 ; 3, 2 et 2,1 mmol/l).

5. Discussion

Notre étude suggère qu'une hypoperfusion colique constatée lors du clampage aortique s'accompagne d'une augmentation des taux de D-lactate postopératoire.

Ces résultats sont en accord avec les données de la littérature concernant l'intérêt du D-lactate dans le diagnostic de l'ischémie digestive [1–4]. Deux études expérimentales, portant sur des modèles de clampage de l'artère mésentérique supérieure, montrent une augmentation de D-lactate après ischémie–reperfusion digestive [1,3]. Des auteurs mesurent les taux de D-lactate préopératoires chez 31 patients opérés en urgence d'un syndrome abdominal aigu [2]. Ces derniers sont significativement augmentés lorsqu'une ischémie digestive est retrouvée. Les auteurs ont ainsi pu préciser qu'un taux de D-lactate supérieur à 0,20 mmol/l est associé à une ischémie digestive avec une sensibilité de 90 % et une spécificité de 87 %. Une équipe évalue de façon prospective le D-lactate comme marqueur de l'ischémie colique chez 24 patients opérés en urgence pour une rupture de l'aorte [4]. Les patients qui développent une ischémie colique postopératoire ont un taux de D-lactate supérieur à ceux qui n'en présentent pas. Dans ce travail, un taux de D-lactate supérieur à 0,20 mmol/l est prédictif de la survenue d'une ischémie colique avec une sensibilité de 82 % et une spécificité de 77 %.

Dans notre étude, le nombre de sujets inclus était trop petit pour calculer la sensibilité et la spécificité du D-lactate comme marqueur de l'hypoperfusion digestive. Pour les mêmes raisons de taille d'effectif, nous ne pouvions pas comparer les taux de D-lactate entre différents sous-groupes. Par exemple, nous ne pouvions pas étudier les patients opérés en urgence d'un anévrisme rompu car leur nombre était trop petit ($n = 3$). Par ailleurs, le paramètre que nous avons choisi (la PrAMI) n'est pas la méthode de référence en matière de diagnostic d'hypoperfusion colique. Ainsi, certaines équipes ne mesurent pas la PrAMI considérant que la réimplantation de l'AMI ne diminue pas l'incidence des ischémies coliques postopératoires [22] Pour une équipe, le seuil de PrAMI définissant l'hypoperfusion colique n'est pas 40 mais 50 mmHg [23]. Cependant, ces auteurs ne démontrent pas que la réimplantation de l'AMI en fonction de ce critère (PrAMI < 50 mmHg) diminue l'incidence des ischémies coliques postopératoires. Enfin, le rapport PrAMI/PAM est proposé pour déterminer le risque d'ischémie colique postopératoire. Lorsque la PrAMI est inférieure à 40 mmHg ou lorsque le rapport PrAMI/PAM est inférieur à 0,4, la réimplantation de l'AMI (plus ou moins artères hypogastriques) peut être bénéfique [19]. Par ailleurs, deux études montrent que la PrAMI est bien corrélée avec la mesure Doppler du flux sanguin de la muqueuse rectale [20,24].

Les taux de D-lactate sont restés bas chez le seul patient ayant développé une ischémie colique postopératoire. Une explication possible de ce résultat pourrait être le mécanisme de l'ischémie colique. En effet, lors de la reprise chirurgicale, une thrombose complète de l'AMI a été constatée. Or, pour être objectivée, toute translocation nécessite la persistance d'un flux sanguin suffisant pour charrier les produits transloqués dans la circulation systémique. Ainsi, le D-lactate pourrait être un marqueur de la souffrance ischémique colique mais serait nul en cas d'occlusion vasculaire complète. Bien sûr cette explication doit être vérifiée et validée par d'autres études qui permettront peut-être de préciser la place du D-lactate dans la stratégie diagnostique des ischémies coliques postopératoires.

Notre étude présente de nombreuses et claires limitations. Premièrement, la taille des effectifs est limitée. Deuxièmement, nous n'avons comparé le D-lactate qu'à un seul autre paramètre de la perfusion colique — la PrAMI — et ce paramètre est discutable. Enfin, le D-lactate n'a pas augmenté chez le seul patient ayant développé une ischémie colique postopératoire.

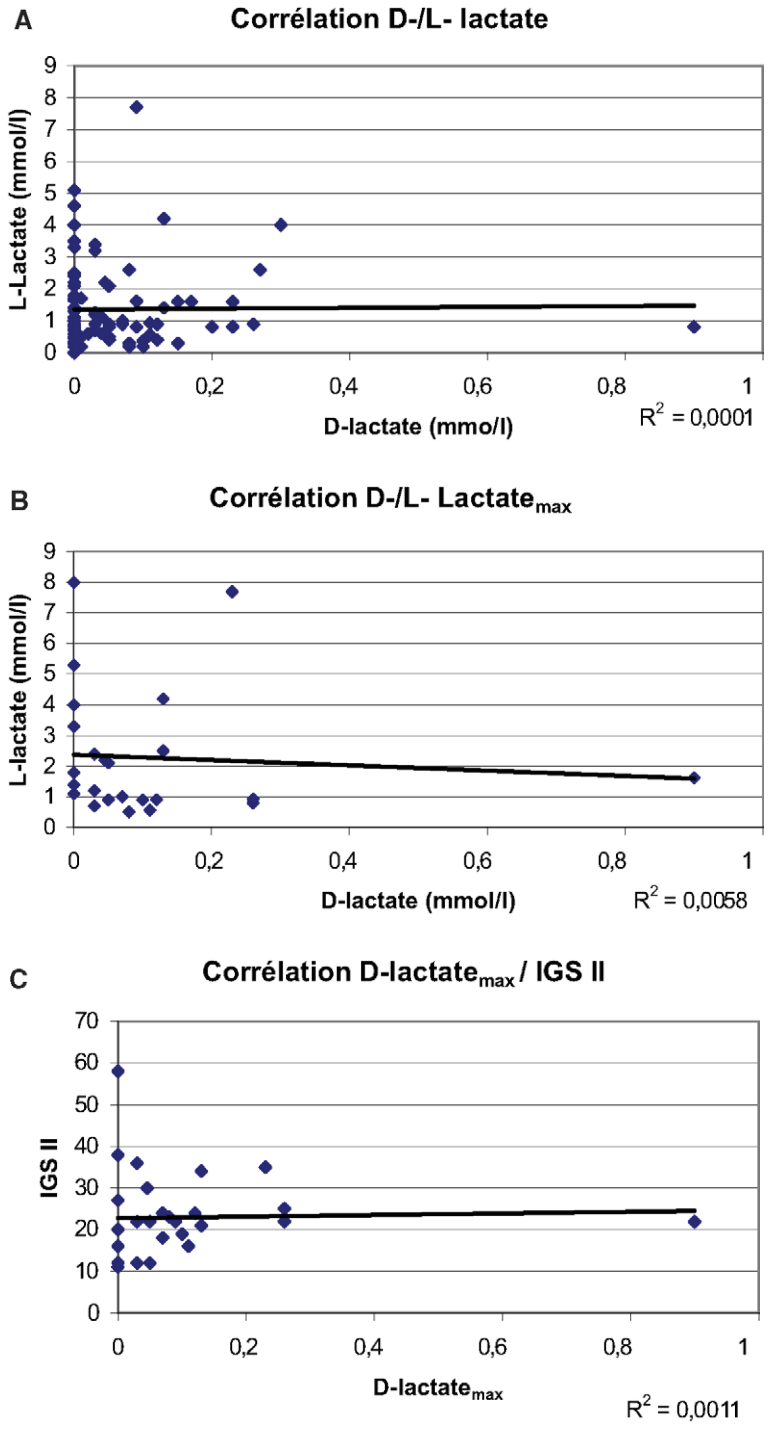

Fig. 3. Courbes de corrélation.
A. Les taux de D-lactate et de L-lactate prélevés au même moment ne sont pas corrélés (coefficient de corrélation : 0,01 ; $p = 0,89$).
B. Les taux de D-lactate et de L-lactate$_{max}$ ne sont pas corrélés (coefficient de corrélation : –0,08 ; $p = 0,72$).
C. Les taux de D-lactate$_{max}$ ne sont pas corrélés aux scores IGSII (coefficient de corrélation : 0,03 ; $p = 0,87$).

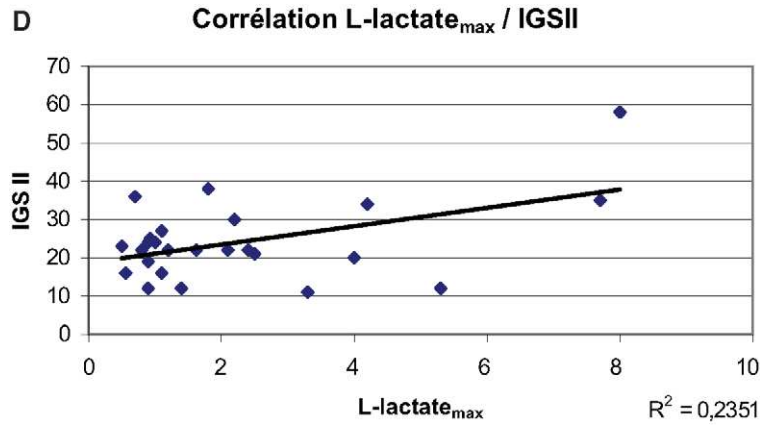

Fig. 3. *(suite)*
D. Les taux de L-lactate$_{max}$ sont positivement corrélés aux scores IGSII (coefficient de corrélation 0,48 ; $p = 0,01$).

Néanmoins, cette étude suggère qu'une « simple » hypoperfusion colique de 69 minutes (médiane de la durée de clampage aortique) peut être suffisante pour augmenter les taux de D-lactate en période postopératoire et traduire un phénomène de translocation d'origine bactérienne. Cette information doit être vérifiée pour préciser d'une part la place du D-lactate dans la stratégie diagnostique des ischémies coliques et d'autre part pour étudier le mécanisme de la translocation.

6. Conclusion

Notre étude suggère que le D-lactate peut être un marqueur postopératoire de l'hypoperfusion colique survenue pendant la chirurgie des anévrismes de l'aorte abdominale. Des travaux complémentaires sont nécessaires pour déterminer la place d'un tel marqueur en pratique clinique.

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Etude N°6

Collange O, Tamion F, Chanel S, Hue G, Richard V, Thuilliez C, Dureuil B, Plissonnier D

D-lactate is not a reliable marker of gut ischemia-reperfusion in a rat model of supraceliac aortic clamping.

Crit Care Med, 2006. 34(5): p. 1415-9.

D-Lactate is not a reliable marker of gut ischemia–reperfusion in a rat model of supraceliac aortic clamping*

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Objective: **D-Lactate is the dextrorotatory form of L-lactate. L-Lactate is the isomer routinely tested in clinical practice to assess cell hypoxemia. D-Lactate has been recently proposed as a specific marker of gut ischemia–reperfusion (IR), particularly after surgery for ruptured aortic aneurysms. We sought to assess D-lactate as a reliable marker of gut IR in a rat model of supraceliac aortic clamping.**

Design: **Prospective, randomized trial.**

Setting: **Animal research center.**

Subjects: **Male Wistar rats.**

Interventions: **After general anesthesia, rats were randomized into two groups (n = 8 in each). The IR group underwent a laparotomy, aortic clamping for 40 mins, and 1 hr of reperfusion. The control group underwent the same procedure, except for aortic clamping.**

Measurements and Main Results: **The following variables were tested after 1 hr of reperfusion (IR group) or after the equivalent time (control group): 1) tissue and cell insult via ileum morphometry and electron microscopy, serum glutamic transaminases (serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase), pH, and L-lactate; 2) systemic inflammatory response via tumor necrosis factor-α; and 3) D-lactate levels. After IR, mucous membrane thickness and villi height decreased significantly, respectively by 30% and 45%, and electron-microscopic examination showed typical IR mucous membrane cell insult. IR also caused lactic acidosis (pH = 7.16 ± 0.05 vs. 7.31 ± 0.02, $p < .01$; L-lactate = 7.1 ± 1.6 vs. 1.6 ± 0.4 mmol/L, $p = .001$) and increased blood levels of transaminases. Concurrently, the inflammatory response was characterized by an increase in tumor necrosis factor-α (213 ± 129 vs. 47 ± 32 pg/mL, $p < .05$). However, blood levels of D-lactate never increased after IR.**

Conclusions: **D-Lactate is not a reliable marker of gut IR in our model of supraceliac aortic clamping in rats. (Crit Care Med 2006; 34:1415–1419)**

KEY WORDS: ischemia–reperfusion; gut; supraceliac aorta; D-lactate; computerized morphometric analysis; inflammatory response

There is no reliable specific biological marker for gut ischemia–reperfusion (IR)-induced alterations. The available markers are rarely used in clinical practice because they are detectable only at a time too late to treat the ischemic injury. For example, elevation of creatine phosphokinase-BB enzyme levels specifically indicating gut muscular insult is only detectable 12 hrs after an ischemic injury of the gut has occurred, by which time the damage is irreversible (1, 2). Several previous animal and human studies have proposed D-lactate as a sensitive, specific, and early marker of gut ischemia (3–6).

D-Lactate is the dextrorotatory form of lactate habitually tested for in the intensive care unit. Its two isomers, (L- and D-lactate) differ in terms of the position of their alpha-hydroxyl radical. Each isomer results from the reduction of pyruvate by a specific lactate dehydrogenase. Therefore, to produce L-lactate from a pyruvate molecule (optically neutral), an L-lactate dehydrogenase is needed, and conversely, a D-lactate dehydrogenase will produce D-lactate.

Mammals only have one type of enzyme: L-lactate dehydrogenase. L-Lactate is a marker of cell hypoxemia, and its level correlates with survival for patients with septic shock (7–9). Microorganisms, particularly bacteria, are equipped with D-lactate dehydrogenase and produce D-lactate during fermentation. D-Lactate is therefore a marker of bacterial infection (10–12). D-Lactate has also recently been proposed as a marker for translocation in gut ischemia (4, 6). However, there is no study comparing the insult of the gut, the inflammatory response, and the D-lactate levels in an IR model.

The objective of our study was to determine whether D-lactate is a reliable marker of gut IR in rats.

MATERIALS AND METHODS

Experimental Groups

This study was performed on 14-wk-old male Wistar rats weighing between 350 and 400 g (Janvier Laboratory, Orléans, France). Rats were treated under institutional guidelines of animal use and care. Animals were allowed unrestricted access to food and water before and after operation.

The rats were divided into two groups of equal size:

- IR group: aortic clamping for 40 mins and 1 hr of reperfusion (n = 8).
- Control group: sham-operated animals (n = 8).

*See also p. 1561.

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Experimental Procedures

The experiments were carried out in accordance with the French code of conduct concerning laboratory animals (university license: A76-450-0). The rats were anesthetized with ketamine (Imalgène 1000) injected at 0.2 mL/100 g into the peritoneum and placed in a supine position. A carotid approach was performed by an anterior cervical incision to insert a 3-Fr catheter into the right carotid artery. Arterial pressure was monitored continuously throughout the procedure by a Gould arterial pressure recorder attached to this catheter. A midline incision was made in the abdomen, and the aorta was visualized between the diaphragmatic cruxes upstream of the celiac trunk. For the IR group, the supraceliac aorta was clamped for 40 mins with an atraumatic vascular clamp. The abdomen was closed, leaving an orifice in the xiphoid region to withdraw the aortic clamp after the 40-min clamping period. The abdominal wall was then completely closed. For the sham-operated group of control animals, the surgical procedure was identical except for aortic clamping.

After 1 hr of reperfusion (IR group) or the equivalent time (control group), the animals were exsanguinated under anesthesia by aortal puncture. Blood samples were obtained for determination of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, pH, bicarbonate, $Paco_2$, tumor necrosis factor-α (TNFα), and L- and D-lactate. Intestinal (ileum) tissue samples were obtained for morphometric analysis and electron microscopy.

Measurements

Hemodynamic Variables. Continuous hemodynamic monitoring was done throughout the procedure. This monitoring enabled us to record the animals' heart rates and blood pressures. Mean arterial pressure (MAP) was calculated using the following formula: MAP = (2 × DAP + SAP)/3, in which DAP is diastolic blood pressure and SAP is systolic blood pressure.

Tissue and Cell Insult After 1 hr of Reperfusion. Gut insult was assessed by computerized morphometric study of ileal mucous membranes and scanning electron microscopy.

- For morphometric study, samples were set in paraffin and cut into 5-μm slices. The slices were scanned (Nikon Super Coolscan 8000 ED, Tokyo, Japan) and analyzed with a computerized image analysis system (ADCIS Aphélion v 3.2, Hérouville Saint-Clair, France). We measured mucous membrane thickness and villi height. Three measurements were made for each animal, and a mean value was calculated for each variable.
- For electron microscopy, ileum tissue was sampled from four randomly chosen animals of each group. Samples were immediately fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 hrs at 22°C, rinsed for 18 hrs (4°C) with 0.05 Tris buffer (pH 7.6), and washed three times for 5 mins each time. An H-2000 transmission electron microscope was used for the examination.

Blood Markers of Cell Insult. We measured transaminases, L-lactate, and blood gas (pH, bicarbonate, $Paco_2$) as potential markers of cell insult.

- Transaminases were measured as a nonspecific marker of cell insult. Serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase tests were performed on the serum obtained after centrifugation (3,500 × *g* for 15 mins) to separate the blood components. Measurements were made, after calibration according to the International Federation of Clinical Chemistry recommendations, on a Synchron IR (Beckman-Coulter, Fullerton, CA).
- L-Lactate and arterial blood gases samples were drawn with Pulsator-type syringes (Portex, Keene, NH). Measurements were made on an ABL 700 (Radiometer, Copenhagen, Denmark).

Systemic Inflammatory Response 1 hr After Reperfusion. The blood TNFα level was used as a marker of systemic inflammatory response to the ischemia–reperfusion insult. A portion of the serum obtained by centrifugation was stored at −80°C to be tested later for TNFα. TNFα was measured using an enzyme-linked immunoassay (Cytoscreen, Biosource International, Camarillo, CA). Specific anti-TNFα antibody was mixed with the sample, and a second marker antibody was then added; after rinsing with phosphate-buffered saline, a developer was added to stain the solution. The stained solution was then analyzed by measuring optical density at 450 nm. Each sample was measured twice. A standard range of TNFα levels was also measured.

D-Lactate Measurement. After sampling, the proteins were removed from 1 mL of whole blood with perchloric acid (1:1 vol). Then, the samples were centrifuged at 4°C at 1500 rpm for 20 mins and stored at −70°C until the spectrochemical D-lactate measurements were made (10).

To avoid missing a transient D-lactate peak during the early reperfusion time, an additional series of eight rats was tested. For this extra series, IR 15, the clamping time was also 40 mins, but the rats were killed for D-lactate testing only 15 mins after reperfusion.

Statistical Analyses

The results are expressed using mean values ± SD and analyzed by a two-sided Mann-Whitney test (significance level of 5%). Values were considered statistically significant at an α of $<.05$. Analyses were performed with StatView for Windows (v 5.0, SAS Institute, Cary, NC).

RESULTS

Hemodynamic Effects of Clamping the Supraceliac Aorta

MAPs in IR and control groups are shown in Figure 1. Before aortic clamping

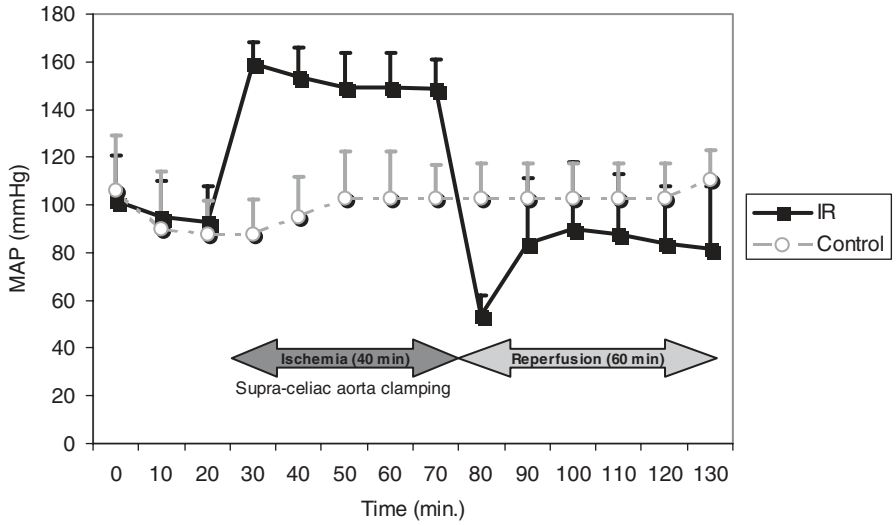

Figure 1. Change in mean arterial pressure (*MAP*) during clamping and unclamping of the supraceliac aorta. During aortic clamping, arterial pressure increased. When the clamp was removed, the arterial pressure decreased. Arterial pressure of the ischemia–reperfusion (*IR*) group was lower than in the control group during all reperfusion. The IR group underwent supraceliac aortic clamping for 40 mins and 1 hr of reperfusion; for the group control, the supraceliac aorta was approached but not clamped.

there was no statistical difference in MAP ($p = .70$) or heart rate between the two groups ($p = .81$). In the control group, blood pressure ($p = .57$) and heart rate ($p = .94$) did not vary significantly during the experiment.

Effects of Clamping/Unclamping. During aortic clamping, MAP increased (159 ± 9 vs. 88 ± 15 mm Hg, $p = .0001$). During the same time, heart rate decreased (312 ± 34 vs. 389 ± 55 beats/min, $p = .016$). Immediately after unclamping of the aorta, MAP decreased to reach its lower value of 54 ± 8 mm Hg. Heart rate was also significantly lower after unclamping. During reperfusion, blood pressure increased gradually. MAP in the IR group was lower than in the control group throughout reperfusion. The reduced MAP in the IR group as compared with the control group was statistically significant until after 10 mins of reperfusion. In the IR group, the heart rate increased gradually but remained significantly lower than in the control group for 30 mins of reperfusion.

Tissue and Cell Insult

The results for both groups are shown in Table 1.

Gut Insult. In morphometric analysis, after IR, mucous membrane thickness and villi height were highly reduced. The mean thickness of the mucous membrane was reduced by 30% in the IR group (307 ± 9 vs. 442 ± 39 μm, $p = .0003$) and height of the villi was reduced by >45% (140 ± 12 μm vs. 266 ± 36 μm, $p = .0028$) (Table 1, Fig. 2).

Electron microscopic examination showed ultrastructural epithelial damage as mitochondrial swelling, disorientation, or disappearance of cristae (Fig. 3).

Cell Insult. One hour after reperfusion, the mean serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase values were significantly increased in the IR group (Table 1). IR also generated metabolic acidosis. This metabolic acidosis was accompanied with an increase in L-lactate of 7.1 ± 1.6 mmol/L (vs. 1.6 ± 0.4 mmol/L control group, $p = .0012$).

Inflammatory Response. After only 1 hr of reperfusion, TNFα blood level was significantly higher in the IR group, with an increase of 350% (213 ± 129 vs. 47 ± 32 pg/mL, $p = .021$).

D-Lactate

There was no significant difference between the D-lactate level in IR and control groups (0.05 ± 0.03 vs. 0.04 ± 0.01 mmol/L, $p = .34$).

In the IR 15 extra series (in which D-lactate was measured 15 mins after reperfusion), the D-lactate level was not significantly different between IR and control group values (0.04 ± 0.03 mmol/L).

Table 1. Results after ischemia (40 mins) and reperfusion (1 hr)

	IR (Mean ± SD)	Control (Mean ± SD)	p
Hemodynamic measures			
Mean arterial pressure, mm Hg	82 ± 26	110 ± 12	.07
Heart rate, beats/min	390 ± 46	370 ± 33	.34
Tissue and cell insult			
Mean thickness of gut mucous membrane, μm	307 ± 9	442 ± 39	.0003
Mean height of gut villi, μm	140 ± 12	266 ± 36	.003
SGOT, IU/L	576 ± 316	179 ± 82	.037
SGPT, IU/L	320 ± 168	72 ± 19	.014
pH	7.16 ± 0.05	7.31 ± 0.02	.0007
$Paco_2$, kPa	4.3 ± 0.8	5.9 ± 0.6	.023
HCO_3^-, mmol/L	11 ± 3	22 ± 2	.0007
L-Lactates, mmol/L	7.1 ± 1.6	1.6 ± 0.4	.001
Inflammatory response			
TNF-α, pg/mL	213 ± 129	47 ± 32	.021
D-Lactate			
D-Lactate, mmol/L	0.05 ± 0.03	0.04 ± 0.01	.34
D-Lactate 15 mins after reperfusion (IR 15), mmol/L	0.04 ± 0.03		

IR, ischemia-reperfusion group (aortic clamping for 40 mins and 1 hr of reperfusion (n = 8); Control, control group (sham-operated animals, which had the supraceliac aorta approached but not clampled; n = 8); SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamic-pyruvic transaminase; TNF, tumor necrosis factor; IR 15, additional group with aortic clamping for 40 mins and only 15 mins of reperfusion (n = 8; D-lactate measures only).

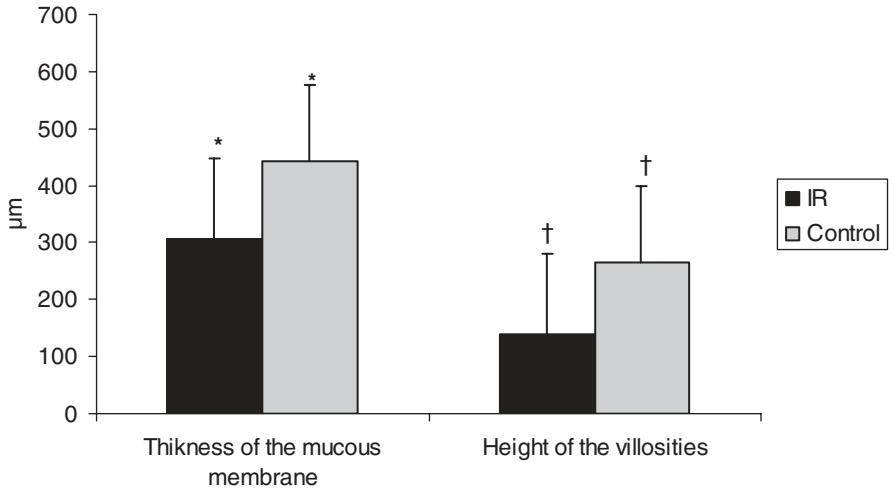

Figure 2. Effect of ischemia–reperfusion (*IR*) on gut: morphometry. The IR group underwent supraceliac aortic clamping for 40 mins and 1 hr of reperfusion; for the group control, the supraceliac aorta was approached but not clamped. *$p = .0003$; †$p = .003$.

DISCUSSION

In our rat model of supraceliac aortic clamping, D-lactate seemed to remain low after 15 mins and 1 hr of reperfusion. During the same time, gut insult was proved by a significant decrease in mucous membrane thickness and in villi height. At the cell level, electron microscopy showed typical ultrastructural epithelial damage as mitochondria swelling and loss of cristae. These findings correlated with a systemic inflammatory response detected by a significant increase in blood TNFα levels.

Model of Supraceliac Clamping. Many gut IR models have been described in the literature, but very few used supraceliac clamping. Several dog (13–16), rabbit (17), or mouse (18, 19) models have been reported. To the best of our knowledge, no rat model of supraceliac aortic clamping has been described until now. This model has two advantages. First, our

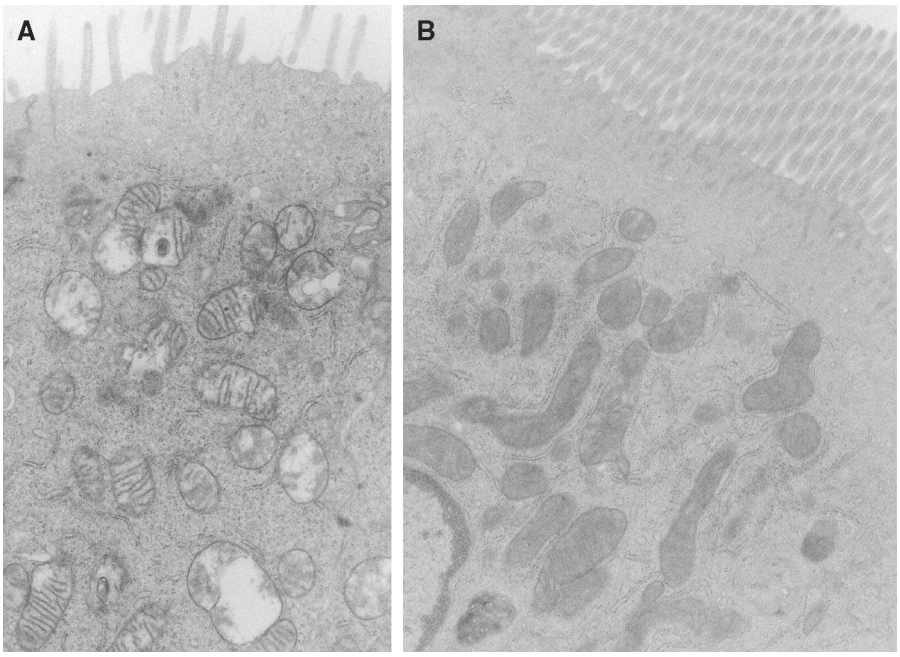

Figure 3. Effect of ischemia-reperfusion (IR) on the ileum's epithelial cells. *A*, IR group (supraceliac aortic clamping for 40 mins and 1 hr of reperfusion). Epithelial damage was demonstrated by swollen mitochondria and loss of cristae. *B*, control group.

model causes an IR of the gut. The IR periods used in our study (40 mins of ischemia and 1 hr of reperfusion) were sufficient to observe a significant inflammatory response. Second, this model has some clinical relevance because, with treatment of a thoraco-abdominal aneurysm, the patient's supraceliac aorta must be clamped.

The main observed result (i.e., absence of D-lactate elevation) could be explained by too early a measurement after reperfusion (1 hr). However, the choice of the other markers seemed to be judicious. Indeed, TNFα increases occurred at the same time when histologic lesions of gut ischemia were evidenced.

D-Lactate and IR. Unlike the Poeze et al. (6), Murray et al. (3, 4) and Gunel et al. (5) studies, our study failed to show any increase in D-lactate after gut IR. Indeed, the D-lactate levels recorded are all lower than the 0.20 mmol/L value defined by Poeze et al. (6) and Murray et al. (4) as being the threshold value for the detection of gut ischemia. The D-lactate levels remained under this threshold and did not differ among our groups (control, IR, or IR 15).

Gunel et al. (5) and Murray et al. (3, 4) assessed the D-lactate in rabbit and rat models of IR by clamping the superior mesenteric artery. The D-lactate increased significantly after 1 hr of reperfusion in the IR groups. In these studies, there was no hemodynamic record and no comparison with other markers of IR, so the gut insult was not characterized. Poeze et al. (6) investigated postoperative colonic ischemia in patients consecutive to surgical repair of a ruptured aneurysm of the abdominal aorta. Their data showed that a D-lactate level of >0.20 mmol/L was a predictive factor for postoperative colonic ischemia (6). Other studies have demonstrated that after aortic repair, there is a correlation between the rate of colonic ischemia and the shock severity observed during surgery (20, 21). Consequently, D-lactate elevation could also be a marker of shock rather than of gut ischemia *per se*. In another study by Murray et al. (4), D-lactate level was >0.20 mmol/L in patients with mesenteric ischemia diagnosed after an emergency laparotomy. Although no hemodynamic data are available in this study, one can hypothesize that such severe patients also had hemodynamic instability and shock, which in turn may account for the increase in D-lactate. A clinical study performed in our department seems to confirm this hypothesis: D-lactate was studied after aortic aneurysm operation, and its level was significantly higher in those cases with shock than in those without (0.21 vs. 0.06 mmol/L, $p = .001$) (unpublished personal data).

Furthermore, D-lactate could also be a marker of bacteriemia. Indeed, D-lactate is a marker of the metabolic activity of bacteria in body fluids such as the synovial fluid, ascites, pleural fluid, or cerebral spinal fluid (10). If bacteriemia or septicemia occur, it is possible that the presence of bacteria in the blood may be sufficient to induce an increase in D-lactate level.

CONCLUSIONS

Our model of clamping the supraceliac aorta caused IR of the gut and a systemic inflammatory response after 1 hr of reperfusion but no change in blood D-lactate level. These results indicate that D-lactate is not a reliable marker of gut ischemia in our animal model.

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Etude N°7

Collange O, Tamion F, Meyer N, Quillard M, Kindo M, Hue G, Veber B, Dureuil B, Plissonnier D,

Early detection of gut ischemia-reperfusion injury during aortic abdominal aneurysmectomy: a pilot, observational study.

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Early Detection of Gut Ischemia-Reperfusion Injury During Aortic Abdominal Aneurysmectomy: A Pilot, Observational Study

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Objective: **D-lactate is the enantiomer of L-lactate, which is measured routinely in clinical practice to assess cell hypoxia. D-lactate has been proposed as a specific marker of gut ischemia-reperfusion (IR), particularly during surgery for ruptured abdominal aortic aneurysms. The aim of this study was to compare the use of D-lactate measurement and colonic tonometry (taken as a reference method) for gut IR detection during elective infrarenal aortic aneurysm (IrAA) surgery.**

Design: **Prospective, monocenter, observational study.**

Setting: **Vascular surgery unit, university hospital.**

Participants: **Candidates for elective IrAA surgery.**

Interventions: **Patients without (controls) and with gut IR (defined as $\Delta CO_2 > 2.6$ kPa) were compared retrospectively.**

Measurement and Main Results: **D-lactate levels were compared with colonic perfusion levels (ΔCO_2), as assessed by colonic tonometry, at 7 time points during surgery and until 24 hours after surgery. D-lactate also was measured in mesenteric vein blood before and after gut reperfusion. Plasma TNF-α level was measured at the same time points to assess systemic inflammatory response. Eighteen patients requiring elective IrAA surgery were included. The ΔCO_2 and TNF-α level varied significantly over time. There was a significant ΔCO_2 peak at the end of clamping (2.6 ± 1.8 kPa, $p = 0.006$) and a significant peak in TNF-α level after 1 hour of reperfusion (183 ± 53 ng/L, $p = 0.05$). D-lactate levels were undetectable in systemic and mesenteric blood in all the patients throughout the study period. Gut IR patients ($n = 6$) experienced a longer overall duration of intraoperative hypotensive episodes and received more catecholamines than the controls ($n = 12$).**

Conclusions: **Compared with colonic tonometry, D-lactate was not a reliable biomarker of gut IR during elective IrAA surgery.**

KEY WORDS: aortic aneurysm, abdominal, mesenteric vascular occlusion, reperfusion injury, inflammation mediators, D-lactate

GUT HYPOXIA (ischemia-reperfusion [IR]) often occurs during aortic aneurysm surgery as a result of clamping of the aorta, reduced intraoperative blood pressure, and anesthesia.[1,2] It is associated with an increased incidence of postoperative complications including systemic inflammatory response and multiple-organ dysfunction syndrome.[3,4] Early detection of gut injury, thus, is essential for the best management.

The diagnosis and assessment of gut hypoxia are challenging. Inadequate splanchnic perfusion can be detected indirectly from an increase in lactate levels. Monitoring of intestinal perfusion by intestinal tonometry can detect early mucosal ischemia, as reflected by a decrease in intramucosal intestinal pH (pHi) or widening of the regional-arterial PCO_2 gradient (ΔCO_2).[5] Both pHi and ΔCO_2 are reliable prognostic indicators for the development of organ failure after major surgery.[6] However, arterial lactate concentrations have poor sensitivity as an index of gut hypoxia and as a predictor of postoperative organ failure.[4] Serum D-lactate levels, on the other hand, may prove to be an early indicator of intestinal ischemia.[7-10]

Each lactate isomer results from the reduction of a pyruvate molecule (optically neutral) by a specific lactate dehydrogenase. An L-lactate dehydrogenase produces L-lactate, and a D-lactate dehydrogenase produces D-lactate. Mammals only possess L-lactate dehydrogenase, whereas micro-organisms, particularly bacteria, have D-lactate dehydrogenase and produce D-lactate during fermentation.[11] A leaky mucosal barrier might cause permanent translocation and accumulation of D-lactate.[10] Thus, D-lactate might be a useful clinical marker for the early diagnosis of acute intestinal insult in clinical practice.[9,10,12]

However, the evidence for an association between circulating D-lactate levels and ischemia-induced intestinal injury is conflicting.[13,14] The aim of this study was to compare D-lactate levels and tonometry ΔCO_2 values as early indicators of postoperative gut hypoxia after elective infrarenal aortic aneurysm (IrAA) surgery.

METHODS

This was a prospective, observational, single-center study conducted in a vascular surgery unit. The study compared 2 diagnostic techniques for gut hypoperfusion; namely, measurement of D-lactate levels and colic tonometry. Both indirectly assess gut mucosal perfusion. The institutional Ethics Committee on Human Research approved the study protocol, and written informed consent was obtained from all the patients before surgery.

Patients requiring elective IrAA surgery between January and September 2002 were enrolled in the study. The main criteria for

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exclusion were age <18 years, recent colonic surgery or pre-existing colonic disease, a diagnosis of mycotic aneurysm, recent inflammatory syndrome (fever above 38°C or recent history of infection), recent gastrointestinal bleeding, pre-existing chronic renal insufficiency (creatinine clearance <15 mL/min), and participation in another clinical trial less than 30 days before enrollment. Patients with iliac aneurysms and in whom a need for bifurcation grafts or suprarenal clamping was foreseen also were excluded.

Patients were premedicated with 1.5 mg/kg of hydroxyzine orally. Anesthesia was induced with sufentanil, propofol, and vecuronium and maintained after orotracheal intubation with isoflurane-nitrous oxide, sufentanil, and vecuronium. The inspired oxygen fraction was maintained at 0.4. Epidural anesthesia was not administered.[15] A triple-lumen catheter was inserted into the internal jugular vein, and a radial artery catheter was used for arterial pressure monitoring and blood sampling. All patients received 10 to 15 mL/kg/h of isotonic sodium chloride intraoperatively. Colloid infusion was given to maintain a stable hemodynamic state (systolic arterial pressure >90 mmHg and urine output >50 mL/h). Solutions containing lactate ions (eg, lactated Ringer's) were not used. Vasopressor agents (dopamine or norepinephrine) were started when fluid resuscitation was not sufficient to correct hypotension (systolic arterial pressure <90 mmHg).[16] The choice of vasoactive agent was free. Blood collected by the cell saver was infused in the normal way. Patients with a hemoglobin concentration <10 g/dL received packed red blood cells to achieve a 10 g/dL concentration.

The aorta was approached via a midline laparotomy. It was clamped below the renal artery and above the inferior mesenteric artery. The inferior mesenteric artery was not reimplanted.

The patients' age, sex, body weight, and comorbidities were recorded. The following intraoperative data were recorded: duration of aortic clamping, duration of surgery, blood transfusion volumes, cell saver transfusion volumes, number of hypotensive episodes, duration of hypotension, fluid management and load (total, crystalloid, and colloid), arterial pressure, cardiac frequency, catecholamine dose, and urinary output. Postoperative data were duration of sedation, duration of mechanical ventilation, blood transfusion and fluid resuscitation, urinary output, unspecific clinical symptoms compatible with ischemic colitis (diarrhea and blood), and in-hospital mortality. The number of hypotensive episodes (systolic arterial pressure <90 mmHg) was noted and their overall duration determined.

To assess the robustness of tonometry, 2 groups of patients were assembled retrospectively according to the ΔCO_2 value measured at the end of aortic occlusion (T2). The control group had a ΔCO_2 below 2.6 kPa and the gut IR above 2.6 kPa at T2. A 2.6-kPa threshold was chosen because outcomes are poor when the value is higher.[4,19-21] The 2 groups of patients were compared retrospectively on the basis of patient demographics, intraoperative data (listed previously), postoperative data, and TNF-α values after 1 hour of reperfusion. The intraoperative variables influencing ΔCO_2 at T2 and the relationship between ΔCO_2 and the overall duration of intraoperative hypotensive episodes were analyzed.

All parameters were performed at 7 time points: before aortic cross-clamping (T1), at the end of aortic cross-clamping time (just before aortic cross-clamp release, T2), 15 minutes (T3), 1 hour (T4), 3 hours (T5), 6 hours (T6), and 24 hours (T7) after aortic clamp release. Ischemia was defined as the period between aortic cross-clamp and aortic cross-clamp release; reperfusion was defined as the period after aortic cross-clamp release.

A TRIP tonometer (Tonometrics Division, Instrumental Corp., Helsinki, Finland) was used to measure the regional CO_2 ($PrCO_2$). The tonometer is a silicon 8-F catheter with a balloon tip and a semipermeable membrane that allows diffusion of gases but not fluids. After induction of anesthesia, the tonometer was introduced intrarectally, positioned in the sigmoid colon, and connected to a Tonocap device (Datex, Helsinki, Finland) for gas tonometry. Correct placement of the catheter was checked by radiography before surgery and de visu by the surgeon during laparotomy. The tonometer was maintained in situ throughout the surgery. It was removed 24 hours after aortic cross-clamp release. After automatic calibration, regional CO_2 gut pressure ($PrCO_2$) was measured by infrared spectroscopy. Arterial blood samples were obtained simultaneously to measure $PaCO_2$. The ΔCO_2 was calculated as the gut-to-arterial PCO_2 difference ($P(r-a)CO_2$) as described elsewhere.[17]

Blood specimens were collected in heparinized plastic tubes. Plasma was separated by centrifugation at 3,500 rpm for 10 minutes and stored at −80°C before analysis. Tumor necrosis factor alpha (TNF-α) was determined on duplicate samples by an automated chemiluminescent immunometric assay (DPC Immulite 1000 analyzer, Siemens Healthcare Diagnostics) (detection threshold, 1.7 ng/L). TNF-α levels were measured at the same 7 time points as for D-lactate determinations (stated previously).

After sampling, 1 mL of whole blood was deproteinized with perchloric acid, centrifuged at 3,500 rpm for 20 minutes at 4°C, and stored at −70°C until enzymatic spectrophotometry.[18] A standard curve was obtained before each set of measurements. The technique's sensitivity threshold was 0.14 mmol/L.[13] Arterial D-lactate was determined at 7 time points; additionally, venous mesenteric D-lactate was measured at T1 and T2.

Results were expressed as means ± standard error or medians with ranges. As the data were not Gaussian, differences in variables across multiple time points were tested using the Friedman repeated-measures analysis of variance on ranks, followed by multiple comparisons corrected for multiplicity. The retrospective comparison between groups was performed using the unpaired t-test, multiple regression analysis, and the Spearman correlation test. The significance threshold was $p < 0.05$. Analyses were performed using SPSS 13.3 for the overall analysis, an Excel spreadsheet for post hoc comparisons, and GraphPad Prism version 5.03 for Windows.

RESULTS

Eighteen patients (mean age, 70 ± 2 years) were included in the study (Table 1). All underwent surgery for uncomplicated, elective IrAA. There were no cases of aneurysm of the iliac vessels. The main comorbidities were arterial hypertension (9/18), moderate cardiac insufficiency classes I and II (New York Heart Association classification system) (6/18), and diabetes mellitus (4/18).

Intraoperative and postoperative data are summarized in Table 1. No patient developed clinical symptoms of colonic ischemia. There were no in-hospital mortalities.

The ΔCO_2 varied significantly over time ($p = 0.014$) (Tables 2 and 3). It was 1.8 ± 0.4 kPa (13.5 ± 3 mmHg) before aortic cross-clamping and peaked significantly at the end of clamping (2.6 ± 0.4 kPa [19.5 ± 3 mmHg]; $p < 0.05$). It decreased below 2 kPa (15 mmHg) by 3 hours of reperfusion, but a second significant increase was observed at 6 hours of reperfusion (2.7 ± 0.5 kPa [20.2 ± 3.7 mmHg]; $p < 0.05$). It decreased again to below 2 kPa (15 mmHg) after 24 hours of reperfusion. Overall, 13 patients had a ΔCO_2 above 2 kPa (15 mmHg). If the ΔCO_2 threshold for detecting gut ischemia was taken as 2.6 kPa (19.5 mmHg), 6 patients may have been considered to have experienced gut ischemia (Fig 1).

TNF-α levels varied significantly over time ($p < 0.001$) (Table 2). The level was 34 + 12 ng/L at baseline and peaked at 1 hour of reperfusion to 161 ± 67 ng/L, a value that was significantly higher ($p < 0.05$) than the values before aortic

Table 1. Perioperative Data

	Total	Control	Gut IR	p
Number (n)	18	12	6	
Demographic Data				
Age (y)	70 ± 2	71 ± 3	67 ± 4	0.45
Weight (kg)	81 ± 4	75 ± 4	93 ± 7	0.06
Comorbidities				
Arterial hypertension (n)	9	6	3	>0.9
Cardiac insufficiency (n)	6	2	4	>0.5
Diabetes mellitus (n)	4	3	1	>0.5
Perioperative Data				
Laparotomy duration (min)	202 ± 16	208 ± 21	190 ± 24	0.37
Aortic occlusion duration (min)	86 ± 9	82 ± 10	95 ± 18	0.57
Blood cell clots (n)	1.1 ± 0.3	1.1 ± 0.4	0.8 ± 0.5	0.88
Cell saver transfusion (mL)	503 ± 119	476 ± 142	558 ± 235	0.75
Fluid load				
Total (mL/kg)	27 ± 2	27 ± 3	25 ± 2	0.81
Crystalloid (mL/kg)	15 ± 1	15 ± 2	15 ± 2	0.85
Colloid (mL/kg)	11 ± 2	12 ± 3	10 ± 2	0.64
Catecholamines				
All (n)	13	7	6	>0.05
Dopamine (n)	6	4	2	>0.9
Norepinephrine (n)	7	3	4	>0.05
Dopamine equivalent dose (μg/kg/min)	5 ± 0.9	3.7 ± 1	7.6 ± 1.3	0.04
Hypotensive episodes				
Number	3.2 ± 0.6	2.5 ± 0.6	4.5 ± 1.4	0.34
Duration (min)	22 ± 4	16 ± 4	33 ± 7	0.04
Urinary output (mL/kg/h)	3.3 ± 1.2	2.7 ± 0.8	4.6 ± 3.4	
Postoperative Data				
Sedation duration (h)	7 ± 1.4	6 ± 1.4	9 ± 3.2	0.32
Mechanical ventilation duration (h)	11.3 ± 1.6	9.3 ± 1.8	15.2 ± 3.2	0.1
Blood product transfusion (n)	1.3 ± 0.4	1.5 ± 0.5	0.8 ± 0.5	0.34
Fluid load				
Total (mL)	104 ± 7	111 ± 9	91 ± 12	0.2
Crystalloid (mL)	64 ± 6	68 ± 8	56 ± 12	0.4
Colloid (mL)	40 ± 2	43 ± 3	35 ± 3	0.12
Catecholamines				
All (n)	14	8	6	>0.1
Dopamine (n)	6	4	2	>0.9
Norepinephrine (n)	8	4	4	>0.1
Dopamine equivalent dose (μg/kg/min)	5.3 ± 1	4.1 ± 1.3	7.6 ± 1.3	0.1
Urinary output (mL/kg/h)	1.6 ± 0.2	1.8 ± 0.3	1.2 ± 0.3	0.24

NOTE. Values are expressed in mean + SE.

cross-clamping, after clamp release, and after 24 hours of reperfusion. After 3 hours of reperfusion, the level decreased gradually to reach 25 ± 5 ng/L at 24 hours (Fig 2).

D-lactate levels in the systemic blood remained below the sensitivity threshold (0.14 mmol/L) at all times (Table 2). The highest level recorded in any patient was 0.12 mmol/L. The values did not vary over time (mean from 0.01-0.03 mmol/L) and, therefore, were taken to be nil for all patients at all times.

Patients with a ΔCO_2 above 2.6 kPa at T2 (gut IR group, n = 6) were compared with patients with a ΔCO_2 below 2.6 kPa (controls, n = 12). The gut IR patients had a longer overall duration of intraoperative hypotensive episodes (16.4 *v* 33 ± 7 min; p = 0.04) and received a higher dopamine equivalent dose (3.7 ± 1 *v* 7.6 μg/kg/min; p = 0.04) (Table 1). There were no significant differences in terms of fluid load or urinary output.

According to the multiple regression analysis, no independent variable influenced ΔCO_2. However, there was a positive correlation between ΔCO_2 and the overall duration of hypotensive episodes (r = 0.51; p = 0.03). There was no significant difference between the 2 groups in terms of TNF-α after 1 hour of reperfusion (T4) (188 ± 104 *v* 107 ± 78, p = 0.42).

DISCUSSION

This study has shown that (1) gut hypoxia, as assessed by colonic tonometry, was associated with elective IrAA surgery, with a systemic inflammatory response occurring during reperfusion; and (2) D-lactate was not a reliable biomarker for assessing impaired intestinal mucosal barrier function during routine IrAA surgery.

Gut (gastric or colonic) tonometry was proposed originally to assess splanchnic perfusion. It has proved to be useful in predicting the inflammatory response status and outcome, especially in critically ill patients, and in detecting early hypovolemia or shock.[17,22] Gut injury occurs during clamping and declamping of the abdominal aorta and can trigger the absorption of bacteria or bacterial endotoxins across the ischemic intestinal wall and the release of cytokines from the intestine. Patients who reach or maintain a normal ΔCO_2 after an aggressive insult such as surgery have a higher probability of survival.[6,23] In addition, gastric intramucosal pH or the mucosal-arterial CO_2 gap may be better endpoints of resuscitation than conventional whole-body parameters. Colonic tonometry is used during emergency surgery for ruptured abdominal aortic aneurysm (AAA), and ΔCO_2 is correlated with the incidence of colonic ischemia episodes, postoperative complications, and mortality.[19,22,24,25]

The increase in ΔCO_2 during a decrease in oxygen delivery can be explained by at least 2 different mechanisms: (1) a decrease in CO_2 washout secondary to a decrease in splanchnic blood flow; and (2) an imbalance between decreased aerobic CO_2 production and increased anaerobic production due to H^+ buffering by bicarbonates.[26] In this study, the ΔCO_2 peak at the end of aortic cross-clamping may be considered as a decrease in splanchnic blood flow.

The ΔCO_2 value is about 1 kPa (7.5 mmHg) in normal volunteers, and a ΔCO_2 above 2.6 kPa (19.5 mmHg) predicted death in patients in intensive care.[19] Lebuffe et al showed that patients with a ΔCO_2 between 1.6 and 2.7 kPa (12 and 20.2 mmHg) stayed longer in intensive care after cardiac surgery. Lee et al observed that aortic occlusion was associated

Table 2. ΔCO2, TNF-α and D-Lactate During Infrarenal Aortic Surgery

			D-Lactate (mmol/L)	
Perioperative Time	ΔCO_2 (kPa)	TNF-α (ng/L)	Systemic Arterial Blood	Mesenteric Vein Blood
T1	1.8 ± 0.4$	34 ± 12*	0.01 ± 0.01	0.01 ± 0.01
T2	2.6 ± 0.4$	61 ± 35*	0.02 ± 0.01	0.02 ± 0.01
T3	2.2 ± 0.3	117 ± 70	0.02 ± 0.01	
T4	2.3 ± 0.3	161 ± 73*	0.03 ± 0.01	
T5	1.9 ± 0.5£	71 ± 17	0.03 ± 0.01	
T6	2.7 ± 0.5£	64 ± 19	0.03 ± 0.01	
T7	1.7 ± 0.1	25 ± 5*	0.02 ± 0.01	
Variation over time (the Friedman test)	Yes, $p = 0.014$	Yes, $p < 0.001$	No	No

NOTE. Results are expressed in mean ± SEM.

Variation of ΔCO_2, TNF-α, and D-Lactate across time for all patients.

All parameters were performed at 7 time points: before aortic cross-clamping (T1), at the end of aortic cross-clamping time (just before aortic cross-clamp release, T2), 15 minutes (T3), 1 hour (T4), 3 hours (T5), 6 hours (T6), and 24 hours (T7) after aortic clamp release. Ischemia was defined as the period between aortic cross-clamp and aortic cross-clamp release; reperfusion was defined as the period after aortic cross-clamp release.

ΔCO_2 was the reference parameter for gut ischemia.

$\Delta CO_2 = PrCO_2 - PaCO_2$. $PrCO_2$ = regional pressure in CO_2, measured by gas tonometry in the colon. ΔCO_2 significantly varied over time ($p = 0.014$). Significant pairwise comparison is indicated by $ and £ ($p < 0.05$).

TNF-α was used as a marker of systemic inflammatory response.

TNF-α significantly varied over time ($p < 0.001$).

D-Lactate was tested as a reliable marker of gut IR.

D-lactate did not vary over time. All D-lactate values were considered as nil (under the sensitivity threshold of the measurement technique, 0.14 mmol/L).

* values significantly different ($p < 0.05$).

with a transient drop in rectal mucosal oxygen saturation (−50% to 75%), without any detectable clinical consequences.[27] Among the 18 patients in this study, 13 had a ΔCO_2 above 2 kPa (15 mmHg) and 6 had a ΔCO_2 above 2.6 kPa (19.5 mmHg), suggesting colonic hypoperfusion.

Other teams have tested the diagnostic accuracy of sigmoid pHi in the evaluation of colon ischemia in patients undergoing AAA surgery.[20,28,29] Sigmoid pHi was related to the severity of colonic damage and the duration of ischemia. Donati et al demonstrated that pHi and ΔCO_2 are the sensitive prognostic indicators during AAA surgery and suggest that tonometry may identify patients at higher risk of postoperative organ failure. The authors' study found a significant ΔCO_2 peak at the end of aortic cross-clamping. It also found that the ΔCO_2 value at T2 was associated with a longer overall duration of intraoperative hypotensive episodes.

During reperfusion, the ΔCO_2 values decreased below 2 kPa (15 mmHg) by 3 hours of reperfusion but a significant increase in ΔCO_2 occurred at 6 hours, which was not associated with any clinical event (hemodynamic changes, use of vasoactive agents, awakening of patient or extubation, etc). However, by 24 hours of reperfusion, the ΔCO_2 value had decreased again below 2 kPa (15 mmHg). These observations were consistent with published results, indicating that the lowest levels of sigmoid pHi are found 4 to 6 hours after aortic clamping.[28,29] It is suggested that the systemic inflammatory response, as shown by the TNF-α value and observed at 1 hour of reperfusion, might explain the ΔCO_2 peak at 6 hours although the data are too weak to establish a clear relationship between these events.

A significant TNF-α peak at 1 hour of reperfusion was observed. The observed variations in colonic perfusion, thus, were sufficiently great to induce a systemic inflammatory response, as already described.[17,22] Thus, TNF-α may play a significant role in the pathogenesis of organ dysfunction through vascular endothelial damage.

Table 3. Hemodynamics During Infrarenal Aortic Surgery

	Cardiac Frequency (beats/min)		Systolic Arterial Pressure (mmHg)		Diastolic Arterial Pressure (mmHg)		Mean Arterial Pressure (mmHg)	
	Control	Gut IR	Control	Gut IR	Control	Gut IR	Control	Gut IR
T1	59 ± 11	62 ± 11	106 ± 32	113 ± 29	62 ± 14	64 ± 17	81 ± 18	83 ± 21
T2	59 ± 13	63 ± 15	109 ± 17	112 ± 15	56 ± 7	56 ± 8	77 ± 10	78 ± 10
T3	68 ± 15	65 ± 14	107 ± 25	109 ± 26	56 ± 13	56 ± 13	75 ± 16	75 ± 18
T4	62 ± 11	61 ± 13	112 ± 14	112 ± 14	57 ± 10	55 ± 12	79 ± 10	78 ± 11
T5	75 ± 19	80 ± 23	131 ± 23	128 ± 20	64 ± 11	62 ± 9	88 ± 18	86 ± 12
T6	77 ± 19	79 ± 19	133 ± 25	130 ± 23	68 ± 17	63 ± 13	91 ± 19	88 ± 18
T7	83 ± 14	85 ± 17	142 ± 20	146 ± 23	69 ± 7	70 ± 9	95 ± 12	97 ± 14

Parameters were recorded at 7 time points: before aortic cross-clamping (T1), at the end of aortic cross-clamping time (just before aortic cross-clamp release, T2), and 15 minutes (T3), 1 hour (T4), 3 hours (T5), 6 hours (T6), and 24 hours (T7) after aortic clamp release. Ischemia was defined as the period between aortic cross-clamp and aortic cross-clamp release; reperfusion was defined as the period after aortic cross-clamp release.

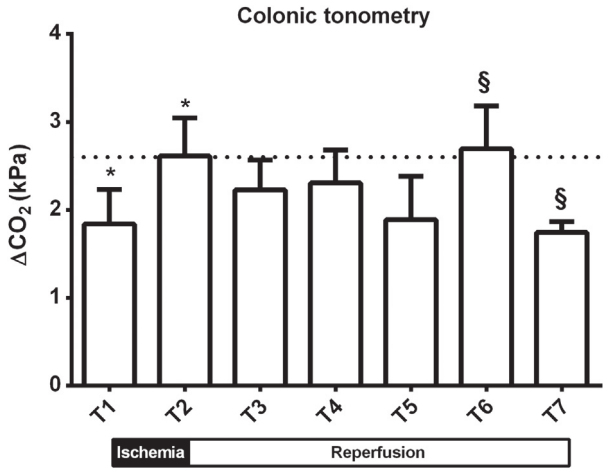

Fig 1. Colonic tonometry values during elective IrAA surgery. Values are means ± SEM. $\Delta CO_2 = PrCO_2 - PaCO_2$ (where $PrCO_2$ = regional pressure in CO_2, measured by gas tonometry in the colon). The dashed line indicates the ΔCO_2 threshold value defining gut ischemia (2.6 kPa [19.5 mmHg]).

Several studies have suggested a significant association between plasma D-lactate levels and ischemic colitis after aortic surgery.[8-10] However, none of the patients in this study had an elevated D-lactate level. The level was undetectable both intraoperatively and postoperatively and, thus, did not follow the increase in colonic tonometry or systemic TNF-α values. According to Poeze et al, a D-lactate level of >0.20 mmol/L is a predictive factor for postoperative colonic ischemia after ruptured aortic aneurysm surgical repair.[9] A significant correlation also has been observed between the rate of postoperative colonic ischemia and severity of shock during aortic surgery.[1] Murray et al found a D-lactate level greater than 0.20 mmol/L in patients with mesenteric ischemia diagnosed after an emergency laparotomy.[12]

Other evidence, however, does not support an association between D-lactate levels and ischemia-induced intestinal injury. The authors have shown, in a rat model of gut IR, with significant IR injuries on gut histology, that D-lactate was not elevated after reperfusion.[14] In a preliminary clinical study, it

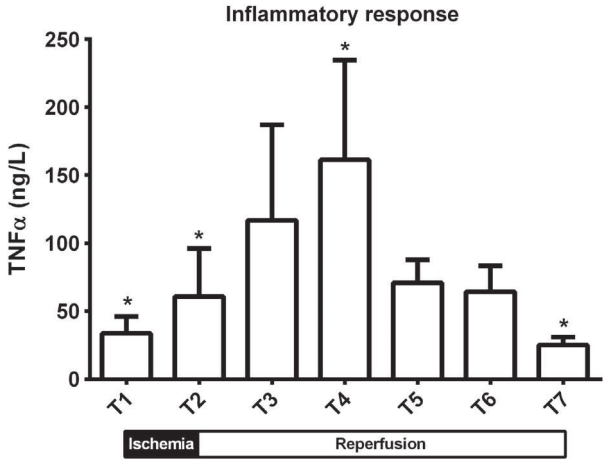

Fig 2. Inflammatory response during elective IrAA surgery. Values are mean ± SEM; $^{*}p < 0.05$.

was found that only hypovolemic or septic shock patients had a significantly higher D-lactate level than other AAA patients (0.21 *v* 0.06 mmol/L, p = 0.001).[13] Increased D-lactate level has been recorded during hemorrhagic shock by Szalay et al, and a decrease in D-lactate level has been found to predict outcome in septic shock patients.[30,31]

This study had some limitations: (1) few patients were included, (2) specific parameters of volemia were not recorded, (3) the use of D-lactate as a biomarker for gut hypoperfusion was investigated, but maybe the observed colonic hypoperfusion was sufficient to give rise to a systemic inflammatory response but not great enough to increase D-lactate. According to Lebuffe et al, intestinal tissue injury, as assessed by biopsies during coloscopy in 8 patients, occurred 48 hours after AAA surgery when the ΔCO_2 was high.[32] Moreover, colonic tonometry may be more sensitive but less specific than D-lactate to detect significant gut hypoperfusion, and (4) there are still no universally accepted normal values for $PrCO_2$ and ΔCO_2. This makes the interpretation of the results and comparison with other trials difficult. These 3 limitations lessen the impact of the negative results of this study, but these should nevertheless prompt reorientation of research on D-lactate. Moreover, even if D-lactate may not be a specific and sensitive marker of gut ischemia during IrAA surgery, it may prove to be an interesting marker of shock or cell injury in other situations.

CONCLUSIONS

Gastrointestinal mucosal microcirculatory perfusion deficits have been associated with a decrease in gut barrier function, possibly enhancing systemic inflammation and distant organ dysfunction. Early detection of gut hypoxia, therefore, is important for optimal management. The results of this pilot study indicated that elective IrAA surgery promoted transient and moderate gut hypoperfusion, as assessed by ΔCO_2 values, which was accompanied by a systemic inflammatory response. However, D-lactate remained undetectable both intraoperatively and postoperatively and, thus, did not vary in line with colonic tonometry or systemic TNF-α measurements. Further larger-scale studies are needed to establish a reliable marker for the early diagnosis of acute intestinal insult in clinical practice.

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Etude N°8

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Pulmonary Apoptosis After Supraceliac Aorta Clamping in a Rat Model[1]

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Background. **Lungs are a major target in several models of systemic inflammation. We investigated the effect of gut ischemia reperfusion on lung injury as apoptotic histological changes and pulmonary dysfunction.**

Materials and methods. **Sixteen Wistar male rats were randomized in two equal groups: a control group and a gut ischemia–reperfusion (IR) group for which gut IR was performed by clamping the supraceliac aorta during 40 min. After 60 min of reperfusion, blood gas, bronchoalveolar liquid (BAL) and pulmonary tissue were sampled for measurements. Acidosis status was used to assess the importance of gut IR. Tumor necrosis factor-α in the BAL reflected the inflammatory pulmonary response. A terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling method was used to quantify the percentage of apoptotic cells in BAL and to visualize apoptotic cells on tissue samples. Pulmonary dysfunction was assessed by PaO_2 measure.**

Results. **Gut IR caused an important metabolic acidosis (pH = 7.19 ± 0.05 *versus* 7.32 ± 0.02, $P = 0.032$ and HCO_{3-} = 10.8 ± 2.54 *versus* 21.1 ± 1.72 mmol/L, $P = 0.027$). At the pulmonary level, there was yet no hypoxemia (paO_2 = 18.1 ± 1.85 *versus* 12.3 ± 1.1 kPa, $P = 0.005$) but a significant inflammatory response (tumor necrosis factor-α in BAL = 7.5 ± 5 *versus* 0 pg/mL). The number of apoptotic cell in BAL more than doubled in the gut IR group (51.3 ± 8 *versus* 23 ± 4.3%, $P = 0.046$). Apoptose involved pneumocytes and bronchiolar epithelial cells.**

Conclusions. **Our rat models of gut IR induced a significant pulmonary injury characterized by a doubling in apoptotic cells but not yet by a functional pulmonary impairment.**

Key Words: **aorta clamping; gut ischemia reperfusion; lung injury; apoptosis.**

INTRODUCTION

The lung appears as a major target in multiple organ dysfunction syndrome (MODS) [1]. It has been shown that the lung can be damaged by indirect injury such as gut [2–5], liver [6], and skeletal muscle reperfusion [7] as well as aortic clamping/unclamping during vascular surgery [8–12].

The pathophysiology of lung injury associated with gut ischemia–reperfusion (IR) probably involves a variety of inflammatory and vasoactive mediators. Polymorphonuclear leukocytes are thought to play a major role as mediators in distant organ injuries. Levels of cytokines, eicosanoids, leukotriene B4 and thromboxane B2, and platelet-activating factor are elevated during reperfusion. These agents are powerful chemoattractants and chemoactivators for polynuclear cells (PMNs). Thus, after gut ischemia, reperfusion is characterized by a local accumulation of PMN, an increase in microvascular permeability, and evidence of mucosal barrier dysfunction. In addition to this local injury, circulating mediators have been related to remote organ injury in the lungs and liver. Evidence is increasing that apoptosis plays an important role in the pathogenesis of IR injury in a variety of organs, such as brain [13, 14], heart [15], kidney [16], liver [17], gut [18, 19], and lung [20]. More importantly, inhibiting apoptosis during IR injury is associated with improved survival and organ function [21, 22]. Therefore, delineating the

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precise mechanisms involved during IR injury may help optimize future therapies designed to abrogate IR injury-induced apoptosis. The aim of this study is to investigate the effect of gut IR on lung injury as apoptotic histological changes and pulmonary dysfunction in rat model of supraceliac aortic clamping.

MATERIALS AND METHODS

Experimental Design and Animals

Male rats from Wistar strains weighting between 350 and 400 g were used (Janvier Laboratories, Orleans, France) to perform a prospective randomized and controlled study. Sixteen rats were randomized in two equal groups: 1) control group (i.e., the rats were subjected to anesthesia and sham surgery) and 2) gut IR group (i.e., the rats were subjected to 40 min of gut ischemia and 1 h of reperfusion by clamping and unclamping the supraceliac aorta).

Procedure

The rats were allowed to unrestricted access food and water before and operation. Anesthesia was performed with intraperitoneal injection of 0.2 mL/100 g of ketamine (Imalgène 1000, Merial, France). All animals were left under spontaneous ventilation. The supraceliac aorta was approached through a mid-line abdominal incision. The abdominal viscera were pushed back to the right. The aorta was approached just below the diaphragm up to the celiac trunk. The aorta was clamped for 40 min with an atraumatic vascular miniclamp. The abdomen was partially closed, except in the xiphoid area by which the aortic clamp was removed after 40 min of clamping. Control animals were subjected to the same protocol, except that the supraceliac aorta was not occluded. After 1 h of reperfusion, the animals were sacrificed, and blood samples were taken from the abdominal aorta. A bronchoalveolar lavage (BAL) fluid was obtained by the infusion of 2 mL of sterile saline solution using a disposable 14-G catheter (Jelco®, Johnson-Johnson Medical, Tampa, FL) inserted into the right bronchus. The instilled fluid was then harvested by aspiration into the syringe. The total BAL fluid harvested from each animal was stored in sterile tubes. After obtaining BAL, pulmonary tissue was extracted from the left lung and plunged in formol 10% for histological analysis.

Assessment of Arterial Blood Gas Value

The blood sample was drawn in a Pulsator® (Portex, Keene, NH) type syringe and was immediately analyzed on an ABL 700® type automate (Radiometer, Copenhagen, Denmark). The PaO_2, $PaCO_2$, HCO_{3-}, and pH values were corrected with the rats' rectal temperature at the end of the test procedure. Throughout the experiment, the rats breathed spontaneously and were given no oxygen support.

Assessment of Tumor Necrosis Factor-α (TNF-α) Production in the BAL

Total BAL fluid was kept at a temperature of 4°C until the TNF-α levels were measured. BAL samples from each animal were adjusted to 10^6 cell/mL. TNF-α concentration was measured with an immunoassay Kit (Biosource International Cytoscreen Rat TNF-α Ultrasensitive Elisa). The BAL fluid was filtered through a 0.22-mm filter before use. The minimal detectable dose of TNF-α is <0.7 pg.mL^{-1}.

Determination of the Number of BAL Cells

To determine the number of BAL cells, 0.1 mL of 0.5% crystal violet solution in 30% acetic acid was added to each BAL. The cell count was performed in a specific computer.

Assessment of Pulmonary Apoptosis

Detection of DNA Fragmentation on the Cells From the BAL by the Terminal Deoxynucleotidyl Transferase-Mediated dUTP-Biotin Nick-End Labeling (TUNEL) Assay

The assay is based on *in situ* incorporation of a labeled terminal nucleotide deoxytransferase in the DNA breaks in the apoptotic cells. The BAL were centrifuged for 15 min at 800 rpm (0.134 *g*) and treated in a 1% formol solution for 5 min. The cells were then spread on SuperFrost/Plus slides (Menzel-Gläser, Germany) and kept at a temperature of –20°C until they were used. The cells were then thawed and rinsed in PBS 1, then treated with proteinase K (20 μg/mL; Sigma, Lezennes, France) for 15 min at 37°C. The slides were incubated for 1 h at 37°C with a mixture containing the terminal deoxynucleotidyl transferase enzyme (Enzyme Solution, Roche Molecular Biology, France) and FITC labeled d-UTP (Label Solution, Roche Molecular Biology). The slides were then counterstained with diluted propidium iodide (0.5*g/mL) in an antifade solution (Adgenix, France). Five hundred cells were analyzed per sample. Only the cells with DNA breakages (in green) not labeled with propidium iodide (in red) can be considered as being apoptotic cells, the others are either alive (those without the propidium iodide and fluoresceine label) or necrotic (labeled both with propidium iodide and fluoresceine). Positive controls (pretreated with Dnase, 1UI) and negative controls (transferase depleted solution) also were prepared. Two different observers analyzed the slides using an epifluorescence microscope (DMRB, Leica, Germany).

Detection of DNA Fragmentation by the TUNEL Assay on Slices of Tissue Embedded in Paraffin

The analysis was performed on two individuals in each group. The fragments of pulmonary tissue were plunged into a buffered formol solution immediately after harvesting, and then set in paraffin. Three to five micrometer slices were then placed on SuperFrost/Plus® slides Menzel-Gläser, Germany) and checked for adhesion. The paraffin was removed from the slices of pulmonary tissue and they were rehydrated in a series of baths of xylene, ethanol and distilled water. After rinsing in PBS 1X, the slices of tissue were treated with proteinase K (20 μg/mL; Sigma) for 15 min at 37°C. The slides were incubated for 1 h at 37°C with a mixture containing the deoxynucleotidyl transferase terminal enzyme (Working Strength TdT enzyme Solution®, Intergen, Burlington, MA) and digoxigenin-labeled d-UTP. The d-UTP was then detected by incubating the slides with an anti-digoxigenin antibody, combined with peroxydase (Antidigoxigenin peroxidase conjugate®, Intergen) for 30 min at 37°C. Finally, the slides were submitted for 10 min to a peroxydase substrate (DAD dilution buffer®, Intergen). After rinsing in PBS 1X, the slices were counterstained with Mayer's Hemalum (Merck, France). The cells with brown colored nuclei were considered to be apoptotic cells. The method was validated by using two controls: a positive control was set up, comprising pulmonary tissue slices previously treated with DNase so that all of the nuclei would present a positive response to the TUNEL assay: a brown stain in all of the nuclei. A negative control comprising pulmonary tissue slices that had not been incubated with the terminal deoxynucleotidyl transferase enzyme so that no nucleus would be stained after revelation: no signal in the nuclei.

Statistical Analysis

The results were expressed in mean values (± SEM) and analyzed with the Mann–Whitney *U* test. a *P* value <0.05 was considered as significant.

TABLE 1

Lung Effects of Gut Ischemia–Reperfusion by Clamping–Unclamping the Supra Celiac Aorta

	Control group	Gut ischemia–reperfusion group	*P* value
Gas exchange*			
pH	7.32 ± 0.02	7.19 ± 0.05	0.032
$PaCO_2$ (kPa)	4.8 ± 0.4	3.1 ± 0.4	0.005
PaO_2 (kPa)	12.3 ± 1.1	18.1 ± 1.85	0.005
HCO_3^- (mmol/L)	21.1 ± 1.72	10.8 ± 2.54	0.027
TNFα in BAL (pg/mL)	0	7 ± 5	
% of BAL apoptotic cells†	23 ± 4.3	51.3 ± 8	0.046

Results in mean ± SEM.
* Measured during spontaneous ventilation in the ambient air (F_iO_2 = 21%), animal temperatures were identical in both group.
† Mean frequency for cells presenting DNA fragmentation.

RESULTS

Effect of Gut IR on the Gas Exchange

Pulmonary dysfunction was assessed by analyzing the blood gases. All of the samples were taken during spontaneous ventilation in the ambient air (FiO_2 = 21%). Compared with the control group, gut IR induced metabolic acidosis, hypocapnia, and no hypoxemia but heperoxemia (Table 1). The pH level in gut IR group (7.19 ± 0.05) was significantly lower than in control rats (7.32 ± 0.02, $P = 0.032$). HCO_{3-} systemic production in gut IR group (10.8 ± 2.54 mmol/L) was significantly lower than in control group (21.1 ± 1.72 mmol/L, $P = 0.027$). PaO_2 concentration in gut IR group (18.1 ± 1.85 kPa) was statistically higher than in control group (12.3 ± 1.11 kPa, $P = 0.005$).

Effect Gut IR on TNF-α Production in BAL

The TNF-α level measured by BAL of clamping rats was 7 ± 5 pg/mL per 10^6 cells. TNF-α was undetectable in the control group.

Effect of Gut IR on Pulmonary Apoptosis

The mean number of cell analyzed by BAL was 467.8 ± 58 in the control group and 464.4 ± 54 in the gut IR group. No difference was observed between the two groups with respect to the number of cells analyzed ($P = 0.49$). The mean frequency for cells presenting DNA fragmentation was significantly higher in the gut IR groups : % DNA $frag_{BAL}$: 51.3% ± 8% *versus* 23 ± 4.3% ($P = 0.046$) (Fig. 1). In gut IR group, the slices of pulmonary tissue showed marked apoptosis of pneumocytes and bronchiolar epithelial cells (Fig. 2 and 3). In the control group, the number of apoptotic cells was visibly smaller (Fig. 2). There was no other visible histological injury in the gut IR group; in particular, there was no edema.

DISCUSSION

Our model of gut IR induced lung injury is assessed by histological evidence of pulmonary apoptosis, lung neutrophilic infiltrates, and local expression of the proinflammatory cytokine. The lung is a major target organ in multiple organ dysfunction syndrome (MODS) caused by severe injury. Acute lung injury is the most common reason for morbidity after aortic surgery. Relevant models of pulmonary injury have resulted from hind limb ischemia, burns, caerulein-pancreatitis, or intestinal IR [23, 24]. Experimental lung injury after gut IR occurs by both cellular and humoral factors of the systemic inflammatory response. The mechanism of respiratory failure after gut IR remains little understood. During inadequate mucosal blood flow, gut barrier function can be impaired and invested by bacteria or endogenous endotoxin, i.e., bacterial translocation [25]. This process is associated with activation of sys-

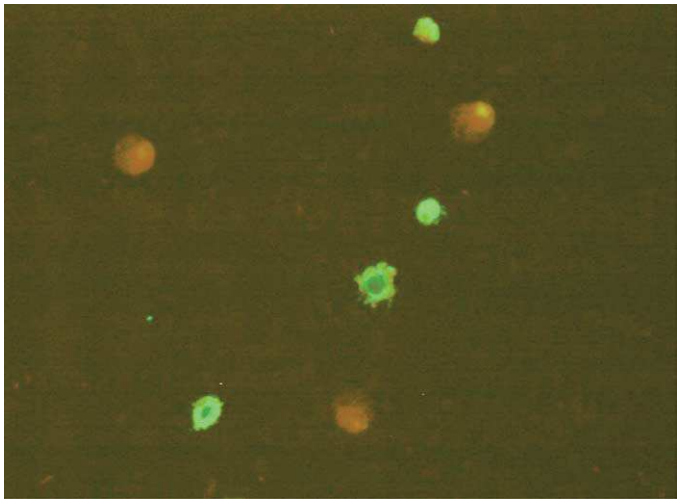

FIG. 1. Tunel assay on BAL. Apoptotic cells are in green; non-apoptotic cells are in red. The number of apoptotic cells was significantly higher in the gut IR group: 51.3 ± 8% *versus* 23 ± 4.3%, $P = 0.046$. (Color version of figure is available online.)

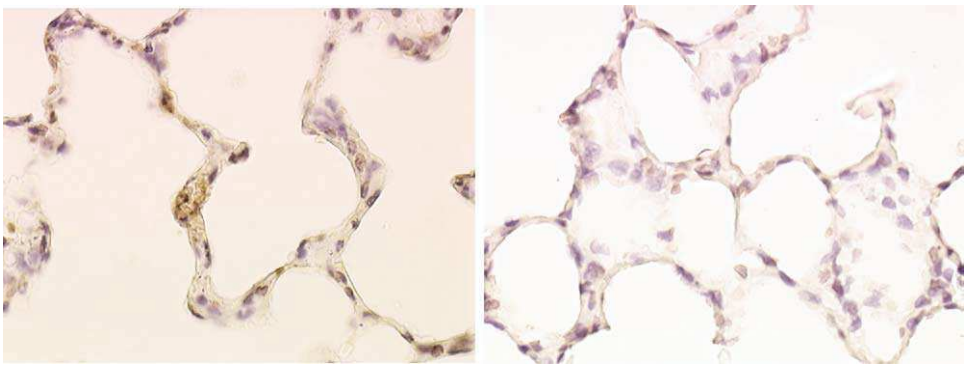

FIG. 2. Tunel assay on pulmonary parenchyma. Apoptotic pneumocyte are in brown. The number of apoptotic pneumocyte was higher in the gut IR group (left picture) compared with the control group (right picture). (Color version of figure is available online.)

temic inflammatory mediators, including toxin, inflammatory mediators (such as TNF-α), and interleukins. Moreover, systemic inflammatory response could be enhanced by endothelial activation, in particular during gut IR. Lung damage appears as increased inflammatory cell infiltration, alveolar endothelial cell damage and enhanced permeability of microcirculation [26]. Activated neutrophils are known to accumulate in the lung after experimental gut IR. Both injuries are PMN-dependent and can be attenuated by prior depletion of circulating PMNs [27].

In a model of mesenteric artery occlusion, gut IR increased gut myeloperoxydase levels, primed circulating neutrophils, increased lung MPO levels, and provoked distant lung injury. In our study, we observed a significant increase in TNF-α rate by BAL in the gut IR group as compared with the control group. Jernigan *et al.* have reported that BAL macrophages play a major role in the mechanism of acute lung injury after hemorrhagic shock [28]. Among cytokines production by macrophages, TNF-α appears to be a major mediator of distant organ injury and lung [29]. Injection of exogenous TNF-α causes lung injury characterized by PMN sequestration in pulmonary microvasculature, increased endothelial permeability, and interstitial edema [30]. The exact mechanism of cytokine (TNF-α)-induced acute lung injury appear to be multifactorial: neutrophil chemotaxis, degranulation, adherence, release of oxygen metabolites, and apoptosis phenomena. Apoptosis can be triggered by different pathways responsible for caspase activation. These include activation of members of the TNF superfamily (TNF receptor, Fas, CD40) located on the plasma membrane, some of which contain a death domain. In this experiment, we demonstrated that the intestinal ischemia followed by 1 h of reperfusion up-regulated significantly lung apoptosis, according to elevation of DNA fragmentations in BAL and pneumocytes. Similarly, extensive alveolar epithelial cell apoptosis has been found in experimental models of pulmonary fibrosis and endotoxin-induced lung injury [31, 32]. Moreover, clinical studies have described histological signs of pulmonary apoptosis occurring when fatal acute respiratory distress syndrome [33].

As a consequence, we examined the gas exchange through lung damage. In the gut IR group, the early blood gas analysis showed hyperoxia and a moderate hypocapnia. Hypocapnia resulted from hyperventilation compensating metabolic acidosis. However, hyperventilation could not be sufficient to explain hyperoxia. We hypothesize that hyperoxia resulted of a change in the respiratory quotient. After ischemia-reperfusion, the gut releases more CO_2 when oxygen uptake is probably impaired. The final result should be an increase in respiratory quotient explaining the significant hyperoxemia in the gut IR group (according to the formula: $PaO_2 = (P_B - P_{H2O})$. FiO2 – $PaCO_2$/RQ, where P_B is the barometric pressure, P_{H2O} the water pressure and RQ the respiratory quotient). In agreement with our results, Fischer *et al.* showed in a rat model of pulmonary transplantation that PaO_2 was conversely

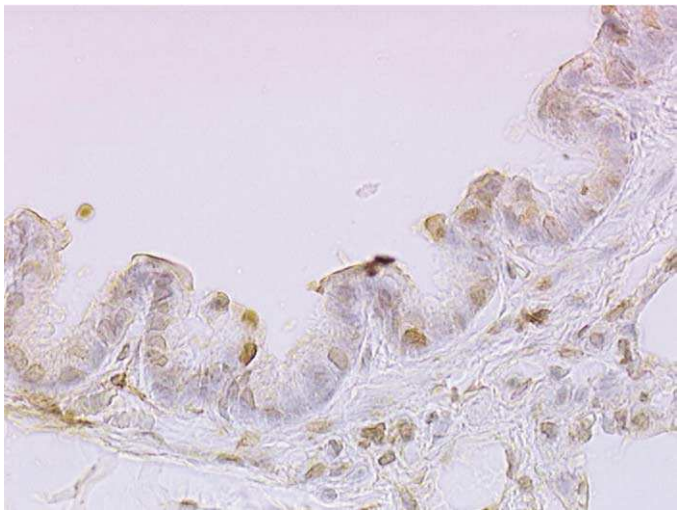

FIG. 3. Tunel assay on bronchiolar parenchyma. Apoptotic epithelial bronchiolar cells are in brown. Tissue sample from the gut ischemia reperfusion group. (Color version of figure is available online.)

correlated with the number of necrotic cells but not with the number of apoptotic cells [34]. Moreover, the statistically insignificant ($P = 0.08$) relation between PaO_2 and apoptose showed that PaO_2 increased with the number of apoptotic cells. During clinical experience, impair gas exchange through microvascular changes in the lung and increased permeability have been observed after clamping of the aorta [11, 12]. Our model showed that gut IR is responsible of a major increase in pulmonary apoptotic cell death when there is no hypoxemia yet. Surprisingly, there was no edema or other histological impairment than apoptose on our tissue sample. This should mean that anti apoptotic treatment should prevent lung dysfunction after a gut IR injury.

CONCLUSIONS

Our rat model of supra celiac aortic clamping showed that gut IR induces pulmonary inflammation, a doubling in pulmonary apoptotic cells but not yet a pulmonary dysfunction. These results partially explain the mechanism of lung injury after clamping aortic and suggest that inhibiting apoptosis may be a novel pharmacologic approach to prevent lung dysfunction after gut ischemia reperfusion.

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Etude N°9

Collange O, Charles AL, Noll E, Bouitbir J, Zoll J, Piquard F, Diemunsch P, Geny B

Isoflurane anesthesia preserves liver and lung mitochondrial oxidative capacity after gut ischemia-reperfusion.

Anesth Analg, 2011. 113(6): p. 1438-41.

Society of Critical Care Anesthesiologists

Section Editor: **Michael J. Murray**

BRIEF REPORT

Isoflurane Anesthesia Preserves Liver and Lung Mitochondrial Oxidative Capacity After Gut Ischemia–Reperfusion

Olivier Collange, MD,*† Anne-Laure Charles, PhD,†‡ Eric Noll, MD,*† Jamal Bouitbir, PhD,†‡ Joffrey Zoll, PhD,†‡ François Piquard, MD, PhD,† Pierre Diemunsch, MD, PhD,*† and Bernard Geny, MD, PhD†‡

BACKGROUND: Lung and liver dysfunction is involved in gut ischemia–reperfusion (IR)–induced multiple organ failure. We compared the effects of ketamine and isoflurane on liver and lung mitochondrial oxidative capacity after gut IR.
METHODS: Adult male Wistar rats were randomized into 4 groups (controls and gut IR receiving either intraperitoneal ketamine or inhaled isoflurane). Maximal oxygen consumption and the activity of respiratory chain complexes were measured on isolated liver and lung mitochondria.
RESULTS: Gut IR significantly impaired liver and lung mitochondrial oxidative capacity when using ketamine but not isoflurane.
CONCLUSIONS: Isoflurane preserved liver and lung mitochondrial oxidative capacity after gut IR. (Anesth Analg 2011;113:1438–41)

Gut ischemia–reperfusion (IR) is a common occurrence after an ischemic event (acute mesenteric ischemia, aortic occlusion, or gut hypoperfusion associated with shock) followed by restoration of bloodflow. All types of shock cause disproportionate splanchnic hypoperfusion, and with resuscitation, gut reperfusion leads to systemic inflammatory response and remote organ injury.[1] In the clinical setting of shock, persistent gut IR episodes are predictive of multiple organ failure and death.[2,3]

Intestinal venous circulation is coupled in series with the hepatic and pulmonary vascular systems. Both the liver and lung are closely involved in the deleterious effects induced by gut IR, partly because they trap circulating activated leukocytes.[4] However, brief exposure to volatile anesthetics, such as isoflurane, can protect against IR-induced injury in many organs, including the liver and lungs.[5,6]

Mitochondria have a key role in cell function and survival and mitochondrial dysfunction would appear to be involved in sepsis-induced multiple organ failure.[7] Previous studies on gut IR have approached mitochondrial function indirectly by measuring serum acetoacetate/3-hydroxybutirate levels as an index of hepatic mitochondrial redox state and pyridine nucleotide (NADH) autofluorescence as an indicator of mitochondrial oxygen consumption and redox status.[4,8] Horie et al. have shown that gut IR significantly increases liver NADH by 52%, a sign of liver mitochondria impairment.[4]

However, the impact of gut IR on both liver and lung mitochondria remains incompletely understood. The objective of our study was to investigate whether gut IR impairs liver and lung mitochondrial function and whether inhaled isoflurane, when compared with intraperitoneal ketamine, can reduce any potential deleterious effects of gut IR on mitochondrial oxidative capacity.

METHODS

Animals and Experimental Design

Male Wistar rats weighing 350 ± 30 g were randomized into 4 groups of 8 animals (2 control groups [C] and 2 gut-IR groups [IR]) receiving either ketamine or isoflurane.

The animals were placed on heating pads to maintain body temperature at 37°C and kept under spontaneous ventilation during the experiment. Anesthesia was induced and maintained by either intraperitoneal ketamine (induction dose: 20 mg/kg) or inhaled isoflurane (1%–2%) from the beginning to the end of the experiment. Gut IR was induced by 60 minutes of superior mesenteric artery clamping followed by 60 minutes of reperfusion, as previously described.[9]

Procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals, and the study was approved by the institutional animal care committee of the University of Strasbourg (CREMEAS authorization no. AL/03/11/06/09).

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The authors declare no conflict of interest.

Reprints will not be available from the authors.

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Table 1. Blood Gases After Gut IR Under Ketamine and Isoflurane Anesthesia

	PaO_2 (mm Hg)		$PaCO_2$ (mm Hg)		pH	
	Controls	Gut IR	Controls	Gut IR	Controls	Gut IR
Ketamine	450 + 25	383 ± 18	45 ± 2	22 ± 2*	7.40 ± 0.05	7.30 ± 0.06*
Isoflurane	423 ± 34	390 ± 26	38 ± 2	29 ± 2*	7.44 ± 0.03	7.36 ± 0.02*

Values are means ± SD. IR = ischemia/reperfusion.
* Significantly different ($P < 0.05$) from controls.

Measurements
All variables were determined at the end of reperfusion (IR) or equivalent time (C).

Arterial Blood Gases
Arterial blood was sampled (Pulsator® syringe, Portex, Keene, NH) after 1 hour of ischemia and 1 hour of reperfusion and immediately analyzed for arterial partial oxygen (Pao_2) and partial carbon dioxide ($Paco_2$) pressure using an automated blood gas analyzer (Radiometer ABL 725®, Copenhagen, Denmark). Blood gases and pH values were corrected to the rats' rectal temperature measured at the end of the experiment. Throughout the experiment, the rats breathed spontaneously with 1 L/min of oxygen support.

Arterial Blood Lactate
Arterial blood lactate levels were used as a quantitative marker of gut IR and measured using a micromethod device (Lactate Pro, LT1710, Arkray, KGK, Japan).[10]

Mitochondrial Oxidative Capacity
The oxidative capacity of isolated liver and lung mitochondria, which were obtained by differential centrifugation in ice-cold buffer, was assessed by measuring oxygen consumption with a Clark-type electrode (Strathkelvin Instruments, Glasgow, Scotland). Maximal mitochondrial respiration (State 3) rates were measured in the presence of a saturating amount of adenosine diphosphate as a phosphate acceptor.

The relative contributions of respiratory chain complexes I to IV to the global mitochondrial respiratory rates were determined. When State 3 was recorded, electron flow went through complexes I, III, and IV. Complex I was blocked with amytal (0.02 mM). Using succinate 25 mM, we obtained the activities of complexes II, III, and IV (State 3_{+Suc}). The activity of complex IV (cytochrome c oxydase) was then determined by the further addition of N,N,N′,N′-tetramethyl-p-phenylenediamine dihydrochloride (TMPD, 0.5 mM) and ascorbate (0.5 mM) as an artificial electron donor to cytochrome c oxydase (State 3_{+TMPD}).[11]

Statistical Analysis
Results were expressed as means ± SD. Two-way analysis of variance was used to compare groups; significance levels were set at $P < 0.05$. We used GraphPad Prism version 5.03 for Windows (GraphPad Software, San Diego, CA).

RESULTS
Arterial Blood Gases
During both ketamine and isoflurane anesthesia, gut IR tended to decrease Pao_2 values ($P = 0.07$) and significantly reduced pH and $Paco_2$ values ($P < 0.05$) (hyperventilation compensating metabolic acidosis) (Table 1).

Blood Lactate
Gut IR led to a similar increase in blood lactate levels whether ketamine (from 1.2 ± 0.5 to 2.9 ± 0.8 mmol/L; +142%; $P < 0.001$) or isoflurane (from 1.2 ± 0.2 to 2.9 ± 0.5 mmol/L; +142%; $P < 0.001$) was used to induce and maintain anesthesia.

Liver and Lung Mitochondrial Oxidative Capacity
There was no significant difference between ketamine and isoflurane control groups.

During ketamine anesthesia, gut IR significantly reduced maximal oxidative capacity (State 3) by 50% in isolated liver mitochondria (from 51 ± 11 to 24 ± 45 $\mu molO_2/min/g$; $P < 0.05$) (Fig. 1A). It also significantly reduced respiratory chain complex activity, by 27% for $State3_{+Suc}$ activity (from 51 ± 8 to 32 ± 13 $\mu molO_2/min/g$; $P < 0.05$) and by 30% for State 3_{+TMPD} activity (from 69 ± 10 to 48 ± 15 $\mu molO_2/min/g$; $P < 0.05$).

In isolated lung mitochondria, maximal oxidative capacity was also reduced (by 30%), but not significantly (from 23 ± 4 to 16 ± 9 $\mu molO_2/min/g$; $P > 0.05$) (Fig. 1B). The decrease in respiratory chain complex activity was, however, significant: activity decreased by 41% for $State3_{+Suc}$ activity (from 27 ± 5 to 16 ± 8 $\mu molO_2/min/g$; $P < 0.001$) and by 17% for State 3_{+TMPD} activity (from 47 ± 8 to 39 ± 13 $\mu molO_2/min/g$; $P < 0.05$).

During isoflurane anesthesia, gut IR did not impair either liver or lung mitochondrial respiration rates (Figs. 1A and 1B).

DISCUSSION
Isoflurane anesthesia preserved liver and lung mitochondrial respiration in our gut IR model using isolated mitochondria. In this model, IR caused the same gut injury, as assessed by lactate levels, regardless of whether intraperitoneal ketamine or inhaled isoflurane was used to induce and maintain anesthesia. However, when ketamine was used, there was significant, early impairment of liver and lung oxidative capacity, whereas this did not occur with isoflurane. Complex I activity in the lung tended to be less impaired than in the liver, but this tendency was not statistically significant. Further studies should determine the protective mechanism specific to each of these organs.

Our experiment simulated clinical practice when hypovolemia triggers mesenteric hypoperfusion during surgery. Our observations are consistent with an indirect protective effect of isoflurane on electron transport in lung and liver

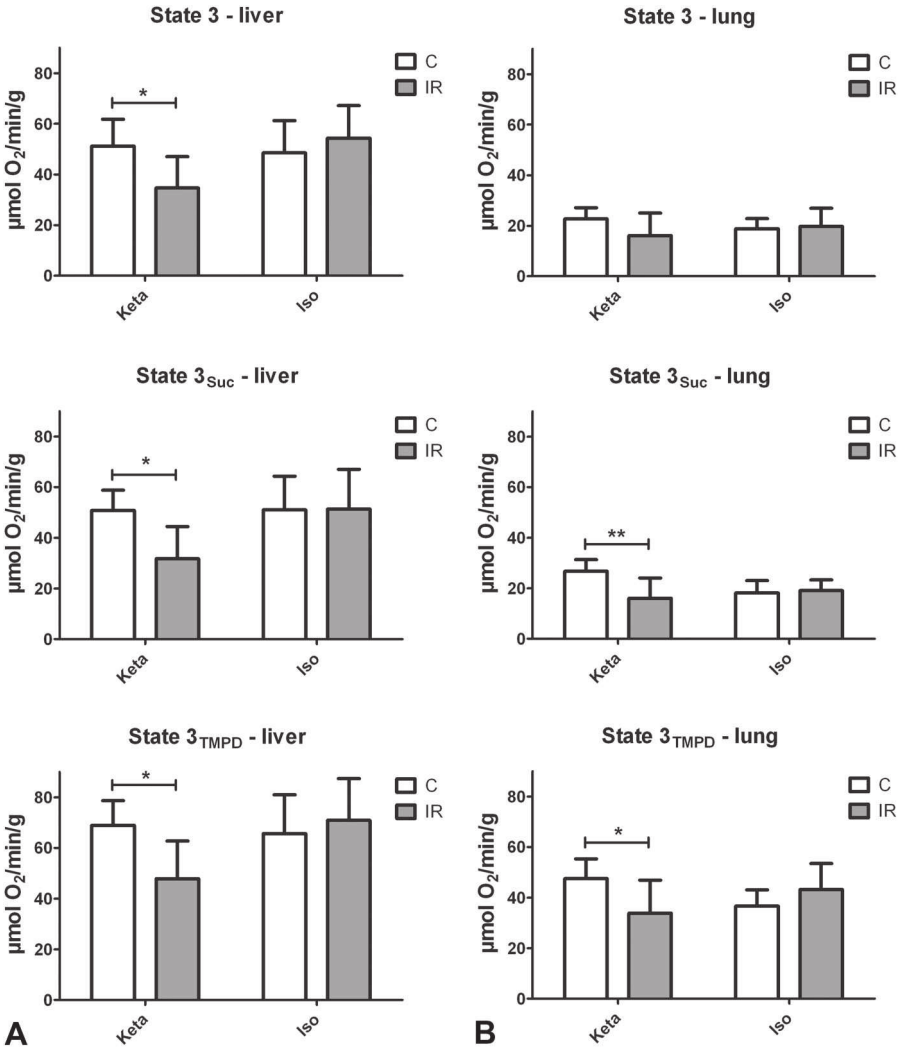

Figure 1. Oxidative capacity of liver (A) and lung (B) mitochondria. Respiration rates of isolated liver (A) and lung (B) mitochondria from control (C) and gut-ischemia–reperfusion (IR) rats were measured in the presence of adenosine diphosphate (State 3), succinate (State 3_{+Suc}), and TMPD (State 3_{+TMPD}). Anesthesia was induced with either intraperitoneal ketamine (Keta) or inhaled isoflurane (Iso). Mean ± SD. $*P < 0.05$. $**P < 0.001$.

mitochondria that can be attributed to either pre- or postconditioning mechanisms.[5,12,13] Isoflurane has been shown to protect against IR injury in multiple organs including the liver and lungs, probably by inhibiting cell death and improving mitochondrial function.[5,6,12] This protection has been demonstrated when isoflurane is given before (preconditioning)[5,12] or after (postconditioning)[13] the ischemic period.

Our direct measurements of mitochondrial respiratory capacity support earlier, indirect findings on mitochondrial function.[4,8] They provide further evidence of mitochondrial involvement in isoflurane-mediated protection of the liver and lung. Future research should further study the clinical significance of isoflurane administration in patients with gut IR and establish whether isoflurane protection can enhance the prognosis of these patients.

DISCLOSURES

Name: Olivier Collange, MD.
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Olivier Collange has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Name: Anne-Laure Charles, PhD.
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Attestation: Anne-Laure Charles has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Attestation: Bernard Geny has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Methylene Blue Protects Liver Oxidative Capacity after Gut Ischaemia—Reperfusion in the Rat

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WHAT THIS PAPER ADDS?

This experimental study is the first to our knowledge to show that early impairment of liver mitochondrial respiration after gut ischaemia/reperfusion (IR) is related to blood lactate levels and that methylene blue (MB) injection restores liver mitochondria oxidative capacity after gut IR. This observation could have clinical implications in situations such as gut IR during vascular surgery. If injected before vascular unclamping, MB might protect liver mitochondria function and minimise the incidence of postoperative multiple organ dysfunction. MB is already used in many indications (e.g., methaemoglobinaemia) and has been proposed as treatment in vasoplegic shock during sepsis, cardiopulmonary bypass surgery and liver transplantation.

Objectives: Mesenteric ischaemia/reperfusion (IR) may lead to liver mitochondrial dysfunction and multiple organ failure. We determined whether gut IR induces early impairment of liver mitochondrial oxidative activity and whether methylene blue (MB) might afford protection.

Design: Controlled animal study.

Materials and methods: Rats were randomised into three groups: controls ($n = 18$), gut IR group (mesenteric ischaemia (60 min)/reperfusion (60 min)) ($n = 18$) and gut IR + MB group (15 mg kg^{-1} MB intra-peritoneally) ($n = 16$). Study parameters were: serum liver function markers, blood lactate, standard histology and DNA fragmentation (apoptosis) on intestinal and liver tissue, maximal oxidative capacity of liver mitochondria (state 3) and activity of complexes II, III and IV of the respiratory chain measured using a Clark oxygen electrode.

Results: Gut IR increased lactate deshydrogenase (+982%), aspartate and alanine aminotransferases (+43% and +74%, respectively) and lactate levels (+271%). It induced segmental loss of intestinal villi and cryptic apoptosis. It reduced liver state 3 respiration by 30% from 50.1 ± 3 to 35.2 ± 3.5 μM O_2 min^{-1} g^{-1} ($P < 0.01$) and the activity of complexes II, III and IV of the mitochondrial respiratory chain. Early impairment of liver mitochondrial respiration was related to blood lactate levels ($r^2 = 0.45$). MB restored liver mitochondrial function.

Conclusions: MB protected against gut IR-induced liver mitochondria dysfunction.

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Mortality and morbidity rates from acute mesenteric ischaemia following impairment of mesenteric circulation are significant and have changed little over the past decade.[1–3] Gut reperfusion leads to the production of numerous mediators such as reactive oxygen metabolites, pro-inflammatory cytokines and nitric monoxide and also activates multiple enzymes.[4] Interactions between polymorphonuclear neutrophils and endothelial cells would be instrumental in inducing secondary, inflammatory injury to distant organs. This would be the leading cause of death in critically ill patients.[5]

The gut—liver axis is thought to play a key role in the deleterious effects induced by gut ischaemia—reperfusion

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(IR).[6] Intestinal circulation is coupled in series with the liver vasculature, which traps circulating leucocytes activated by gut IR and, following intestinal ischaemia, Kupffer cells are the primary source of pro-inflammatory cytokine release.[7,8] Although gut IR is not characterised by early histological liver lesions, a subcellular approach might nevertheless throw light on a role of the liver in the deleterious effects induced by gut IR.[9] The study of liver mitochondria, in particular, might be useful as mitochondria are the main energy source in cells and convert nutrients into energy through cellular respiration.[10] Impaired mitochondrial function is strongly involved in IR physiopathology.[11]

Methylene blue (MB) is a candidate substance for reducing the effects of gut IR on remote organs such as the liver. It is an inexpensive drug used to treat diseases such as methaemoglobinaemia and Alzheimer's and has been proposed for use in vasoplegic shock during sepsis, cardiopulmonary bypass surgery and liver transplantation.[12] The only histological study of MB action on the intestine after gut IR has shown no alleviation of IR-related intestinal lesions. However, MB is known to enhance key biochemical pathways in mitochondria, and cycling between oxidised and reduced MB forms might block oxidant production.[13–15] The aim of this study was to determine, first, whether gut IR induces early impairment of liver mitochondrial function and, second, whether MB might reduce such impairment.

MATERIALS AND METHODS

Experimental design

The study was performed in adult male Wistar rats (330–380 g) (Depré, Saint Doulchard, France). The rats were housed at a temperature of 22 ± 2 °C, kept on a 12:12-h photoperiod, and provided with food and water *ad libitum*. Procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and the study was approved by the institutional animal care committee of the University of Strasbourg (CREMEAS authorisation N° AL/03/11/06/09).

Fifty-four rats were randomised into three groups: (i) a gut IR group ($n = 18$) which underwent gut ischaemia (60 min) and reperfusion (60 min) by superior mesenteric artery (SMA) clamping/unclamping and received 2 ml saline serum intra-peritoneally (IP) 10 min before reperfusion; (ii) a gut IR + MB group ($n = 16$), treated as the gut IR group but with 15 mg kg^{-1} MB diluted in saline serum before injection; (iii) a control group (C, $n = 18$) which underwent anaesthesia and sham surgery and received 2 ml of saline serum at a time-point corresponding to 10 min before reperfusion.

Experimental procedures

Anaesthesia was induced and maintained with IP ketamine (Imalgène 1000®, Merial, France). The rats were placed in a supine position on heating pads to maintain body temperature at 37 °C (rectal temperature probe) and kept under spontaneous ventilation during the experimental procedure. After a midline abdominal incision, the SMA was isolated at its origin and occluded with an atraumatic clamp for 60 min. This was followed by reperfusion for 60 min. Gut IR was confirmed by complete pulse cessation and restoration in the mesenteric arcades and by a change in intestinal colour. Control animals underwent the same protocol but their SMAs were not occluded.

All tests were performed at the end of reperfusion (treated groups) or an equivalent time (controls).

Circulating liver function markers and blood lactate

Blood obtained by abdominal aorta puncture was centrifuged (3500 *g* for 15 min) to obtain serum. Serum markers of liver insult (lactate dehydrogenase (LDH), aspartate and alanine aminotransferases (AST and ALT)) and markers of cholestasis (bilirubin and alkaline phosphatase (ALP)) were measured on a Synchron IR (Beckman-Coulter, Fullerton, CA, USA) after calibration according to the International Federation of Clinical Chemistry recommendations. Blood lactate levels were measured by a micromethod on a Lactate Pro device (LT1710, Arkray, KGK, Japan).

Standard gut and liver histology and DNA fragmentation (TUNEL) assay

Fragments of the last ileal loop and liver segment VI (eight samples) were placed into a buffered formol solution immediately after harvesting, then processed, embedded in paraffin and sectioned (3–5 μm). A pathologist blinded to the study protocol analysed the sections under a light microscope.

For standard histology, tissue sections were stained with haematoxylin–eosin and intestinal tissue injury was graded according to the Chiu/Park scale:[16] Grade 0, normal mucosa; Grade 1, development of a subepithelial space at the tips of the villi; Grade 2, more extensive subepithelial space than in Grade 1; Grade 3, massive epithelial lifting down the sides of the villi; Grade 4, villi denuded of epithelium; Grade 5, loss of villi; Grade 6, crypt layer injury; Grade 7, transmucosal injury; and Grade 8, transmural infarction.

In the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assay, after paraffin removal, the gut and liver tissue sections from eight samples were rehydrated in a series of xylene, ethanol and distilled water baths, rinsed in phosphate buffered saline (PBS) 1×, treated with proteinase K (20 μg ml^{-1}) (SIGMA, France) for 15 min at 37 °C, and then incubated for 1 h at 37 °C with a mixture containing deoxynucleotidyltransferase terminal enzyme (*In Situ* Cell Death Detection Kit, POD, Roche Diagnostics, Penzberg, Germany). Incorporated fluorescein was detected by anti-fluorescein antibody Fab fragments from sheep, conjugated with horseradish peroxidase (POD) and incubated for 30 min at 37 °C.

Lastly, tissue sections were exposed for 10 min to a peroxidase substrate, rinsed in PBS 1×, and counterstained with Mayer's Hemalum (Merck, France). Cells with brown-coloured nuclei were considered to be apoptotic.

Isolation of mitochondria and mitochondrial respiratory chain complex analysis

Liver mitochondria were isolated on ice from 8 (IR + MB group) or 10 (control and gut IR groups) animals (Fig. 1). Tissue was placed in isolation buffer A containing 70 mM sucrose, 210 mM mannitol, 1 mM ethylene glycol tetra-acetic acid (EGTA) in 50 mM Tris/HCl pH 7.4. The tissue was finely minced with scissors and then homogenised in the same buffer using a Potter Elvejem. The homogenate was centrifuged at 3000 rpm for 3 min at 4 °C and the resultant supernatant at 10,000 rpm for 10 min at 4 °C. The mitochondrial pellet was washed in isolation buffer B containing 70 mM sucrose and 210 mM mannitol in 50 mM Tris/HCl pH 7.4 before being re-suspended in this buffer. Aliquots were then removed for protein measurement by the Bradford method (Biomate 3, Thermo Fisher Scientific, Inc.).

Mitochondrial respiration was measured (oxygen consumption) by methods adapted from a previously described procedure using a Clark oxygen electrode (Strathkelvin Instruments Limited, North Lanarkshire, Scotland) in a 3 ml volume chamber at 25 °C with continuous stirring of the respiratory medium (100 mM KCl, 50 mM Mops, 1 mM EGTA, 5 mM Kpi, 0.1% fatty acid-free bovine serum albumin (BSA) at pH 7.4).[17,18,22] Basal oxygen consumption ('state 4') was measured after addition of glutamate—malate as substrate. Maximal respiration rate ('state 3') was measured after addition of adenosine diphosphate (ADP), which causes a sudden burst of oxygen uptake as the diphosphate (ADP) is converted into the triphosphate (ATP).

The relative contribution of respiratory chain complexes to the global mitochondrial respiratory rate was measured by adding specific blockers and activators. During state 3, electrons flow through each of the complexes I, III and IV. Complex I was blocked with amytal (0.02 mM) and complex II was stimulated with succinate (25 mM). Under these conditions, oxygen consumption reflects the activity of complexes II, III and IV (state 3_{+Succ}). Then, the artificial electron donor N,N,N′,N′-tetramethyl-*p*-phenylenediaminedihydrochloride (TMPD) and ascorbate (0.5 mM), which maintains TMPD in a reduced state, were added. As reduced *TMPD* donates electrons to *cytochrome c*, this enabled determination of the activity of cytochrome c oxidase (complex IV) alone (state 3_{+TMPD}).

Statistical analysis

Results were expressed as means ± standard error of the mean (SEM). Groups were compared by one-way analysis of variance (ANOVA) and the Tukey *post hoc* test. The relationship between lactate and liver mitochondria parameters was tested by Pearson correlation and regression analysis. *p*-values <0.05 were considered significant. Statistical analysis was performed using GraphPad Prism software (version 5.03 for Windows) (GraphPad Software, San Diego, CA, USA).

RESULTS

Liver function tests and blood lactate levels

Gut IR induced liver injury as evidenced by a significant increase in LDH (+982%, 235 ± 41 vs. 2308 ± 530 UI l^{-1}; $P < 0.05$), AST (+43%, 86 ± 10 vs. 199 ± 20 UI l^{-1}; $p < 0.05$) and ALT (+74%, 43 ± 1 vs. 158 ± 21 UI l^{-1}; $P < 0.05$) (Fig. 2). Injury was not associated with cholestasis as there was no significant change in conjugated bilirubin (1.4 ± 0.2 vs. 1.9 ± 0.2 µmol l^{-1}) or in ALP (126 ± 22 vs.

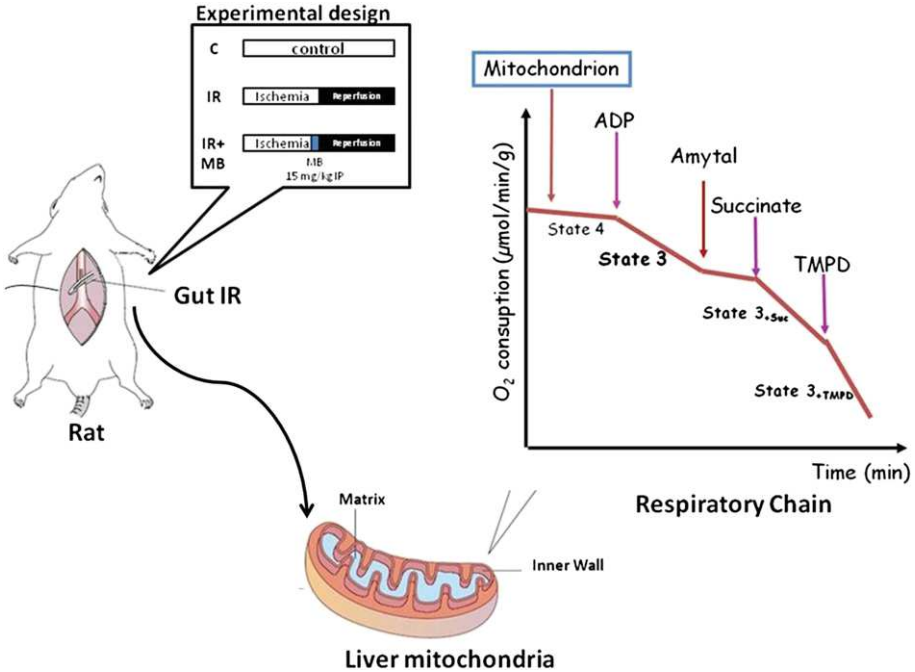

Figure 1. Isolation of liver mitochondria and substrates used to measure respiratory chain activity (modified from Charles et al.[33]).

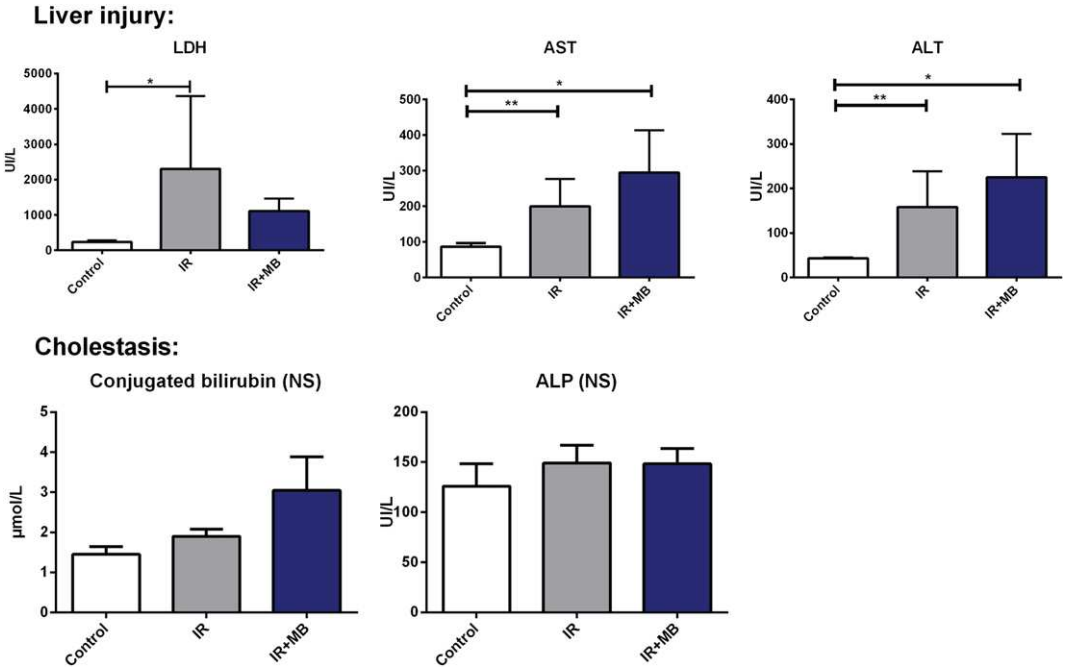

Figure 2. Liver function. LDH, lactate deshydrogenase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Mean ± SEM. NS, not significant, *$p < 0.05$, **$p < 0.01$.

149 ± 17 UI l^{-1}). MB did not impact on the results of these liver function tests.

Blood lactate level was significantly higher in the IR and IR + MB groups (3.3 ± 1 and 3.8 ± 1.9 mmol l^{-1}, respectively) than in controls (1.4 ± 04 mmol l^{-1}) ($P < 0.001$). The difference in level between the IR and IR + MB groups was not significant ($p = 0.58$).

Histology and DNA fragmentation

The intestine of control rats was normal with no signs of ischaemic injury and presented incidental apoptotic cells which were generally found among the epithelial cells of the villi tips (Fig. 3). Gut IR induced focal injuries ranging in grade from 0 (normal mucosa) to 5 (loss of villi) and an increase in DNA fragmentation in intact mucosal structures, especially in the crypt layer. The extent of focal injury and apoptosis was similar in the IR and IR + MB groups.

The livers of the control and the IR groups showed normal architecture and cellularity on histological examination, with no signs of ischaemia or inflammation in the IR group, and rare (<1%) apoptotic hepatocytes in the DNA fragmentation assay. Histology and DNA fragmentation results did not differ in the IR and IR + MB groups (normal in both).

Liver mitochondrial function

Significant impairment of liver mitochondrial oxidative capacity occurred after gut IR (Table 1, Fig. 4). Basal mitochondrial respiration (state 4) decreased by 28%, maximal oxidative capacity (state 3) by 30%, complex II, III and IV activity (state 3_{+Succ}) by 35% and complex IV activity (state 3_{+TMPD}) by 29%. MB treatment restored all these activities (Table 1, Fig. 4).

Relationship between gut IR injury and liver mitochondrial impairment

To steer clear of any possible interference by MB, the relationship between liver mitochondrial impairment and gut insult, as assessed by blood lactate, was investigated by combining data for the control and IR groups only. Liver mitochondrial respiration was significantly and inversely correlated with lactate level for state 3 ($60 \pm 5.1 - (0.11 \times \text{lactates})$ µM O_2 min^{-1} g^{-1}, $r^2 = 0.45$, $P = 0.001$) (Fig. 5), state 3_{+succ} ($r^2 = 0.54$, $P = 0.002$) and state 3_{+TMPD} ($r^2 = 0.45$, $P = 0.001$).

DISCUSSION

In our study, gut IR was characterised by villi necrosis, crypt apoptosis and increased blood lactate. Gut IR significantly decreased the activity of each complex of the liver mitochondrial respiratory chain but these decreases were counteracted by pharmacological post-conditioning with MB.

Intestinal histology after gut IR revealed typical focal gut mucosal necrosis without transmucosal infarction ranging in grade from 0 (normal) to 5 (loss of villi) within a sample.[16] In addition, as already reported, the mucosal cells of the crypt layer were subject to DNA fragmentation processes.[19] These histology and biology data thus confirm that our gut IR model induced moderate yet significant impairment as can be observed clinically in non-lethal gut IR.

Gut IR increased transaminases and LDH, a marker of ischaemic damage, but did not affect serum bilirubin nor

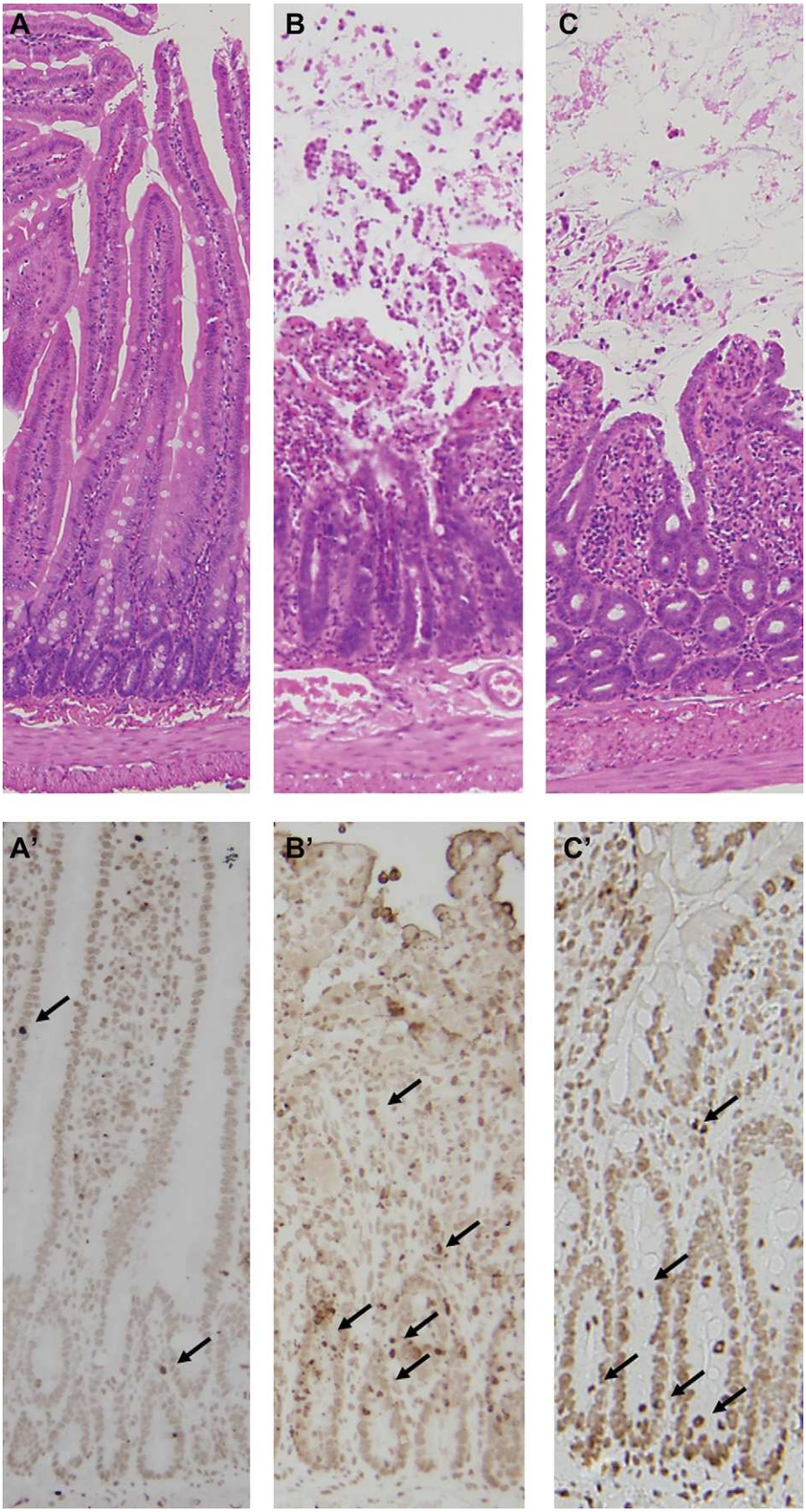

Figure 3. Gut histology and apoptosis. Representative small intestines from rats: (A—C) Haematoxylin—eosin stained sections (×10). (A) Controls: normal mucosa (grade 0); (B) IR: loss of villi (grade 5); (C) IR + MB: as in B. (A′—C′) Apoptotic cells in DNA fragmentation assay (see arrows) (×20). (A′) Controls: few apoptotic cells; (B′) IR: apoptosis in all intact villi, particularly in crypt layer; (C′) IR + MB: as in B′.

Table 1. Liver mitochondria oxidative capacity and respiratory chain complexes.

	Substrates/ Respiratory chain complexes	Mean O_2 consumption (μM/min/g)			ANOVA *P* value	Percent change *P* value[a]		
						C vs. IR	C vs. IR + MB	IR vs. IR + MB
State 4 (basal)	Glutamate—malate/ I—III—IV	4.8 ± 03	3.5 ± 0.2	5.2 ± 0.2	<0.002	−27 <0.01	— > 0.05	+48 <0.05
State 3 (max)	Glutamate—malate/ I—III—IV	50 ± 3	35.2 ± 3.5	49.1 ± 2.7	<0.004	−29 <0.01	— > 0.05	+39 <0.05
State 3 $_{+Succ}$	Succinate/ II—III—IV	50 ± 2.4	32.5 ± 3.7	47.3 ± 3.1	<0.001	−35 <0.01	— > 0.05	+45 <0.01
State 3 $_{+TMPD}$	TMPD/ IV	67.1 ± 3	47.9 ± 4.2	62.8 ± 3.9	<0.003	−29 <0.01	— > 0.05	+31 <0.05

Means are given with ±SEM.
[a] Tukey post-hoc test.

did induce liver lesions, suggesting early liver insult.[20] About 80% of patients with ischaemic injury have substantially elevated LDH (ALT/LDH ratio <1) yet low serum bilirubin.[21]

Liver subcellular dysfunction may precede histological lesions after gut IR.[11] In our study, gut IR significantly decreased liver maximal oxidative capacity and also impaired the activity of complexes II, III and IV of the liver mitochondrial respiratory chain. These results support data obtained by indirect assessment of mitochondrial function. Poggetti et al. demonstrated gut IR-induced impairment of the liver mitochondrial redox state by using serum acetoacetate/3-hydroxybutyrate levels as indicator.[22] Horie et al. found that gut IR caused liver cell hypoxia and mitochondrial impairment by using autofluorescence of a hepatic pyridine nucleotide (reduced nicotinamide adenine dinucleotide phosphate, NADH) as an indicator of mitochondrial O_2 consumption and redox state.[7] Increased lactate may also signal liver mitochondrial dysfunction. In our study, blood lactate was inversely correlated with liver maximal oxidative capacity ($r^2 = 0.45$). In humans, splanchnic hypoperfusion is correlated with hepatic cellular damage.[23] As decreased oxidative capacity is generally associated with reduced ATP production and subsequent organ dysfunction, impairment of liver mitochondrial respiration during gut IR might contribute towards a poor prognosis and warrant effective treatment.

We confirmed that MB does not alleviate IR-induced intestinal lesions.[15] However, MB can prevent haemodynamic instability, base deficit and lung injury after gut or liver IR.[24] It attenuates liver damage in extrahepatic cholestasis and protects against different types of liver injury.[24,25]

We observed no decrease in transaminases on MB injection, maybe because we chose a moderate dose (15 mg kg^{-1}) with known benefits and no major adverse effects. MB doses of 2 and 60 mg kg^{-1} had no beneficial effects in a similar rat model of gut IR whereas 6 and 20 mg kg^{-1} reduced haemodynamic deterioration and metabolic acidosis.[26] During vasoplegic shock, patients typically receive an intravenous (IV) bolus of 1—2 mg kg^{-1} MB.[27,28] A decrease in transaminases might have been missed because reperfusion time was too short (1 h) with regard to transaminase kinetics in hepatic disease which is apparently slow.[29] Alternatively, MB was perhaps just unable to normalise transaminases. It should be stressed, however, that a decrease in aminotransferase levels alone after a marked increase has no prognostic meaning as both resolution and massive hepatic necrosis may show a similar biochemical pattern.[30]

In our study, MB counteracted the deleterious effects of gut IR on liver oxidative capacities and restored cellular energy homeostasis. The underlying mechanisms warrant study. MB might modulate the nitric oxide (NO)/guanylate cyclase pathway and reduce oxidative stress and/or might interact directly with mitochondria.[13,14,31] MB passes through both cellular and mitochondrial membranes, accumulates within mitochondria and improves mitochondrial respiration at low concentrations (0.5—2 mM) by shuttling electrons to oxygen in the electron transport chain. MB also counteracts perturbed mitochondrial metabolism induced by mutagens, acts as an alternative electron acceptor in mitochondria and might inhibit the production of superoxide by competing directly with molecular oxygen.[14]

Our study has several limitations. First, we used a mild gut IR model (SMA ischaemia (1 h)/reperfusion (1 h)) simulating certain clinical and surgical situations of gut suffering (vascular surgery, intestinal surgery, shock…) but this model cannot be generalised to all situations. An earlier model using supra-coeliac ischaemia (40 min)/reperfusion (1 h) was associated with higher intestinal injury (lactate 7.1 ± 1.6 mmol l^{-1}).[32] One hour of ischaemia was associated with >50% lethality within the first hour of reperfusion (unpublished data). Our present model induced significant histological injury, apoptosis and an increase in lactate (3.3 ± 1 mmol l^{-1}) but was not lethal, and is moreover the most widely used experimental model.

Second, we used a single MB dose (15 mg kg^{-1} IP) based on published work.[26] This dose restored 100% of liver mitochondria oxidative capacities and has no known adverse effects. However, the lowest MB dose that protects liver mitochondria needs to be determined. We injected MB 10 min before reperfusion because onset of ischaemia is rarely predictable in clinical practice. MB injection

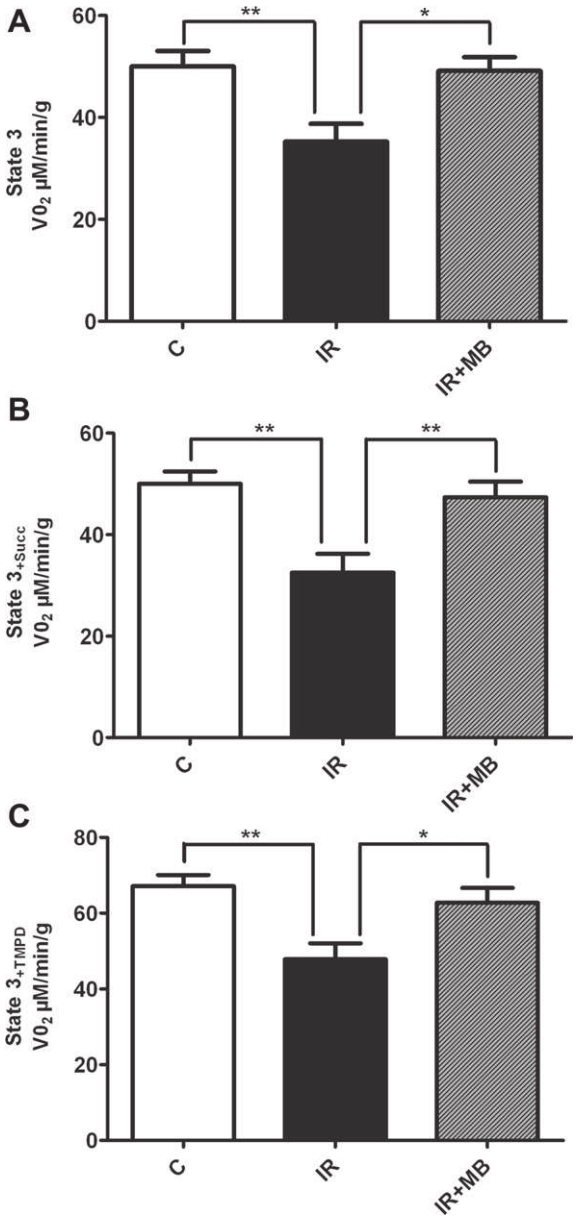

Figure 4. Respiration rates in isolated liver mitochondria. Mitochondria isolated from control group (C) ($n = 10$), IR group ($n = 10$), and IR + MB group ($n = 8$) with added (A) glutamate–malate and ADP (state 3), (B) succinate (state 3_{+Succ}), (C) TMPD/Ascorbate (state 3_{+TMPD}). Mean ± SEM. *$p < 0.05$, **$p < 0.01$.

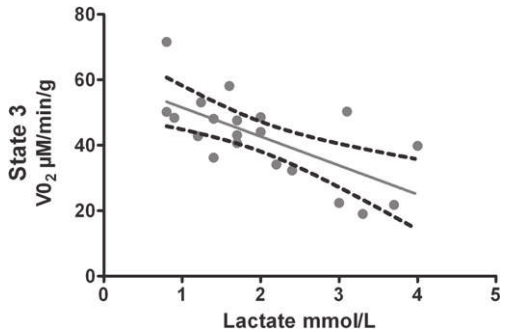

Figure 5. Relationship between blood lactate and maximal liver mitochondria oxidative capacity (state 3) for control and IR groups combined. $r^2 = 0.48$; $p = 0.001$.

immediately after ischaemia seemed clinically sound and relevant. Such post-conditioning has been shown to reduce reperfusion-induced injury on occlusion of other vascular beds.[33]

Third, we measured mitochondrial activity after gut IR in liver only. A multi-organ approach is severely hampered by the need to extract mitochondria from fresh tissue and measure oxygen consumption immediately. However, we used the same protocol as in an earlier study on pulmonary mitochondria.[34] Gut IR induced a 50% decrease in oxidative capacity in the liver and a 30% decrease in the lung. Finally, we addressed only a small piece of a complex situation.

In conclusion, the liver–gut axis has a key role in the deleterious effects of gut IR. We have demonstrated early impairment of the complexes of the liver mitochondrial respiratory chain after gut IR. MB suppressed this remote impairment. If injected before vascular unclamping in a clinical setting, MB might protect liver mitochondria function and minimise the incidence of postoperative multiple organ failure. Clinical studies on how to best use MB to limit the deleterious effects of gut IR during vascular surgery are thus warranted.

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CONFLICT OF INTEREST

None.

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Etude N° 11

Zheng F, Barthel G, Collange O, Montemont C, Thornton SN, Longrois D, Levy B, Audibert G, Malinovsky JM, Mertes PM

Methylene blue and epinephrine: a synergetic association for anaphylactic shock treatment.

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Methylene Blue and Epinephrine: A Synergetic Association for Anaphylactic Shock Treatment*

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Background: Severe hypotension resulting from anaphylactic shock may be refractory to epinephrine and impair cerebral oxygenation and metabolism contributing to anaphylactic shock morbidity and mortality. Refractoriness to epinephrine could be corrected by nitric oxide pathway inhibitors such as methylene blue.

Objectives: To compare the systemic and regional (brain and skeletal muscle) effects of epinephrine and methylene blue given alone or in combination in a rat model of anaphylactic shock.

Design: Prospective laboratory study.

Setting: University laboratory.

Subjects: Male Brown-Norway rats ($n = 60$).

Interventions: After sensitization and induction of anaphylactic shock by ovalbumin, animals received either vehicle (ovalbumin group) or a 3-mg/kg methylene blue bolus (methylene blue group) or epinephrine (epinephrine group) or both (methylene blue–epinephrine group). Sensitized control rats received only vehicle and no ovalbumin (control group).

Measurement and Main Results: Mean arterial pressure, cardiac output, cerebral blood flow, skeletal muscular oxygen partial pressure, cerebral oxygen partial pressure, skeletal muscular, and cerebral interstitial lactate/pyruvate ratio were measured. Cleaved caspase 3 and hypoxia-inducible factor-1α expression were analyzed in the cerebral cortex by Western blot. Without treatment, rats died rapidly within 15 mins from a decrease in cardiac output and mean arterial pressure, whereas treated rats survived until the end of the experiment. Methylene blue alone extended survival time but without significant improvement of hemodynamic variables and tissue perfusion and did not prevent neuronal injury. Epinephrine restored partially systemic hemodynamic variables and cerebral perfusion preventing glutamate-induced excitotoxicity. Compared with epinephrine alone, the methylene blue–epinephrine association avoided neuronal excitotoxicity and had an additive effect both on hemodynamic variables and for prevention of brain ischemia. Neither treatment could significantly restore cardiac output or prevent muscular compartment ischemia and microvascular leakage.

Conclusions: Anaphylactic shock is associated with severe impairment of cerebral blood flow despite correction of arterial hypotension. Epinephrine must still be considered as the first-line vasoconstrictive agent to treat anaphylactic shock. The epinephrine–methylene blue association was the most effective treatment to prevent cerebral ischemia and could be used in anaphylactic shock refractory to epinephrine. (*Crit Care Med* 2013; 41:195–204)

Key Words: anaphylaxis; cerebrovascular circulation; epinephrine; ischemia; methylene blue; microdialysis; nitric oxide; shock

***See also p. 359.**

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Drs. Zheng and Barthel contributed equally to this study.

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Anaphylactic shock (AS) is a relatively common problem in the perioperative period with a prevalence of one to nine per 10,000 general anesthetic procedures worldwide (1). AS is an acute, potentially fatal reaction within the spectrum of generalized immediate-type hypersensitivity (i.e., anaphylaxis). During anesthesia, this shock is lethal in 3% to 10% of patients (2, 3) or can lead to severe morbidity (cerebral anoxia). Epinephrine (EPI), recommended by guidelines, may fail to restore rapidly adequate organ perfusion or can be completely ineffective; in this case, the shock is considered refractory to catecholamines for reasons that are not understood (4, 5).

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AS is commonly classified as a distributive shock characterized by hypovolemia resulting from increased capillary permeability and arterial hypotension resulting from excessive vasodilatation. However, recent reports indicate that the pathophysiology of anaphylaxis is more complex than initially thought.

Impaired myocardial contractility, an increase in venous tone in the splanchnic and portal vascular beds or in the pulmonary veins, and a severe decrease in blood perfusion in skeletal muscles have been demonstrated (6–10). On the contrary, the consequences on cerebral vasculature and brain perfusion remain to be determined, whereas these changes may be of critical importance in delineating optimal therapeutic goals and strategies (11).

The mentioned effects of AS on organ function are related to the explosive release of mediators such as histamine, platelet activating factor, leukotrienes, and tumor necrosis factor-α (12, 13) leading to the activation of multiple pathways that include but are not limited to the nitric oxide (NO) pathway (14). Despite the documented myocardial depressive effect of NO (15) and the potential beneficial effect related to its inhibition, the effect of pharmacological inhibition of the NO pathway on early survival in AS remains controversial, depending on experimental models, animal species, and NO pathway inhibitors used (16–18). It is possible that inhibition of the NO pathway could have beneficial effects on one organ (e.g., the heart) but be deleterious on other organs (e.g., the brain).

Furthermore, the interactions between EPI and the different drugs known to pharmacologically inhibit the NO pathway have not been thoroughly investigated. Therefore, the purpose of our study was to investigate the beneficial or detrimental role of methylene blue (MB, a substance known to inhibit one of the final effectors of NO, i.e., guanylyl cyclase) alone or in association with EPI, on early survival, hemodynamic, tissue oxygen availability, and metabolic disturbances, with special emphasis on brain consequences, in an anesthetized rat model of Ig-E–mediated AS.

Because long-term consequences of treatments cannot be investigated because of the surgical procedure required in our experiments, brain-cleaved caspase 3 and hypoxia-inducible factor (HIF)-1α expressions were analyzed as surrogate markers of AS-induced delayed injury in the cerebral cortex.

MATERIALS AND METHODS

Animals and Sensitization Protocol

After approval by our institutional Animal Care Committee, all animal procedures and care were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC). The animals had free access to water and food.

Ten-week-old Brown-Norway rats weighting 250 g–300 g (Janvier, Le Genest-St-Isle, France) were used for these experiments. They were kept under standard conditions (temperature 21 ± 1°C; light from 6 AM to 6 PM) and given a standardized diet (A04; UAP, Villemoisson-sur-Orge, France) and water (Aqua-clear; Culligan, Northbrook, IL) ad libitum. On days 0, 4, and 14, rats were sensitized by subcutaneous administration of 1 mg grade VI chicken egg albumin (ovalbumin [OVA]; Sigma-Aldrich, Saint-Quentin Fallavier, France) and 4 mg aluminum hydroxide in adjuvant (Sigma, St. Louis, MO) diluted in 1 mL 0.9% saline solution, as previously described (7).

Surgical Procedure, Measurement of Hemodynamic Variables, Tissue Oxygen Partial Pressure, and Interstitial Microdialysis

The surgical procedure was performed on day 21 after the initial sensitization. Anesthesia was induced with 3% isoflurane and maintained with 1% isoflurane. The trachea was cannulated, and the lungs were mechanically ventilated with 100% oxygen using a Harvard rodent respirator (Model 683; Harvard Apparatus, Cambridge, MA). Tidal volume was 2.5 mL, and respiratory rate was adjusted to maintain $Paco_2$ between 38 mm Hg and 42 mm Hg. Rectal temperature was maintained by an intermittent heating light at 38 ± 0.5°C throughout the experiment. A fluid-filled polyethylene catheter (inside diameter, 0.58 mm; outside diameter, 0.96 mm; Biotrol Diagnostic, Chennevières Les Louvres, France) was inserted in the left femoral artery for pressure monitoring. Mean arterial pressure (MAP) and heart rate were recorded continuously using a strain gauge pressure transducer (DA-100; Biopac Systems, Northborough, MA). Another fluid-filled catheter was inserted in the left femoral vein for administration of drugs and a basal physiological need perfusion of 0.9% saline solution at 10 mL/kg·hr. Skeletal muscle and cerebral metabolisms were studied in two independent experimental series.

Specific Skeletal Muscle Procedure

A thermistor catheter connected to a thermodilution cardiac output (CO) computer (Cardiomax III, Columbus Instrument, Columbus, OH) was inserted in the left carotid artery and moved into the left ventricle for CO monitoring.

A flexible Clark-type polarographic oxygen electrode (diameter, 500 μm; length, 200 mm) computer-supported LICOX system (GMS, Mielkendorf, Germany) was introduced in the right quadriceps muscle for tissue oxygen partial pressure measurement ($mPtio_2$).

In the left quadriceps muscle, a liner flexible microdialysis probe (polyarylethersulfone membrane; membrane length, 10 mm; outside diameter, 0.5 mm; molecular weight cutoff, 20 kd; CMA/Microdialysis AB, Kista, Sweden) was inserted as previously described (7). The probe was connected to a 2.5-mL CMA/106 microsyringe mounted on a microinjection pump (CMA 107 Microdialysis Pump, Stockholm, Sweden) and infused at ambient temperature with a lactate-free Ringer's solution (Na^+ 147 mmol/L, K^+ 4 mmol/L, Ca^{2+} 2.3 mmol/L, Cl^- 156 mmol/L) at a flow rate of 2 μL/min. A period of 30 mins was allowed for equilibration before beginning sample collection. Microdialysates were collected in microvials (CMA) every 10 mins until the end of the experiment, starting 10 mins before shock induction. Samples were kept on ice initially and then stored at –20°C. The interstitial lactate, glucose, pyruvate,

and glutamate were measured with the CMA 600 microdialysis analyzer.

Specific Cerebral Procedure

Cerebral $PtiO_2$ ($cPtiO_2$) was monitored by a flexible Clark-type polarographic oxygen electrode (outside diameter, 0.5 mm, membrane length 200 mm, 4-mm sensitive area; Licox CC1.R, Integra NeuroSciences, Sophia Antipolis, France) connected to LICOX CMP instrument (Integra NeuroSciences) and inserted into the hippocampus (4.6 mm anterior and 2 mm lateral to the λ to a depth of 3 mm).

Cerebral blood flow (CBF) was monitored using a laser-Doppler needle probe (PERIFLUX systems, probe 411, diameter 450 μm) connected to a PeriFlux PF 5010 laser Doppler monitor (Perimed AB, Stockholm, Sweden) inserted into the right cerebral cortex 2 mm anterior to the oxygen electrode.

A central nervous system-designed microdialysis probe (CMA/12, polyarylethersulfone membrane, length 3 mm, outside diameter, 0.5 mm, cutoff 20 kd, CMA/Microdialysis AB) was implanted through a hole placed 7.3 mm anterior and 4 mm lateral to the λ in the striatum to a depth of 6 mm, and probes were infused with CMA isotonic sterile perfusion fluid (NaCl 147; KCl 2.7; $CaCl_2$ 1.2; $MgCl_2$ 0.85 mmol/L) at a flow rate of 2 μL/min.

Induction of Shock and Treatment With MB and/or EPI

After a 30-min stabilization period, AS was induced by intravenous injection of 1 mg ovalbumin (T0). Animals were randomly assigned to five different groups: vehicle in control (CON) group nonshocked, no-treatment in OVA group, MB only, EPI only, and MB–EPI. The investigator was not blinded to the drugs used. A single bolus of MB (Sigma) of 3 mg/kg was injected 3 mins after shock induction (T3). The first bolus of EPI of 2.5 μg was injected at T3 and a second bolus at T5, continuous infusion of EPI 10 μg/kg·min, was initiated immediately after the first bolus. In the group receiving the combined treatment, MB bolus was injected immediately after the first EPI bolus. MAP, heart rate, CO, CBF, $mPtiO_2$, and $cPtiO_2$ were recorded at the following time points: T0 (before shock induction), T1, T2.5, T5, T7.5, T10, T12.5, T15, T17.5, T20, and then every 5 mins until the end of experiment. Arterial blood samples were taken from each animal before the shock induction and at the end of the experiment for measurement of arterial blood gas and plasma sodium, chloride, lactate, nitrite, nitrate, and hemoglobin. Samples for plasma nitrite and nitrate analysis were stored at −80°C. They were then deproteinized before analysis using Vivaspin 500 centrifugal concentrators (Sigma-Aldrich, St. Louis, MO). Plasma concentrations of nitrite and nitrate were measured according to Griess reaction by enzyme-linked immunosorbent assay (Parameter colorimetric competitive enzyme-linked immunosorbent assay kit; R&D Systems, Abingdon, UK) according to the manufacturer's instructions. The animals were euthanized by an overdose of thiopentone sodium after the last blood sample collection.

Western Blot Analysis

Cerebral cortex tissues were homogenized with a Polytron homogenizer (Tissue-Lyser II; Qiagen, Courtaboeuf, France). Protein content was measured by the Bradford method (Pierce, ThermoScientific, Brebières, France). Protein extracts from cerebral cortex tissues (20 μg) were separated by a 4% to 12% Criterion XT Bis-Tris Gel (BioRad, Marnes-la-Coquette, France) and subjected to Western blotting for rabbit anti-HIF-1α (1:2000; Abcam, Paris, France) and rabbit anticleaved caspase 3 (1:1000; Cell Signaling, Ozyme, Saint-Quentin en Yvelines, France), and β-actin was used as a protein loading CON. Bound antibody density analyses were performed by LAS-4000 imager (FSVT, Courbevoie, France) and Multi-Gauge software (LifeScience, Fujifilm, France). After densitometry analyses, optical density values were expressed as arbitrary units (AUs).

Statistical Analysis

Results are expressed as mean ± SEM. Intragroup and among-group comparisons were performed using one-way and two-way analysis of variance for repeated measures. Differences

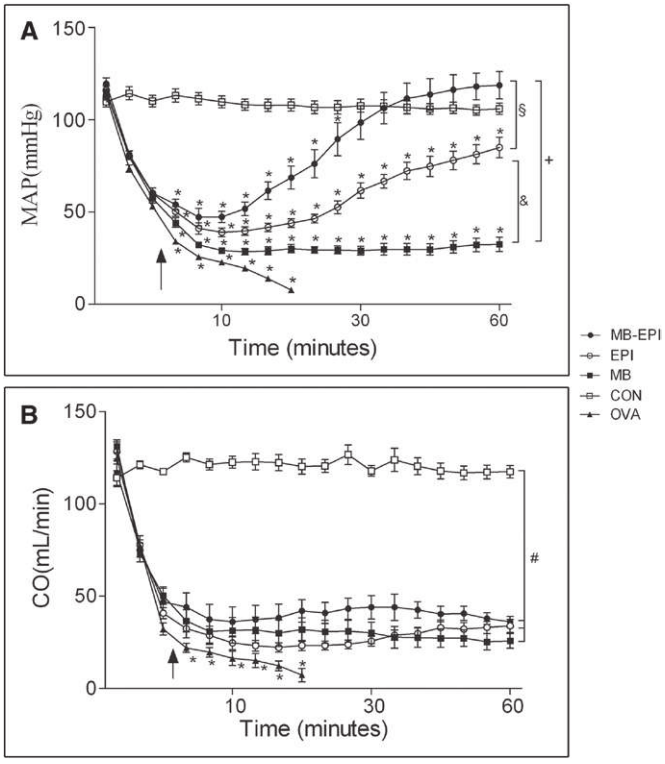

Figure 1. Time course of systemic mean arterial pressure (MAP) (**A**, n = 12 rats/group) and cardiac output (CO) (**B**, n = 6 rats/group) values in ovalbumin (OVA), control (CON), methylene blue (MB), epinephrine (EPI), and epinephrine associated with methylene blue (MB–EPI) groups. Time 0 corresponds to the injection of OVA. Values are presented as mean ± SEM. ↑ corresponds to the beginning of treatment. $^{*}p \leq 0.05$ vs. the CON group, $^{+}p \leq 0.05$ between-group differences MB–EPI group vs. MB group, $^{\&}p \leq 0.05$ between-group differences EPI group vs. MB group, $^{\S}p \leq 0.05$ between-group differences MB–EPI group vs. EPI group, $^{\#}p \leq 0.05$ between-group differences MB–EPI or EPI or MB groups vs. CON group. Triangle = OVA group; white square = CON group; black square = MB group; white circle = EPI group; black circle = MB-EPI group.

between groups at a time period were analyzed using the unpaired *t* test for nonrepeated measures with Statview 5.0 software (Deltasoft, Meylan, France). When a significant interaction was observed with two-way analysis of variance, paired comparisons were made with the Fisher's post hoc test.

RESULTS

Sixty OVA-sensitized Brown-Norway rats (274 ± 4 g) were studied and randomly allocated to the CON group (no-shock, $n = 12$), OVA group (no-treatment, $n = 12$), MB group ($n = 12$), EPI group ($n = 12$), and MB–EPI group ($n = 12$). Because animals from the OVA group died within 15 mins, data from microdialysis, Western blot, and plasma biochemistry were not obtained from this group.

Systemic Hemodynamic Variables

Time-course profiles for MAP (**Fig. 1*A***) and CO (Fig. 1*B*) were different among the groups. Hemodynamic variables remained relatively stable throughout the entire study period in CON animals, whereas OVA injection resulted in a similar rapid and profound decrease of MAP and CO in the other groups. Without treatment, rats died within15 mins with a dramatic decrease in CO and MAP, whereas treated rats survived until the end of the experiment. Treatment resulted in stabilization of MAP at a low value in the MB group. MAP increased progressively, reaching 70% of the basal value at the end of the experiment in the EPI group, and was completely restored as of T30 min in the MB–EPI group. The three treatments enhanced CO as compared with the OVA group; after the initial decrease, CO remained stable at low values in the MB group. EPI alone or in association with MB restored poorly CO at 26% and 31% of basal value, respectively but without a significant difference among these three groups.

Effects of the Different Treatments in the Cerebral Compartment

Cerebral hemodynamic and metabolic variables remained stable during the entire study period in the CON rats. In the absence of treatment, a rapid and profound decrease in CBF and $cPtiO_2$ was observed after shock induction (**Fig. 2A–*B***). A similar decrease in CBF was observed in the treated groups up to 12.5 mins after shock induction. Subsequently, CBF stabilization occurred at low values in the MB group from 106 ± 2 to 22 ± 3 PU, whereas EPI alone or in association with

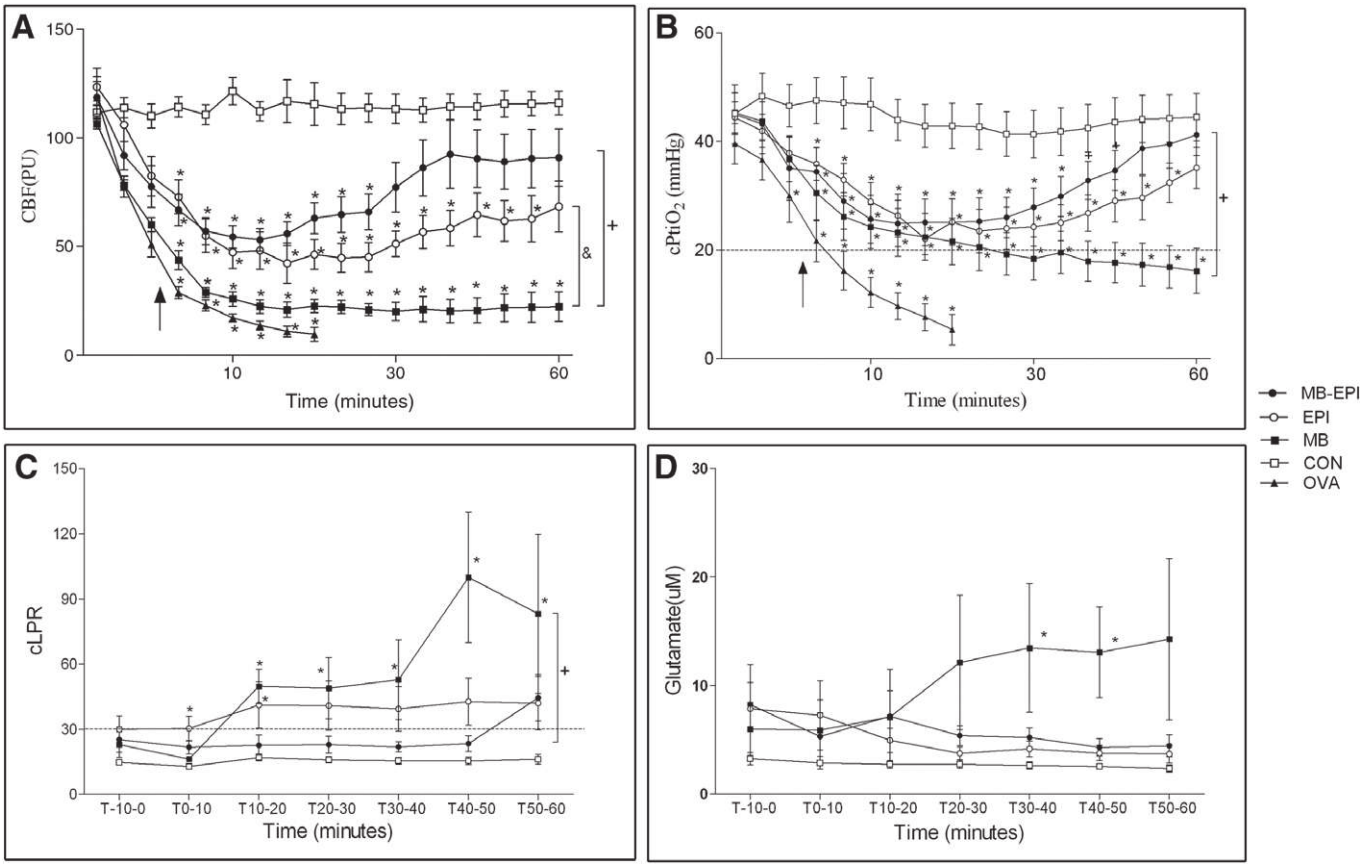

Figure 2. Time course of cerebral cortical blood flow (CBF) (**A**), tissue oxygen partial pressure ($PtiO_2$) in the hippocampus (**B**), lactate/pyruvate ratio (cLPR) in the striatum (**C**), and interstitial glutamate (**D**) in striatum values in ovalbumin (OVA), control (CON), methylene blue (MB), epinephrine (EPI), and epinephrine associated with methylene blue (MB–EPI) groups ($n = 6$ rats/group). Because of the microdialysis flow rate, samples were collected every 10 mins, starting 10 mins before shock induction (T0). OVA rats died within 15 mins, so their data were not measured. Values are presented as mean ± SEM. ↑ corresponds to injection of treatment; the *dotted line* corresponds to the ischemic threshold. $^{*}p \leq 0.05$ vs. the control group, $^{+}p \leq 0.05$ between-group differences MB–EPI group vs. MB group, $^{\&}p \leq 0.05$ between-group differences EPI group vs. MB group. Triangle = OVA group; white square = CON group; black square = MB group; white circle = EPI group; black circle = MB-EPI group.

TABLE 1. Time Course of Interstitial Lactate, Pyruvate, and Glucose Concentrations in the Brain ($n = 6$ rats/group)

Group		Control Group	Methylene Blue Group	Epinephrine Group	Epinephrine Associated With Methylene Blue Groups
Lactate (mM)	T10 to T0	0.32 ± 0.07	0.20 ± 0.04	0.34 ± 0.14	0.27 ± 0.04
	T0 to T10	0.23 ± 0.05	0.23 ± 0.04	0.32 ± 0.08	0.23 ± 0.01
	T10 to T20	0.30 ± 0.05	0.59 ± 0.11[a]	0.40 ± 0.02	0.43 ± 0.06
	T20 to T30	0.25 ± 0.02	0.55 ± 0.12[a]	0.40 ± 0.04	0.47 ± 0.10
	T30 to T40	0.24 ± 0.02	0.50 ± 0.13[a]	0.36 ± 0.04	0.33 ± 0.08
	T40 to T50	0.24 ± 0.03	0.50 ± 0.14	0.31 ± 0.01	0.36 ± 0.08
	T50 to T60	0.24 ± 0.03	0.39 ± 0.12	0.27 ± 0.03	0.31 ± 0.06
Pyruvate (mM)	T10 to T0	0.020 ± 0.003	0.012 ± 0.004	0.011 ± 0.003	0.015 ± 0.004
	T0 to T10	0.017 ± 0.002	0.015 ± 0.003	0.011 ± 0.002	0.012 ± 0.002
	T10 to T20	0.017 ± 0.001	0.014 ± 0.004	0.012 ± 0.003	0.023 ± 0.006
	T20 to T30	0.016 ± 0.002	0.014 ± 0.003	0.011 ± 0.003	0.022 ± 0.005
	T30 to T40	0.016 ± 0.002	0.014 ± 0.003	0.012 ± 0.003	0.016 ± 0.004
	T40 to T50	0.017 ± 0.001	0.007 ± 0.002[a]	0.010 ± 0.003	0.017 ± 0.005[b]
	T50 to T60	0.016 ± 0.002	0.008 ± 0.003	0.010 ± 0.003	0.010 ± 0.003
Glucose (mM)	T10 to T0	0.22 ± 0.03	0.21 ± 0.06	0.23 ± 0.07	0.26 ± 0.05
	T0 to T10	0.25 ± 0.09	0.17 ± 0.05	0.26 ± 0.05	0.25 ± 0.03
	T10 to T20	0.35 ± 0.09	0.071 ± 0.03[a]	0.23 ± 0.01	0.28 ± 0.04[b]
	T20 to T30	0.23 ± 0.08	0.11 ± 0.05	0.23 ± 0.04	0.30 ± 0.05[b]
	T30 toT40	0.23 ± 0.05	0.11 ± 0.05	0.30 ± 0.03[b]	0.34 ± 0.05[b]
	T40 to T50	0.27 ± 0.04	0.13 ± 0.05	0.28 ± 0.04[b]	0.38 ± 0.06[b]
	T50 to T60	0.25 ± 0.03	0.11 ± 0.06	0.31 ± 0.03[b]	0.28 ± 0.07[b]

Time 0 (T0) corresponds to injection of 1 mg ovalbumin.
[a]$p \leq 0.05$ vs. the control group.
[b]$p \leq 0.05$ vs. the methylene blue group.

MB restored partially CBF (55% and 77% from basal values, respectively; Fig. 2*A*).

After shock induction, a significant decrease in $cPtiO_2$ was initially observed in each treatment group. Subsequently, in the MB group, $cPtiO_2$ decreased slightly with time, reaching values below the cerebral ischemic threshold ($cPtiO_2$ <20 mm Hg) as of T25 mins. These low $cPtiO_2$ values in the MB group, reflecting ischemia, were in agreement with the decrease of interstitial glucose concentrations (**Table 1**), the progressive increase in L/P ratio (Fig. 2*C*), and the release of glutamate (Fig. 2*D*).

In contrast, EPI alone or in association with MB restored progressively O_2 availability (79% and 92%, respectively, of basal values; Fig. 2*B*). This was confirmed by cerebral microdialysis results confirming a relative stability of glucose interstitial concentrations and L/P ratio (Fig. 2*C*) and the absence of increased glutamate release reflected by a moderate increase in interstitial glucose concentrations (Table 1) and glutamate decrease (Fig. 2*D*).

Effects of the Different Treatments in the Muscular Compartment

The time-course profiles of $mPtiO_2$ (**Fig. 3*A***) were similar in all treated groups. A rapid and sustained decrease in $mPtiO_2$ was observed after shock induction. The basal values were approximately 40 mm Hg, decreased rapidly after shock induction, and stabilized finally at low values without restoration. The effects of EPI alone, MB–EPI, and MB (6.8 ± 1.6 mm Hg, 6.8 ± 1.6 mm Hg, and 4.2 ± 2.0 mm Hg, respectively) on oxygen availability were not statistically different.

Interstitial muscular lactate concentrations increased progressively and interstitial pyruvate concentrations decreased progressively with time without differences among treatment groups (**Table 2**). A progressive increase in interstitial muscular

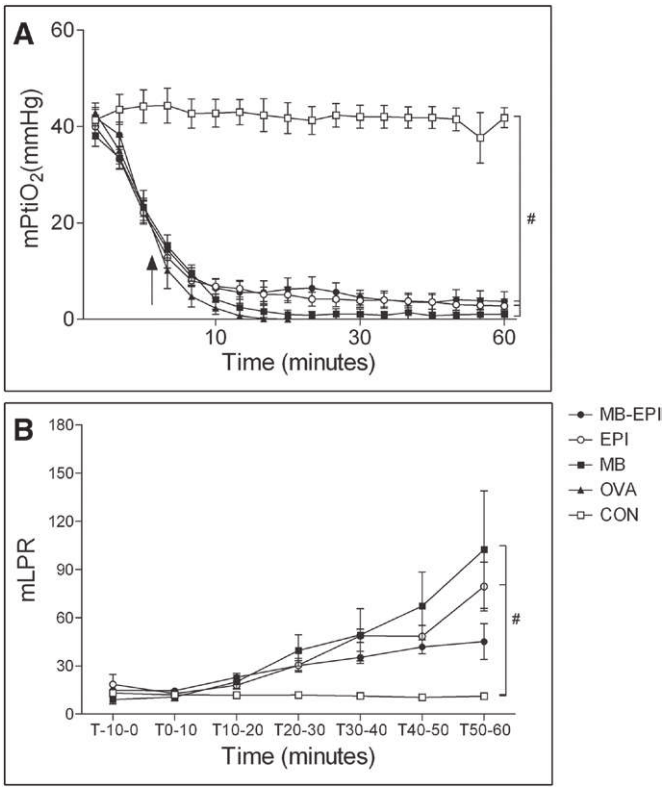

Figure 3. Time course of tissue oxygen partial pressure ($mPtiO_2$) (**A**) and lactate/pyruvate ratio (mLPR) (**B**) values in skeletal muscle in ovalbumin (*OVA*), control (CON), methylene blue (MB), epinephrine (EPI), and epinephrine associated with methylene blue (MB–EPI) groups ($n = 6$ rats/group). Because of the microdialysis flow rate, samples were collected every 10 mins, since 10 mins before shock induction (T0). OVA rats died within 15 mins, so the data were not measured. Values are presented as mean ± SEM. ↑ corresponds to injection of treatment bolus. $^{\#}p \leq 0.05$ between-group differences MB–EPI or EPI or MB groups vs. CON group. Triangle = OVA group; white square = CON group; black square = MB group; white circle = EPI group; black circle = MB-EPI group; $mPtiO_2$ = muscular oxygen partial pressure.

L/P ratios was observed in all treatment groups; values were significantly different in the group MB alone and EPI alone as compared with the CON group (Fig. 3*B*). However, maximal values obtained at the end of the experiment tended to be lower in the MB–EPI-treated animals, although this difference failed to reach statistical significance among treatment groups.

Time Course of Systemic Biochemistry Variables

The time course of the main systemic biochemistry variables is summarized in **Table 3**. No differences were observed among groups before OVA injection (T0). Data from the OVA group are not shown because of animals' death after 15 mins, and so biochemistry results were not measured. Sixty mins after onset of shock, no significant differences were observed among treated groups, except for $Paco_2$, nitrate, and nitrite. In each group, AS induced a severe lactic acidosis and hemoconcentration (hemoglobin: from 12.1 ± 0.3 to 14.6 ± 0.4 g/dL).

Expression of Activated Caspase 3 and HIF-1α in the Cerebral Cortex

Sixty mins after the onset of shock, there was a significant increase in the expression of activated caspase 3 in the cerebral cortex in the MB and EPI groups compared with the MB–EPI group. It was moderately underexpressed in the MB–EPI group compared with the CON group but not significantly. With regard to HIF-1α, changes were not significantly different among groups. It was underexpressed in the MB and EPI groups and similar to the CON group in the MB–EPI group (**Fig. 4**).

DISCUSSION

According to our knowledge, this is the first report describing the highly deleterious consequences of AS on the brain and their attenuation by three different treatment regimens that are routinely (EPI) or potentially (MB) used in humans who have AS.

Under physiological conditions, the cerebral circulation is intimately involved in adequate blood distribution (19–22). Maintenance of CBF during hypotension is essential for homeostasis and prevention of irreversible brain damage. It is assumed, but not demonstrated, that even in severe AS states, cerebral circulation is maintained (11). Meanwhile, a severe decrease in regional flow is expected to occur in skeletal muscle. Our results demonstrate that in untreated AS, the dramatic decrease in CO and CBF precluded any redistribution of blood flow to the brain and resulted in severe brain hypoxia and metabolic disturbances (Figs. 1*B* and 2*A*).

Concerning the pharmacological interventions that could attenuate the decrease in CBF and its deleterious cerebral consequences, the main findings of this study on the treatment of AS were as follows:

1. In this model of lethal AS, administration of MB alone extended survival time but did not prevent neuronal injury, as demonstrated by a major glutamate release; this is probably the result of poor improvement of hemodynamic parameters and tissue perfusion. Therefore, MB alone cannot be recommended as monotherapy.
2. EPI, the recommended treatment of AS, partially restored systemic hemodynamic variables and cerebral perfusion, thus preventing glutamate-induced excitotoxicity.
3. The MB–EPI association, just like EPI alone, avoided neuronal excitotoxicity and had an additive effect on hemodynamic variables and for the prevention of brain ischemia and neuronal apoptosis compared with individually administered drugs.
4. Neither treatment significantly restored CO nor prevented muscular compartment ischemia and microvascular leakage.

A key reason for studying the effect of MB in AS is the suggestion that MB might be a potential therapeutic drug in catecholamine-refractory vasoplegia associated with sepsis and the systemic inflammatory reaction syndrome in a variety of clinical contexts including cardiac surgery (23–25).

In addition, several clinical case reports have been published supporting the clinical interest of MB in AS. In most cases, MB was administered either as a bolus or as a continuous infusion in combination with EPI (14). In our study, we used a single 3-mg/kg bolus of MB, similar to the therapeutic doses, which have been reported as safe and efficient in other types of refractory hypotension (26–28). Although doses as high as 5

TABLE 2. Time Course of Interstitial Lactate and Pyruvate Concentrations in the Skeletal Muscle ($n = 6$ rats/group)

Group		Control Group	Methylene Blue Group	Epinephrine Group	Epinephrine Associated With Methylene Blue Groups
Lactate (mM)	T10 to T0	0.37 ± 0.04	0.38 ± 0.12	0.69 ± 0.14	1.13 ± 0.17[a,b]
	T0 to T10	0.42 ± 0.03	0.47 ± 0.13	0.63 ± 0.12	0.98 ± 0.16[a,c]
	T10 to T20	0.48 ± 0.05	0.79 ± 0.13	0.92 ± 0.13	1.63 ± 0.22[a,b,c]
	T20 to T30	0.43 ± 0.05	1.20 ± 0.18[c]	1.64 ± 0.22[c]	2.13 ± 0.22[a,c]
	T30 to T40	0.47 ± 0.04	1.72 ± 0.29[c]	2.59 ± 0.41[c]	2.29 ± 0.25[c]
	T40 to T50	0.46 ± 0.04	1.91 ± 0.31[c]	2.35 ± 0.32[c]	2.26 ± 0.25[c]
	T50 to T60	0.48 ± 0.04	2.46 ± 0.53[c]	2.70 ± 0.39[c]	2.35 ± 0.56[c]
Pyruvate (mM)	T10 to T0	0.028 ± 0.003	0.047 ± 0.011	0.056 ± 0.014	0.078 ± 0.012
	T0 to T10	0.036 ± 0.004	0.052 ± 0.013	0.055 ± 0.011	0.070 ± 0.013[c]
	T10 to T20	0.036 ± 0.010	0.049 ± 0.011	0.056 ± 0.011	0.073 ± 0.010[c]
	T20 to T30	0.037 ± 0.006	0.043 ± 0.012	0.056 ± 0.007	0.075 ± 0.012[c]
	T30 to T40	0.043 ± 0.006	0.057 ± 0.021	0.053 ± 0.007	0.068 ± 0.010
	T40 to T50	0.045 ± 0.005	0.043 ± 0.012	0.051 ± 0.008	0.057 ± 0.009
	T50 to T60	0.044 ± 0.004	0.033 ± 0.005	0.041 ± 0.010	0.055 ± 0.007[a]

Time 0 (T0) corresponds to injection of ovalbumin.

[a]$p \leq 0.05$ vs. methylene blue group.

[b]$p \leq 0.05$ vs. epinephrine group. [c]$p \leq 0.05$ vs. control group.

to 7.5 mg/kg are usually well tolerated (14), the use of doses of ≤4 mg/kg has been recommended (29), the 50% lethal dose in sheep being estimated at 40 mg/kg (30).

In the present investigations, a single bolus of MB significantly enhanced survival time but stabilized hemodynamic variables at low values as compared with baseline. This confirms results published by other groups (17). In our experiments, treatment with MB alone did not prevent cerebral ischemia attested by decreased $cPtiO_2$ values and neuronal injury, demonstrated by the major glutamate release observed in microdialysate samples. Although clinically insufficient, these results clearly support the interest in NO pathway modulation and the use of MB for the treatment of severe AS, as reported by other groups (17, 31). Indeed, several studies conducted in different species have demonstrated that NO is an important mediator in anaphylaxis. However, the beneficial vs. detrimental effects on hemodynamics, organ dysfunction, and survival associated with modulation of the NO pathway remain unclear.

Studies conducted in mice models reported beneficial effects of N^G-nitro-L-arginine-methyl ester administration on both hypotension and survival (14, 16, 32) and demonstrated the role played by endothelial NO synthase in murine anaphylaxis (13). On the contrary, MB only partially prevented PAF-induced shock (13) or failed to prevent hypotension in OVA-induced shock (18), suggesting that effects of NO, independent from soluble guanylyl cyclase activation, might play a role.

However, studies conducted in rabbits reported decreased survival resulting from worsened bronchospasm and more severe cardiac depression (33). Similar results were observed in IgE-mediated anaphylaxis in dogs, in which N-nitro-L-arginine methyl ester, a nonselective inhibitor of NO synthase isoforms, corrected the vasodilatation and hemoconcentration but did not improve cardiac function (34). This was also reported by our group in this model of anaphylaxis in Brown-Norway rats, showing that constitutive NO synthase inhibition combined with histamine and serotonin receptor blockade improved the initial OVA-induced arterial hypotension but not survival (35). In contrast, 7-nitroindazole, a putative selective NO synthase inhibitor, but not L-NAME or aminoguanidine, has been shown to attenuate arterial hypotension during AS in rats (36). Finally, as observed in the present study, improved MAP and prolonged survival have been reported with the use of MB during experimental anaphylaxis in rabbits (17).

Part of the differences observed might be species-specific. It is probable, but this requires demonstration, that the degree of cardiac dysfunction in different models of AS can also play a role. Extrapolating from experimental models of sepsis and from human studies, it is conceivable that in the presence of severe myocardial dysfunction, NO synthase inhibitors may worsen survival by further alteration of right ventricular function through increased right ventricular afterload (37). From a clinical point of view, all these results are not an incentive to the use of MB alone to treat AS mainly because of reduced

TABLE 3. Biochemistry Variables in Control, Methylene Blue, Epinephrine, and Epinephrine Associated With Methylene Blue Groups (n = 12 rats/group)

Group	Time (mins)	Control Group	Methylene Blue Group	Epinephrine Group	Epinephrine Associated With Methylene Blue Groups
pH	T0	7.44 ± 0.02	7.41 ± 0.02	7.42 ± 0.01	7.42 ± 0.01
	T60	7.44 ± 0.02	7.16 ± 0.07[a,b]	7.17 ± 0.04[a,b]	7.09 ± 0.04[a,b]
P_{CO_2} (mm Hg)	T0	27.2 ± 1.7	28.5 ± 1.7	29.9 ± 1.1	30.2 ± 1.2
	T60	29.3 ± 1.4[c]	17.0 ± 2.0[a,b]	27.0 ± 4.0[c]	31.8 ± 4.3[c]
P_{O_2} (mm Hg)	T0	254.5 ± 18.3	283.3 ± 19.4	294.0 ± 12.4	269.3 ± 15.3
	T60	272.2 ± 12.0	289.5 ± 39.2	287.4 ± 28.2	228.8 ± 31.1
H_{CO_3} (mmol/L)	T0	17.8 ± 0.7	18.3 ± 0.7	19.3 ± 0.5	18.9 ± 0.4
	T60	19.4 ± 0.6	7.0 ± 1.7[a,b]	9.1 ± 0.7[a,b]	9.2 ± 1.0[a,b]
Lactate (mmol/L)	T0	2.7 ± 0.2	2.5 ± 0.2	2.6 ± 0.3	2.7 ± 0.2
	T60	3.2 ± 0.3	8.3 ± 0.8[a,b]	7.0 ± 0.5[a,b]	7.6 ± 0.7[a,b]
Hemoglobin (g/dL)	T0	12.3 ± 0.4	12.1 ± 0.3	12.9 ± 0.2	12.5 ± 0.2
	T60	12.0 ± 0.3	14.6 ± 0.4[b]	14.9 ± 0.5[b]	15.2 ± 0.5[b]
Glucose (mmol/L)	T0	13.6 ± 0.4	12.1 ± 1.2	12.5 ± 0.7	14.2 ± 1.1
	T60	10.1 ± 0.5	11.8 ± 1.5	13.8 ± 1.6	15.7 ± 2.0[a]
Sodium (mmol/L)	T0	137.2 ± 1.2	137.8 ± 0.9	136.4 ± 0.5	136.6 ± 0.6
	T60	137.0 ± 0.6	138.9 ± 1.9	137.9 ± 1.0	131.4 ± 1.4
Nitrite (μmol/L)	T60	2.4 ± 0.8	4.1 ± 1.4	4.1 ± 0.9	8.7 ± 1.5[a,c,d]
Nitrate (μmol/L)	T60	28.4 ± 1.7	22.4 ± 2.5[a]	28.0 ± 1.6[c]	31.7 ± 1.7[c]

Time 0 corresponds to the injection of ovalbumin. All biochemistry variables were measured in plasma. Values are presented as mean ± SEM. To avoid massive blood spoiling before injection of ovalbumin, we measured nitrite and nitrate only at the end of the experiment.

[a] $p \leq 0.05$ vs. control group.

[b] $p \leq 0.05$ vs. time 0.

[c] $p \leq 0.05$ vs. methylene blue group.

[d] $p \leq 0.05$ vs. epinephrine group.

effectiveness. Furthermore, direct inhibitors of NO synthase isoforms are not available in clinical practice.

As could be expected, EPI, the recommended treatment of AS, restored partially systemic hemodynamic variables and cerebral perfusion thus preventing cerebral ischemia and glutamate-induced excitotoxicity as demonstrated by our brain microdialysis results. However, EPI failed to normalize cerebral oxygen availability and could not prevent a significant increase in caspase 3 and a trend in increased HIF-1α expression. This increase in HIF-1α expression failed to reach statistically significant values, probably because of the early tissue sampling time in this study (38).

Interestingly, in this model of lethal anaphylaxis, the combination of EPI and MB was significantly more effective than EPI alone. Although this association failed to normalize CO values, it restored MAP, CBF, and brain oxygen availability. It also prevented any increase in microdialysis L/P ratio and glutamate concentrations as well as caspase 3 and HIF-1α expression arguing against the presence of severe ischemic brain injury. Several hypotheses might explain this apparent synergistic effect. Apart from the direct hemodynamic effects of both drugs and the interaction between NO and catecholamines classically described (39–41), other factors directly related to concomitant MB and EPI administration might also be involved.

Indeed, the discordance between the improved survival associated with MB alone and the lack of correction of tissue perfusion and abnormalities can be related to the NO pathway-independent effects of MB. After systemic administration, MB can be rapidly and extensively accumulated in the nervous system (42). It has been used as a neuroprotective agent in drug-induced encephalopathy, dementia, and manic–depressive psychosis (38, 43, 44). Furthermore, MB exhibits promising cardio- and neuroprotective properties in experimental cardiac arrest and is effective in both attenuating ischemia–reperfusion syndrome and increasing short-term survival after resuscitation (45, 46). MB exerts neuroprotection by regulation of the expression of soluble guanylyl cyclase and diverse biological processes ranging

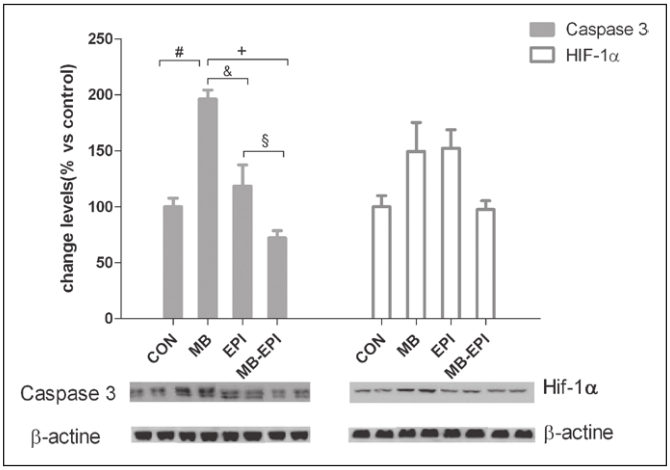

Figure 4. Expression of activated caspase 3 (*gray column*) and hypoxia-inducible factor (HIF)-1α (*white column*) in the cerebral cortex in control (CON), methylene blue (MB), epinephrine (EPI), and epinephrine associated with methylene blue (MB–EPI) groups ($n = 6$ rats/group). Because all rats in the ovalbumin group died within 15 mins, tissues were not used. $^{+}p \leq 0.05$ between-group differences MB–EPI group vs. MB group, $^{\&}p \leq 0.05$ between-group differences EPI group vs. MB group, $^{\S}p \leq 0.05$ between-group differences MB–EPI group vs. EPI group, $^{\#}p \leq 0.05$ between-group differences MB group vs. CON group.

from inhibition of apoptosis and reversal of the shutdown of translation to restoration of functional cellular trafficking and activation of brain repair/regeneration genes as well as induction of critical neuroprotective proteins (47).

Another important finding of our study concerns the discordance between correction of arterial hypotension and persistence of a very low CO after treatment of AS. The consequences of this discordance are illustrated by the persistence of muscular and brain hypoxia with its deleterious metabolic consequences (increased lactate/pyruvate ratio and glutamate release). This observation suggests that on partial or complete correction of arterial hypotension, one should anticipate persistence of low CO. Furthermore, in cases of AS refractory to conventional treatment (lack of correction of MAP), a very low CO may continue and place patients at risk for severe brain dysfunction. This could be attributable to severe hypovolemia (demonstrated in our experiments by hemoconcentrations), a plausible cause in patients without prior cardiac disease, or severe cardiac dysfunction. In such cases, therapeutic measures other than EPI should be considered.

Limitations of the Study

In the present study, we did not observe any alterations in the plasma nitrite level after the different treatment regimens. These results are consistent with previous reports. NO production can be detected in tissue using an NO-sensitive electrode in anaphylactic rabbits (33), but not in plasma (17). A lack of increase in plasma nitrate concentrations has also been reported in 48–80 induced AS in pigs (23). Similar results have been obtained in men, in whom increased NO production has been detected in exhaled breath (48), whereas no significant increase in NO levels was detected in plasma (49). Taken together, these results suggest that NO production can be detected in tissue but not in plasma.

Another potential limitation is the absence of a high level of volume expansion, which was limited to the injection of a bolus of 1 mL of saline followed by a continuous infusion at a dose of 10 mL/kg/hr. Further studies are required to investigate. The additional effect of various volume expansion protocols in our model of lethal anaphylaxis remains to be investigated.

Further studies are required to identify the mechanisms underlying the potential beneficial effects of the MB–EPI association. Although addition of MB in cases of AS refractory to conventional treatment cannot be recommended, such an intervention may be considered in difficult cases.

CONCLUSIONS

Our results suggest that association of MB to EPI but not MB alone may have beneficial effects in severe AS through mechanisms that remain to be elucidated. Furthermore, on treatment of AS, the dissociation between corrected MAP and persistently low CO continues to place the subject at risk for systemic and neurologic hypoperfusion because brain ischemia, hypoxia, and metabolic dysfunction are major characteristics of AS.

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