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Mortality following Lower Limb Ischemia-Reperfusion: A Systemic Inflammatory Response?

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Abstract. Restoration of blood flow to an acutely ischemic lower limb may paradoxically result in systemic complications and unexpected mortality. It has been suggested that lower limb ischemia reperfusion alters gut permeability. In this study, using a rat model, we determined the effect of acute lower limb ischemia-reperfusion on mortality rate, bowel morphology, and circulating concentrations of endotoxin and the proinflammatory cytokine interleukin-6. Survival rate was compared in two groups of adult Wistar rats: (1) control group ($n = 10$); and (2) animals subjected to 3 hours of bilateral hind limb ischemia followed by reperfusion ($n = 10$). Both groups were observed under standard conditions for 4 days. In a second experiment three groups of animals were studied: (I) control ($n = 12$); (II) 3 hours of bilateral hind limb ischemia alone ($n = 12$); and (III) 3 hours of bilateral hind limb ischemia followed by 2 hours of reperfusion ($n = 12$). Animals subjected to bilateral hind limb ischemia followed by reperfusion had a significantly higher mortality rate (70%) than controls (0%) ($p < 0.005$). Morphometric assessment of the small bowel showed a significant decrease in mean mucosal thickness in the ischemia-reperfusion group compared with that in the group of controls and the ischemia-alone group ($p < 0.05$). Bilateral hind limb ischemia followed by reperfusion was associated with significantly increased plasma concentrations of endotoxin ($p < 0.05$) and interleukin-6 ($p < 0.0001$) compared with that of controls and ischemia alone. These results indicate that reperfusion of the acutely ischemic lower limb is accompanied by structural changes in the gut mucosa associated with increased systemic endotoxin concentrations and cytokine activation. Mortality following reperfusion of the acutely ischemic limb may be related to a systemic inflammatory response triggered by endotoxin of gut origin.

Acute ischemia of the lower limb is a common clinical event that may be caused by embolism, acute atherosclerotic thrombosis, traumatic or iatrogenic arterial injury, or arterial clamping during arterial reconstructive surgery such as abdominal aortic aneurysm repair. Reperfusion of the acutely ischemic limb may paradoxically lead to systemic complications such as adult respiratory distress syndrome, renal dysfunction, hepatic dysfunction, or occasionally the full-blown scenario of multiple organ dysfunction syndrome [1–3].

Despite recent advances in surgical technique, anesthesia, and intensive care, elective aortic aneurysm repair is associated with significant mortality (3–5%), and multiple organ dysfunction accounts for 20% of all deaths [3]. Clinical evidence has accumulated demonstrating that many of the patients with multiple organ dysfunction show no evidence of infection, and that many of those with severe infection do not develop multiple organ dysfunction, the inference being that bacterial infection is not necessarily an essential element in the pathogenesis of multiple organ dysfunction [4–6].

Alternatively, it has now become widely accepted that the multiple organ dysfunction syndrome (MODS) following surgery and trauma is a manifestation of a systemic reaction to the original insult and tissue injury. This reaction, the systemic inflammatory response syndrome (SIRS), involves activation of cellular and humoral host responses with the production of an array of inflammatory mediators [7] that eventually precipitate the clinical picture of MODS.

It has been reported that lower limb ischemia-reperfusion causes disruption of gut mucosal tight junctions and an elevation in systemic endotoxin concentrations in rats, suggesting that endotoxemia is caused by a "leaky" bowel mucosa [8]. Endotoxin has a wide range of toxic biologic actions it exerts through the activation of various pathways (e.g., complement pathways [9, 10], coagulation cascade [11, 12], neutrophil activation [13]) and the production of proinflammatory cytokines [e.g., tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, and IL-8] [14, 15]. IL-6 is an important inflammatory mediator produced by a variety of cells including macrophages, B cells, T cells, and endothelial cells [16, 17]. It induces the secretion of acute-phase proteins by hepatocytes, and its concentration has been shown to increase during inflammation, sepsis [18], and trauma [19, 20].

The possible contribution of a systemic inflammatory response to the morbidity and mortality observed after lower limb ischemia-reperfusion has not been elucidated. The aim of this study was to determine the effect of lower limb ischemia-reperfusion on mortality rate, intestinal morphology, systemic endotoxin concen-

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tration, and plasma concentration of the proinflammatory cytokine IL-6 in a rat model.

Materials and Methods

Animal Model

Adult male Wistar rats weighing 300 to 350 g obtained from our breeding colony were used in the study. Animals were kept under standard conditions and had free access to standard rat chow (R. Morton, Ballymena, UK) and tap water until the morning of the experiment. The animals were anesthetized with subcutaneous ketamine (80 mg/kg) and xylazine (8 mg/kg). Hydration of the animals was maintained by injection of sterile 0.9% Na Cl (Antigen Pharmaceuticals, Roscrea, Ireland) 3 ml/kg/hr subcutaneously. Using a heating lamp, body temperature was kept at 37°C. Bilateral hind limb ischemia was induced by applying rubber band tourniquets high around each thigh as originally described by Rothenthal [21]. At the end of the ischemia period reperfusion of the limbs was achieved by releasing the tourniquets. All procedures involving animals were carried out in accordance with the regulations of the United Kingdom Animals (Scientific Procedures) Act, 1986.

Experiment 1: Survival Study

In the first experiment survival rate was compared in two groups of animals: (1) a control group ($n = 10$) and (2) an ischemia-reperfusion group ($n = 10$). The control group had 3 hours of general anesthesia only, whereas the reperfusion group had 3 hours of bilateral hind limb ischemia (under general anesthesia) followed by reperfusion. Animals in both groups were allowed to recover from the anesthetic and kept under standard conditions with free access to food and water. Survival times of these animals were noted over a period of 4 days.

Experiment 2: Effect of Ischemia and Reperfusion on Gut Mucosa and Plasma Endotoxin and IL-6 Concentrations

In the second experiment three groups of animals (12 in each group) were studied: (I) a control group that had 6 hours of general anesthesia only; (II) an ischemic group that had 3 hours of bilateral hind limb ischemia under general anesthesia; and (III) an ischemia-reperfusion group that had 3 hours of bilateral hind limb ischemia (under general anesthesia) followed by 2 hours of reperfusion. Blood samples were obtained at various time points—at the end of general anesthesia in group I, at the end of ischemia in group II, and at the end of reperfusion in group III—for measurement of systemic endotoxin and IL-6 concentrations. Tissue samples were obtained from the small bowel for morphometric assessment of the bowel mucosa.

Blood and Tissue Sampling. At the time of blood sampling the chest wall was painted with chlorhexidine in spirit, a 1 cm disc of skin was excised, and a sterile butterfly needle connected to a sterile 10 ml syringe was used to obtain a blood sample by direct cardiac puncture. Blood samples were collected into heparinized (20 unit heparin/ml blood) sterile pyrogen-free tubes. Samples were transferred on ice to be centrifuged at 2000 rpm (at 4°C) for 10 minutes. Plasma was then aliquoted into sterile cryotubes (Nunc; Intermed, Roskilde, Denmark) and stored at −70°C until the time of assay for endotoxin and IL-6. In each animal, immediately after blood sampling, a midline laparotomy was performed and a 5 cm long intestinal segment was obtained from the terminal ileum. It was opened lengthwise with scissors, washed with 0.9% sodium chloride solution, and immediately fixed in 10% formalin.

Morphometric Assessment of Intestinal Mucosa. A morphometric method was employed to assess the intestinal mucosa. Histologic evaluation was performed independently by a pathologist who had no knowledge of the experimental group from which specific samples were taken. Tissue samples were fixed for at least 48 hours in a formaldehyde fixative. Subsequently the tissue was embedded in paraffin wax and 5-μm sections were cut and stained with hematoxylin and eosin. The sections were carefully examined microscopically, and well orientated areas showing good preservation of structural and cytological details were selected for further study. Using Kontran Image Analysis System, morphometric assessment of the small bowel mucosa was carried out by measuring the total mucosal thickness. Twenty measurements were made for each rat and the average calculated.

Endotoxin Assay. Bacterial endotoxin was quantified using an endpoint chromogenic Limulus Amoebocyte Lysate (LAL) test (Quadratech, Epson, UK). Samples were diluted (1:10) with sterile pyrogen-free water and heat-treated (75°C for 10 minutes). A standard curve was generated in 10% normal rat plasma using *Escherichia coli* endotoxin (0111:B4) supplied with the kit and similarly treated. Aliquots of 25 μl of each standard and sample were dispensed in duplicate into a 96-well microtiter plate and warmed to 37°C (5 minutes). LAL 25 μl reconstituted in sterile pyrogen-free water was added to each well and incubated (37°C for 30 minutes). A chromogenic substrate/buffer mixture 50 μl was added to each well and the reaction stopped 7 minutes later with 50 μl 20% acetic acid. The amount of yellow product formed is directly proportional to the amount of endotoxin in the sample and is determined photometrically at 405 nm. The assay had a sensitivity of 8.3 pg/ml and range of 8.3 to 100.0 pg/ml.

Interleukin-6 Assay. Biologically active IL-6 was measured using a bioassay based on the proliferation of IL-6-dependent B9 hybridoma cells (a generous gift of L. Aarden, Amsterdam). Samples were serially diluted with IL-6-free B9 cell growth medium and dispensed in duplicate into 96-well microtiter plates. Similarly, a standard curve ranging from 0 to 500 pg/ml was generated using recombinant human IL-6 (British Biotechnology, Cowley, UK) and plated out in duplicate. B9 cells were washed free of IL-6 and resuspended in IL-6-free B9 cell growth medium. Standard cell suspension (2.5×10^4/ml) 100 μl was plated into all wells and incubated at 37°C for 4 days. MTT (3-[4,5-dimethylthiazol-2y1]-2,5 diphenyltetrazolium bromide) in phosphate-buffered saline (0.5 mg/ml) was then added to each well followed 5 hours later by 50 ml sodium dodecyl sulfate (20% in 0.01 M HCl). The plates were incubated for another 24 hours. Absorbance was then read at 570 nm, and the amount of IL-6 in each sample was computed from the standard curve. Interassay and intraassay coefficients of variation were less than 10%. The assay has a sensitivity of 30 to 44 pg/ml.

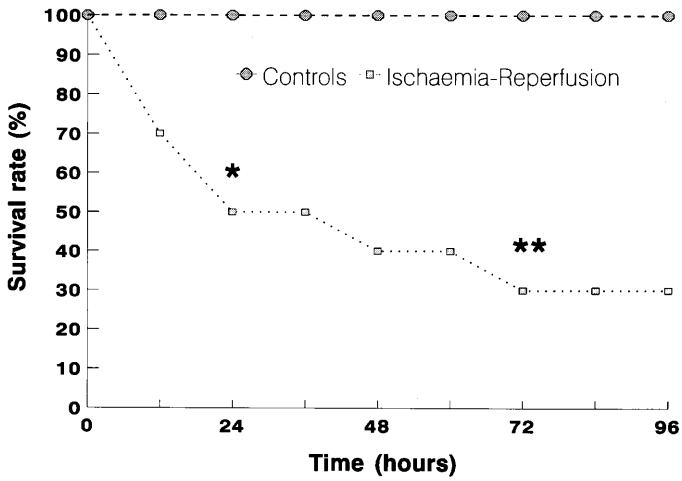

Fig. 1. Comparison of survival rates between the control animals (3 hours of general anesthesia only; $n = 10$) and rats subjected to 3 hours of bilateral hind limb ischemia followed by reperfusion ($n = 10$) observed over a period of 96 hours. $^{*}p < 0.05$, ischemia-reperfusion versus control at 24 hours; $^{**}p < 0.005$, ischemia-reperfusion versus control at 72 hours (chi-square test).

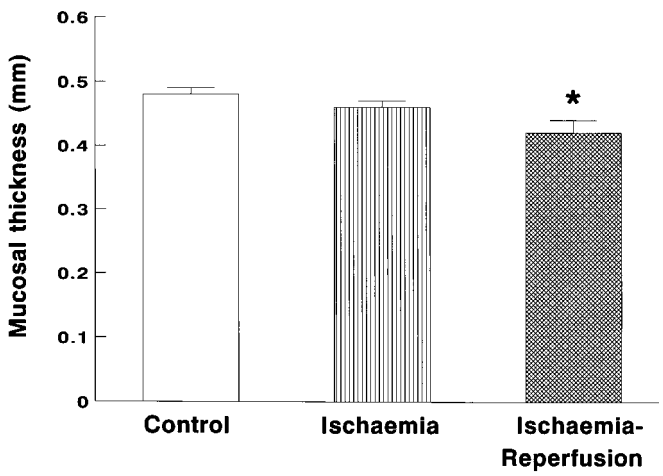

Fig. 2. Mean (± SEM) mucosal thickness in the control animals ($n = 12$), rats after 3 hours of ischemia without reperfusion ($n = 12$), and rats after 3 hours of ischemia plus 2 hours of reperfusion ($n = 12$). $^{*}p < 0.05$ versus control and ischemia alone (Mann-Whitney U test).

Statistical Analysis

Results were expressed as the mean ± the standard error (SEM). A one-way analysis of variance (ANOVA) was used to detect differences between groups, and statistical comparisons were made using the Mann Whitney U test. The chi-square test was used to compare the two groups in the survival study. A p value of 0.05 or less was considered to indicate statistical significance.

Results

Survival Study

Reperfusion after 3 hours of bilateral hind limb ischemia led to 30% mortality within 12 hours. The mortality rate increased significantly (to 50%) within 24 hours after reperfusion ($p < 0.05$), and to 60% within 48 hours. A further significant increase in mortality (to 70%) was observed within 72 hours of reperfusion ($p < 0.005$), and the cumulative mortality rate remained at that level until the end of the study period. By contrast, there was no mortality in the control group during the 4 days of the study (Fig. 1).

Morphometric Assessment of Small Bowel Mucosa

The mean mucosal thickness of the small bowel was 0.48 ± 0.01 mm in the control animals. No significant change in the mean mucosal thickness was detected after 3 hours of bilateral hind limb ischemia alone (0.46 ± 0.01 mm) compared to the controls. However, 3 hours of bilateral hind limb ischemia followed by 2 hours of reperfusion caused a significant decrease in the mean mucosal thickness (0.42 ± 0.01 mm) compared to that in the controls ($p < 0.01$) (Fig. 2).

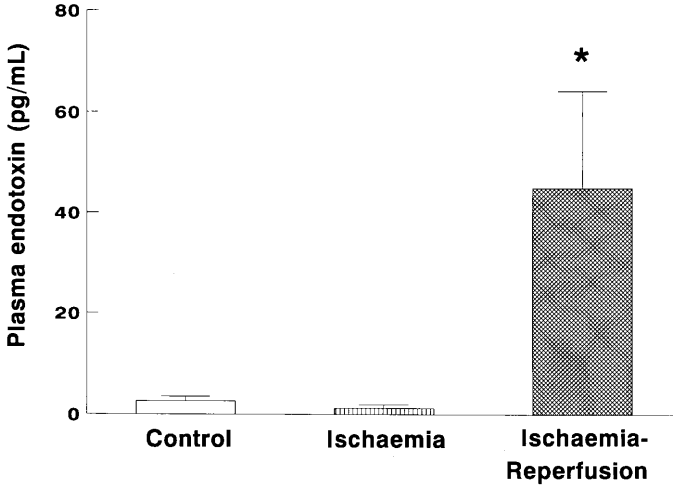

Fig. 3. Mean (± SEM) plasma concentration of endotoxin in control animals ($n = 12$), rats after 3 hours of ischemia without reperfusion ($n = 12$), and rats after 3 hours of ischemia plus 2 hours of reperfusion ($n = 12$). $^{*}p < 0.05$ versus control and ischemia only (Mann-Whitney U test).

Plasma Endotoxin

Significant concentrations of endotoxin were not detected in control animals (2.5 ± 0.9 pg/ml). Three hours of bilateral hind limb ischemia alone did not cause significant alterations in systemic endotoxin concentrations (1.2 ± 0.6 pg/ml) compared to that in controls. Animals subjected to 3 hours of bilateral hind limb ischemia followed by 2 hours of reperfusion had significantly higher endotoxin concentrations (44.8 ± 19.2 pg/ml) than did the control animals or animals subjected to bilateral hind limb ischemia alone ($p < 0.05$) (Fig. 3).

Plasma Interleukin-6

Three hours of bilateral hind limb ischemia alone did not significantly alter plasma concentrations of IL-6 compared with those in

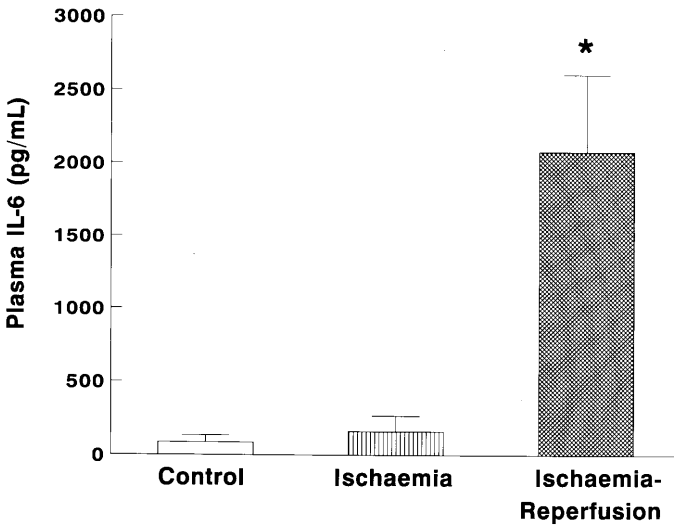

Fig. 4. Mean (± SEM) plasma concentration of interleukin 6 (IL-6) in the control animals ($n = 12$), rats after 3 hours of ischemia without reperfusion ($n = 12$), and rats after 3 hours of ischemia and 2 hours of reperfusion ($n = 12$). $^*p < 0.0001$ versus controls and ischemia only (Mann-Whitney U test).

the controls (140 ± 55 pg/ml versus 104 ± 43 pg/ml, respectively). However, 3 hours of bilateral hind limb ischemia followed by 2 hours of reperfusion increased plasma IL-6 concentrations significantly (1987 ± 489 pg/ml) compared to those in control animals or animals subjected to bilateral hind limb ischemia alone ($p < 0.0001$) (Fig. 4).

Discussion

The reported mortality rate associated with elective aortic aneurysm repair ranges between 1% and 10% with an average incidence in the order of 3% to 5% in most of the recent studies [1]. After surgery for ruptured abdominal aortic aneurysms the death rate is often in excess of 50% [2]. Mortality following peripheral arterial embolectomy averages around 10% [22]. Many of these groups of patients undergoing major arterial surgery die of noncardiovascular systemic complications, such as respiratory, renal, or hepatic failure subsequent to technically successful procedures. In a series reported by Campbell et al. [1], most deaths (71%) in this group of patients were caused by cardiovascular disease. It is noteworthy that MODS accounted for 20% of all deaths. Similarly, in a series of 400 peripheral arterial embolectomies, noncardiovascular systemic complications (e.g., pulmonary edema, hepatic coma, and sepsis) accounted for 18.2% of all deaths [22].

Waydhas et al. [23] studied the relation of MODS to the release of inflammatory mediators in patients with multiple trauma. Significant elevations of inflammatory mediators and other indicators of inflammatory response including polymorphonuclear neutrophil (PMN) elastase, C-reactive protein, antithrombin III, and phospholipase A_2 were observed, which showed a high correlation with patient outcome, namely, the development of MODS and diminished survival. About 63% of patients developed single or multiple organ failure, and 94% of those who developed MODS died. No evidence of infection, however, was detected in 24% of patients who developed MODS; and those who developed bacterial infection or sepsis did so at least 2 days or more after the development of MODS. Systemic endotoxin concentrations, however, were not measured in that study. Similar findings in patients with MODS were reported by other investigators [5, 6].

In our study reperfusion of the acutely ischemic limbs led to cytokine activation and significant mortality. Postmortem histology showed significant interstitial capillary congestion and PMN infiltration suggestive of an early pneumonitis. Examination of the liver revealed nonspecific sinusoidal congestion, and the kidney was histologically unremarkable. In a recent study (manuscript in preparation) reperfusion of the ischemic lower limb in rats was associated with biochemical evidence of hepatic and renal dysfunction.

Morphometric evaluation of intestinal mucosa has been used by several workers as an objective and reliable method for assessing the degree of bowel mucosal injury [24, 25] and has been shown to correlate strongly with histologic mucosal damage [26]. The decrease in the intestinal mucosal thickness and the increase in systemic endotoxin concentration observed in our study suggests that endotoxin permeated an altered gut mucosa and moved into the systemic circulation. Although the gut is the most likely source for the endotoxin, other sources such as the lung are possible. Endotoxin has been shown to alter intestinal permeability, so it may be argued that the changes in the intestine were caused by endotoxin of nongut origin [27]. We have demonstrated an increase in bowel permeability to macromolecules following lower limb ischemia-reperfusion in rats [28], supporting experimental observations by others that reperfusion of the ischemic lower limb caused disruption of mucosal tight junctions and systemic endotoxemia [8]. Another study reported the detection of variable concentrations of endotoxin in the plasma of patients undergoing elective abdominal aortic aneurysm repair [29].

The link between reperfusion of the ischemic extremities and the intestinal changes observed in this study is not clear. Reperfusion of the ischemic lower limb is associated with the generation of oxygen-free radicals, which can cause cell membrane damage and increased permeability [30, 31]. In addition, these toxic oxygen metabolites activate inflammatory cascades, such as the arachidonic acid cascade, with the production of potent vasoactive and chemoattractant mediators (e.g., thromboxane A_2 and leukotriene B_4 [32, 33]). These proinflammatory mediators have been implicated in causing remote lung injury following reperfusion of the acutely ischemic lower limb [30]. It has been shown that the administration of the oxygen-radical scavenger superoxide dismutase decreases the release of inflammatory mediators and attenuates organ failure in multiple-trauma patients [34]. It would be reasonable to speculate that these agents, which reach the systemic circulation after reperfusion, cause intestinal changes directly or indirectly via activation of PMNs or locally resident cells such as macrophages and mast cells [33, 35].

Endotoxin is an important generator of an array of proinflammatory and inflammatory mediators; and by interacting with mononuclear cells it can induce the production of various cytokines such as TNF, IL-1, IL-6, and IL-8. It has been shown that extremely low concentrations of endotoxin can stimulate significant IL-6 release [36]. IL-6 has also been shown to be released directly from the endothelium of injured tissue [37]. The significant elevation of plasma IL-6 concentrations that we observed in this study suggests activation of the cytokine system in response to

reperfusion of the acutely ischemic limb. This activation could be triggered by endotoxin or other mediators generated following reperfusion, such as oxygen-free radicals. Hoch et al. [19] examined the effect of accidental trauma on endotoxin and cytokine production. They reported a rapid and significant increase in circulating concentrations of IL-6 and IL-8, which correlated with the severity of trauma, but they did not detect endotoxin in any of these patients. IL-6 has a wide range of biologic activities, including the induction of acute-phase protein synthesis by hepatocytes and stimulation of immunoglobulin synthesis by B cells [38, 39]. In another study of cytokine production (TNF, IL-1, IL-2, IL-6) in patients with multiple trauma only IL-6 concentrations were elevated at admission, and significant correlation with injury severity score (ISS) was observed [40]. Moreover, when multiple organ dysfunction developed, none of the patients with IL-6 concentrations greater than 400 pg/ml survived.

In conclusion, reperfusion of an acutely ischemic limb represents a form of acute trauma predisposing to significant morbidity and mortality similar to that observed with other forms of trauma. Whether IL-6 production is beneficial during the early mounting of the acute-phase response or causes harm by fueling the systemic inflammatory response remains unclear.

Résumé

Restaurer la circulation sanguine dans un membre inférieur peut, paradoxalement, provoquer des troubles systémiques et être, contre toute attente, à l'origine d'une certaine mortalité. On a suggéré que la séquence ischémie / reperfusion d'un membre inférieur pouvait être responsable d'une altération de la perméabilité de la muqueuse intestinale. Dans cette étude, en utilisant le rat comme modèle, nous avons déterminé l'effet de l'ischémie et de la reperfusion du membre inférieur sur le taux de mortalité, la morphologie intestinale, ainsi que les taux circulants d'endotoxines et des cytokines pro-inflammatoires comme l'interleukine-6. Nous avons comparé la survie de deux groupes de rats adultes Wistar, un groupe de contrôle ($n = 10$) et un groupe d'animaux soumis à 3 heures d'ischémie suivie de reperfusion ($n = 10$). Les deux groupes ont été observés dans les conditions standards pendant une période de quatre jours. Dans une deuxième expérience, trois groupes d'animaux ont été étudiés, un groupe I dit de contrôle ($n = 12$), un groupe II ayant eu trois heures d'ischémie d'un membre inférieur ($n = 12$) et un groupe III ayant subi trois heures d'ischémie suivies de deux heures de reperfusion ($n = 12$). La mortalité des animaux ayant eu une ischémie suivie de reperfusion était significativement plus élevée (70%) comparée à celle des contrôles (0%)($p < 0.005$). Du point de vue morphologique de l'intestin grêle on a démontré une diminution significative de l'épaisseur moyenne de la muqueuse dans le groupe «ischémie/reperfusion» par rapport au groupe «contrôle» et au groupe «ischémie seule» ($p < 0.05$). L'ischémie bilatérale des membres inférieurs suivie de reperfusion était associée à une augmentation significative des concentrations plasmatiques d'endotoxines ($p < 0.05$) et d'interleukine-6 ($p < 0.0001$) comparées à celles du groupe «contrôle« et du groupe «ischémie seule». Ces résultats indiquent que la reperfusion du membre ischémique est accompagnée de changements structuraux de la muqueuse intestinale avec augmentation des concentrations systémiques d'endotoxines et de l'activation des cytokines. La mortalité suivant la reperfusion d'un membre ischémique peut être en rapport avec une réponse inflammatoire systémique déclenchée par les endotoxines d'origine intestinale.

Resumen

La restauración del flujo circulatorio en casos de isquemia aguda de la extremidad inferior, paradójicamente puede resultar en complicaciones sistémicas y mortalidad inesperada. Se ha sugerido que la reperfusión de la extremidad inferior altera la permeabilidad de la pared intestinal. En el presente estudio en ratas determinamos el efecto de la reperfusión de la extremidad con isquemia aguda sobre la tasa de mortalidad, la morfología intestinal, las concentraciones de endotoxina circulante y la citocina proinflamatoria interleucina-6. La tasa de sobrevida fue comparada en dos grupos de ratas Wistar adultos: (a) grupo control (n3D10) y (b) animales sometidos a 3 horas de isquemia bilateral seguida de reperfusión de las extremidades posteriores (n3D10). Ambos grupos fueron observados bajo condiciones estándar por un período de 4 días. En un segundo experimento se estudiaron tres grupos de animales: (I) control (n3D12), (II) 3 horas de sólo isquemia bilateral de las extremidades posteriores (n3D12) y (III) 3 horas de isquemia bilateral seguida de 2 horas de reperfusión (n3D12). Los animales sometidos a isquemia bilateral seguida de reperfusión exhibieron una mortalidad significativamente mayor (70%) frente a los controles (0%) ($P < 0.005$). La valoración morfométrica del intestino delgado demostró disminución significativa del espesor de la mucosa en el grupo con isquemia-reperfusión frente al grupo control y al grupo con isquemia sólamente ($P < 0.05$). La isquemia bilateral de las extremidades seguida de reperfusión se correlacionó con concentraciones plasmáticas de endotoxina significativamente más altas ($P < 0.05$) y de interleucina-6 ($P < 0.0001$) en comparación con los controles y con la isquemia sola. Tales resultados indican que la reperfusión de una extremidad con isquemia aguda se acompaña de cambios estructurales en la mucosa intestinal, asociados con aumento de las concentraciones sistémicas de endotoxina y con activación citocńica. La mortalidad subsiguiente a la reperfusión de la extremidad con isquemia aguda puede estar relacionada con una respuesta inflamatoria sistémica generada por endotoxina de origen intestinal.

Acknowledgments

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Invited Commentary

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Yassin et al. have used a rat model of lower limb ischemia to investigate the systemic effects of skeletal muscle ischemia-reperfusion. Specifically, they postulate that mortality following acute lower extremity ischemia-reperfusion injury might be related to a systemic inflammatory response triggered by endotoxin of gut origin. To test this hypothesis the authors used a standard model of lower extremity ischemia-reperfusion injury and measured circulating levels of endotoxin and interlukin-6 (IL-6) and performed morphometric analyses of microscopic sections of small bowel. The authors found that bilateral hind limb ischemia-reperfusion resulted in a 70% mortality rate at 72 hours, and that plasma concentrations of endotoxin and IL-6 increased significantly after 3 hours of ischemia followed by 2 hours of reperfusion. Postmortem histology demonstrated interstitial capillary

congestion and leukocyte infiltration in the lungs and a decrease in intestinal mucosal thickness. The authors' conclusions are conservative and appropriate for their observations, and their methods are reasonable although somewhat superficial.

Yassin and associates have provided us with an additional and important line of evidence concerning the systemic inflammatory response to skeletal muscle ischemia-reperfersion injury. Although this study has raised more questions than it has provided answers, it nonetheless has offered us several observations suggesting that increased intestinal permeability and gut mucosal damage might be related to the systemic inflammatory response of ischemia-reperfusion injury. The most important question that remains unanswered is whether the increased gut mucosal permeability is a cause or an effect of the systemic inflammatory response to skeletal muscle ischemia-reperfusion injury. To answer this question, detailed time sequence studies would have to be carried out at physiologic and molecular levels and would require extremely large numbers of animals.

The authors' observations are in keeping with a number of other recent publications that have suggested ischemia-reperfusion injury of skeletal muscle causes a systemic inflammatory response. The latter might be related to tumor necrosis factor [1], oxygen-derived free radicals [2], neutrophils and products of lipid peroxidation [3], and a variety of other mediators [4]. I hope the authors can extend their work and demonstrate some ways of diminishing the observed mortality rate following skeletal muscle ischemia-reperfusion injury by blocking some of the putative inflammatory mediators.

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