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Lower Limb Ischemia-Reperfusion Injury Triggers a Systemic Inflammatory Response and Multiple Organ Dysfunction

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Abstract. Restoration of blood flow to an acutely ischemic lower limb may, paradoxically, result in systemic complications and unexpected mortality. We investigated the effect of acute ischemia-perfusion of the lower limb on cytokine production and end organ function. Plasma concentrations of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) were determined in five groups of male Wistar rats: control, 3 hours of bilateral hind limb ischemia alone, and 3 hours of bilateral hind limb ischemia followed by 1 hour, 2 hours, or 3 hours of reperfusion, respectively. In a second experiment, the effect of lower limb ischemia-reperfusion on remote organs (lung, liver, and kidney) was assessed biochemically and histologically. There was a significant increase in plasma concentrations of TNF-α in plasma of animals subjected to 3 hours of bilateral hind limb ischemia followed by 1 hour of reperfusion, 40.1 ± 4.4 pg/ml, when compared with controls, 22.6 ± 4.4 pg/ml, or animals in the ischemia-alone group, 16.3 ± 5.2 ($p < 0.05$). Plasma concentration of IL-6 increased progressively and significantly in animals subjected to bilateral hind limb ischemia followed by 1 hour of reperfusion, 720 ± 107 pg/ml; 2 hours of reperfusion, 1987 ± 489 pg/ml; or 3 hours of reperfusion, 6284 ± 1244 ($p < 0.0001$), compared with controls, 104 ± 43 pg/ml; or animals in the ischemia-alone group, 140 ± 55 pg/ml. In the study comparing portal and systemic concentrations of IL-6, systemic concentrations of IL-6, 967 ± 184 pg/ml were significantly higher than those in the portal circulation 577 ± 127 pg/ml ($p < 0.05$). There was a significant increase in plasma concentrations of urea, creatinine, aspartate transaminase, alanine transaminase, and lactic dehydrogenase in reperfused animals compared with controls ($p < 0.001$). Morbidity and mortality following reperfusion of the acutely ischemic limb may be a manifestation of multiple organ dysfunction caused by a systemic inflammatory response triggered by reperfusion of the ischemic extremities.

Reperfusion of the acutely ischemic limb may, paradoxically, lead to systemic complications that account for significant morbidity and mortality [1–5]. We have previously reported that lower limb ischemia-reperfusion injury alters intestinal structure and permeability [6]. In a subsequent study using the same model, we found that reperfusion of the acutely ischemic limb is associated with endotoxemia in the absence of bacterial translocation [7]. On reaching the circulation, endotoxin can initiate a cascade of events that may lead to sepsis syndrome and in some cases septic shock and multiple organ dysfunction syndrome (MODS) [5–8]. Endotoxin stimulates cellular targets such as monocytes, macrophages, neutrophils, and endothelial cells to produce a variety of inflammatory mediators [9]. Although inflammation is an important immune response vital to the host survival following various forms of injury, uncontrolled production and release of cytokines and other pro-inflammatory mediators following trauma and major surgery may lead to systemic inflammatory response syndrome (SIRS) and MODS [10]. Cytokines can modulate cell function both in the cell of origin (autocrine effect) and neighboring cells (paracrine effect) [11]. In addition, cytokines can circulate to affect different parts of the body in an endocrine fashion. Cytokines can promote neutrophil adherence, by stimulating both endothelial cells and neutrophils, and mediate acute vascular injury and increased vascular permeability [12, 13]. Of particular interest are the cytokines tumor necrosis factor (TNF-α) and interleukin-1-beta (IL-1β), which are believed to be the primary mediators of SIRS [14–16], and interleukin-6 (IL-6), which is a secondary mediator responsible for the acute phase protein response [17–19].

The purpose of this study was to determine whether lower limb ischemia-reperfusion is associated with a systemic inflammatory response and, secondly, to determine the effect of acute lower limb ischemia-reperfusion on remote organs (lung, liver, and kidney) structure and function 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 and Co. Ltd., Ballymena, UK) and water *ad libitum* 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% sodium chloride solution (Antigen Pharmaceuticals Ltd.,

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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 previously described by our group [20]. At the end of the ischemic 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: Cytokine Production

In this experiment, the production of the cytokines IL-6 and TNF were studied in five groups of animals ($n = 12$ in each group): group I, a control group which had 6 hours of general anesthesia only; group II, an ischemic group which had 3 hours of bilateral hind limb ischemia under general anesthesia; groups III, IV and V, ischemia-reperfusion groups which had 3 hours of bilateral hind limb ischemia followed by reperfusion for 1 hour, 2 hours, or 3 hours, respectively.

Blood Sampling

Blood samples were obtained at various time points: at the end of general anaesthesia in group I; at the end of ischemia in group II; and at the end of the specific reperfusion period in each of the reperfusion groups III, IV, and V, respectively. At the time of blood sampling, the chest wall was cleansed with chlorohexidine in spirit, a 1 cm disc of skin excised, and a sterile butterfly needle connected to a sterile 10 ml syringe was then used to obtain a blood sample by direct cardiac puncture. Blood samples for cytokine assay were collected into heparinized (20 unit/ml blood) sterile, pyrogen-free tubes and immediately transferred on ice to be centrifuged at 2000 rpm (at 4°C) for 10 minutes. Plasma was then aliquoted into sterile cryotubes (Nunc; Intermed, Roskidle, Denmark) and stored at −70°C until the time of assay for TNF-α and IL-6. Blood samples for biochemical analyses were collected in sterile tubes and left to clot for 2 to 3 hours. Samples were then centrifuged at 900 × g (at 4°C) for 6 minutes and the separated serum was analyzed for the following parameters: urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), and lactic dehydrogenase (LDH).

TNF-α Assay

Maxisorb 96 well immunoassay plates were coated with TN3 19.12 monoclonal antibody (3.5 mg/ml) in 0.05 M carbonate buffer, pH 9.6, and incubated overnight at 4°C. Before the addition of standard or sample, the plate was blocked with 1% bovine serum albumin (BSA) in phosphate buffer solution (PBS) for 30 minutes at room temperature and washed once with Milli-Q deionized H_2O. A standard curve was prepared by serially diluting rMu TNF in the range 0.039–5.0 ng/ml in pooled normal rat plasma. Fifty microliters of sample diluent (0.5 M phosphate buffer, pH 7.2, containing 1.5 M sodium chloride and 0.5% Tween 20) was added to each well followed by 50 μl of standard or sample (in duplicate). The plate was then incubated while shaking for 4 hours at room temperature after which it was washed once using Milli-Q deionized H_2O. TNF-α was assessed using the P350 rabbit polyclonal anti-murine TNF antibody and donkey anti-rabbit HRP conjugate (Endogen, UK). Before use, these agents were diluted and pre-incubated for 30 minutes at room temperature in PBS containing 1.0% BSA and 5.0% normal donkey serum at 1:100 and 1:1000, respectively. One hundred microliters of polyclonal antibody (P350) was added to each well and allowed to react for 1 hour. The plate was washed once more using Milli-Q deionized H_2O and then 100 μl of horseradish peroxidase (HRP) conjugate added to each well for a further 1 hour incubation. The plate was finally washed three times using Milli-Q deionized H_2O and 100 μl of substrate (O-phenylenediamine/H_2O_2) added to the wells. The reaction was stopped after 30 minutes by adding 50 μl of 2M H_2SO_4 and the plate read at 490 nm using a T-Max plate reader (Alpha Laboratories Ltd, Hampshire, UK). Unknown samples were read directly off the standard curve and results calculated in 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 (donated by University of Amsterdam, Holland). Sample, in serial dilution, were added with IL-6–free B9 cell growth medium to 96 well microtitre plates. A standard curve was generated using recombinant human IL-6 (British Biotechnology, Cowley, UK). B9 cells were washed free of IL-6 and resuspended in IL-6–free B9 cell growth medium. One hundred microliters of standard cell suspension was aliquoted into the wells and incubated at 37°C for 4 days. MTT (3-[4,5-dimethylthiazol-2y1]-2,5 dimethyltetrazolium bromide) in PBS was then added to each well followed 4 hours later by sodium dodecyl sulphate. The plates were further incubated for 24 hours. Absorbance was then read at 570 nm. Interassay and intra-assay coefficients of variation were both less than 10%.

Experiment 2: Portal Versus Systemic Cytokines

To determine whether the gut is the source of pro-inflammatory cytokines, a further experiment was carried out in which we compared plasma concentrations of IL-6 in the portal circulation to those in the systemic circulation. Two groups of six animals each were studied: a control group which had 5 hours of general anesthesia only and an ischemia-reperfusion group which had 3 hours of bilateral hind limb ischemia followed by reperfusion for 2 hours. At the end of the reperfusion period, a midline laparotomy was performed, using aseptic techniques, and portal and systemic blood samples were obtained from the portal vein and inferior vena cava, respectively.

Experiment 3: Assessment of Remote Organs Function

In this experiment five groups of animals (similar to experiment 1) were studied ($n = 5$ in each group) to determine the effect of lower limb ischemia-reperfusion on end organs (lung, liver and kidney) structure and function.

Functional (Biochemical) Assessment

Plasma concentrations of AST, ALT, LDH, urea, and creatinine were measured by means of standard spectrophotometric procedures.

Histological Assessment

In each animal, immediately following blood sampling, a midline laparotomy was performed, and the right kidney and right lobe of the liver were removed and fixed in 10% formalin for histological examination.

Perfusion fixation of the lung. Immediately following harvesting of the kidney and liver, a midline thoracotomy was performed, the trachea was exposed and a fine polythene tube inserted into the trachea through a small tracheostomy incision. A linen ligature was passed and tied around the trachea and the tube. The free end of the tube was inserted into a 20-cm long glass tube filled with formalin and connected to a stand to adjust the perfusion pressure (at 20 cm H_2O). This glass manometer was kept full of formalin by connecting it through a fine polythene tube to a bottle filled with formalin. At the end of the perfusion-fixation period (20 minutes) a linen thread was passed around the hilum of the right lung and tied as the polythene tube was withdrawn from the trachea. The right lung was then fixed in 10% formalin with which it was inflated.

Tissue samples from the different organs were fixed in 10% formalin fixative for at least 48 hours before being processed for histological examination. Three portions from each organ were embedded in paraffin wax (at least 50% of the tissue was embedded in each case) and 5-μm sections were cut and stained with hematoxylin and eosin. The sections were then carefully examined microscopically for evidence of injury.

Statistical Analysis

Results were expressed as the mean (standard error mean). Kruskal-Wallis analysis of variance was used to detect differences between groups and statistical comparisons were made using the Mann-Whitney U test. Any p value of 0.05 or less was considered to indicate statistical significance.

Results

Tumor Necrosis Factor-α

There was a significant increase in plasma concentrations of TNF-α in plasma of animals subjected to 3 hours of bilateral hind limb ischemia followed by 1 hour of reperfusion, 40.1 ± 4.4 pg/ml, when compared with controls, 22.6 ± 4.4 pg/ml, or animals in the ischemia alone group, 16.3 ± 5.2 ($p < 0.05$). By 2 hours following reperfusion, plasma TNF-α levels returned to control levels (Fig. 1).

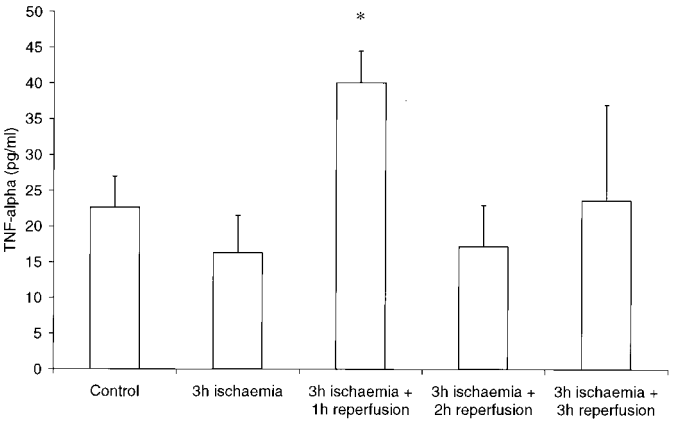


Fig. 1. Mean (± SEM) plasma concentration of tumor necrosis factor-alpha (TNF-α) in control animals ($n = 12$), rats after 3 hours of ischemia without reperfusion ($n = 12$), and rats after 3 hours of ischemia with 1 hour ($n = 12$), 2 hours ($n = 12$), and 3 hours ($n = 12$) of reperfusion, respectively. $^*p < 0.05$ versus control or ischemia alone (Mann-Whitney U test).

Interleukin-6

Plasma concentration of IL-6 increased progressively and significantly in animals subjected to bilateral hind limb ischemia followed by 1 hour of reperfusion, 720 ± 107 pg/ml; 2 hours of reperfusion, 1987 ± 489 pg/ml; or 3 hours of reperfusion, 6284 ± 1244 ($p < 0.0001$), compared with controls, 104 ± 43 pg/ml, or animals in the ischemia-alone group, 140 ± 55 pg/ml (Fig. 2). In the study comparing portal and systemic concentrations of IL-6, systemic concentrations of IL-6, 967 ± 184 pg/ml, were significantly higher than those in the portal circulation, 577 ± 127 pg/ml ($p < 0.05$) (Fig. 3).

Biochemical Assessment of Hepatic and Renal Function

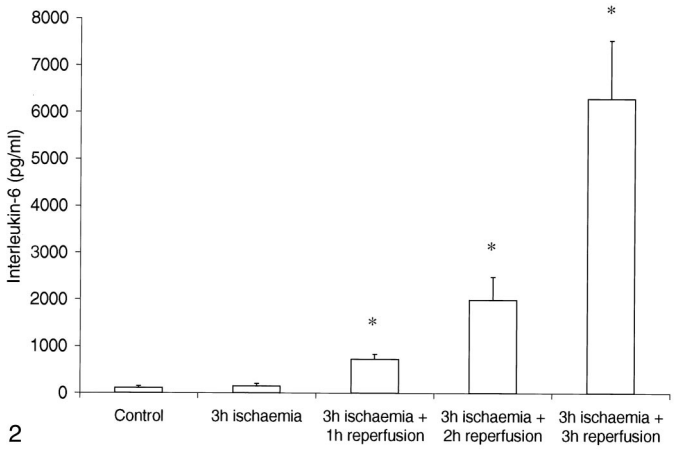
Biochemical parameters of hepatic and renal function in animals subjected to 3 hours of bilateral hind limb ischemia-alone did not differ from those of control animals. A significant increase in serum ALT, AST and LDH was observed in animals subjected to ischemia followed by reperfusion (groups III–V, $p < 0.001$) (Table 1). Serum urea and creatinine concentrations were significantly higher in the reperfusion groups (groups III–V) than in control animals or animals in the ischemia-only group ($p < 0.001$) (Table 2).

Histological Assessment

Light microscopic examination of the liver and kidney was unremarkable. Examination of the lung revealed significant capillary congestion and polymorphonuclear infiltration within the lung tissue only in animals subjected to ischemia followed by 3 hours of reperfusion compared with control animals or animals in the other experimental groups.

Discussion

The findings in this study indicate that reperfusion of the ischemic limb leads, within 1 hour of reperfusion, to a systemic inflammatory response as demonstrated by the increase in plasma TNF-α and IL-6 concentrations. An increase in cytokine production has been observed in patients following major surgery and trauma [19]. While the early increase in circulating cytokines concentrations could be beneficial to the host by initiating the acute phase response, their uncontrolled production may induce a systemic inflammatory response that could inflict fatal damage and contribute to the development of MODS [21, 22]. In support of this concept is the observation that cytokine administration produces many of the pathophysiological features of septic shock [18] and



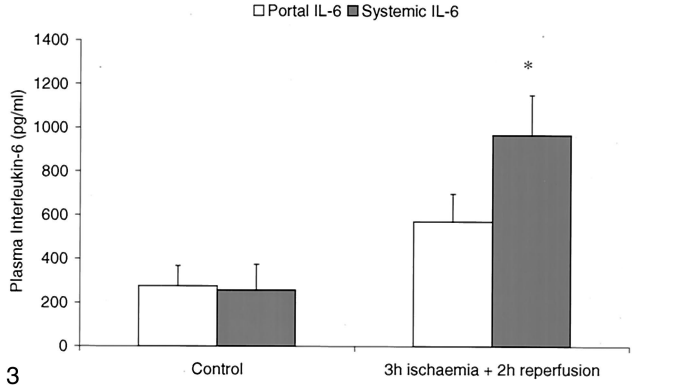


Fig. 2. Mean (± SEM) plasma concentration of interleukin-6 (IL-6) in control animals ($n = 12$), rats after 3 hours of ischemia without reperfusion ($n = 12$), and rats after 3 hours of ischemia with 1 hour ($n = 12$), 2 hours ($n = 12$), and 3 hours ($n = 12$) reperfusion, respectively. $^*p < 0.0001$ versus control or ischemia alone (Mann-Whitney U test).

Fig. 3. Comparison of mean (± SEM) plasma concentration of IL-6 in the portal and systemic circulation in control animals ($n = 6$), and rats after 3 hours of ischemia with 2 hours of reperfusion ($n = 6$), respectively. $^*p < 0.05$ systemic versus portal circulation at 2 hours following reperfusion (Mann-Whitney U test).

Table 1. The effect of ischemia reperfusion on liver function.

	AST (IU/L)	ALT (IU/L)	LDH (IU/L)
Control	150 (15)	55.4 (4.3)	2583 (349)
3 hours of ischemia	316(110)	78.6(14.2)	4129 (524)
3 hours of ischemia + 1 hour of reperfusion	900(179)*	181 (27.2)*	8865(2172)*
3 hours of ischemia + 2 hours of reperfusion	3186(413)*	410 (39.3)*	22,452(3566)*
3 hours of ischemia + 3 hours of reperfusion	2618(133)*	381 (24.3)	21,440(2378)*

Values are mean (SEM).
$^*p < 0.001$ versus control or ischemia alone (Mann-Whitney U test).

specific anti-sera against cytokines exert protective effects against endotoxin injection and sepsis [23, 24]. Furthermore, anti-TNF antibodies have been shown to reduce pulmonary injury following lower limb ischemia-reperfusion [16, 25]. In contrast to IL-6, the increase in plasma TNF concentrations was short-lived and less pronounced. Increased plasma TNF-α, an early pro-inflammatory

Table 2. The effect of ischemia reperfusion on renal function.

	Urea (mmol/L)	Creatinine (mmol/L)
Control	5.38 (0.8)	46.2 (1.9)
3 hours of ischemia	7.0 (0.6)	71.2 (21.1)
3 hours of ischemia + 1 hour of reperfusion	9.0 (0.5)*	210.0 (16.0)*
3 hours of ischemia + 2 hours of reperfusion	11.9 (0.3)*	230.0 (45.0)*
3 hours of ischemia + 3 hours of reperfusion	15.3 (0.6)*	287.0 (33.0)*

Values are mean (SEM).
$^*p < 0.05$ versus control or ischemia alone (Mann-Whitney U test).

cytokine seen in the 1 hour reperfusion group, was not witnessed in the 2- and 3-hour reperfusion groups. TNF-α has previously been shown to appear rapidly after major limb reperfusion injury; and after the early stimulus on reperfusion of the limb [16], TNF-α secretory-cell refractory period and transient immunosuppression [21] may limit further production returning levels to baseline after 2 hours. In the presence of ongoing inflammation, a later TNF-α peak would be expected as when sepsis syndrome is established [24]. Similar findings were observed when studying plasma IL-1β concentrations in patients undergoing abdominal aortic aneurysm surgery [26].

Although from this data it is not possible to confirm the source of the cytokines observed, local production in the limb is the most likely source. It has been suggested that cytokine production in patients undergoing aortic aneurysm surgery is a response to occult ischemic injury of the colon during aortic cross-clamping [27, 28]. Lower limb ischemia reperfusion is associated with systemic endotoxemia, hence a possible second source of cytokines is activation of enterocytes and gastrointestinal macrophages [29, 30]. A recent study using a porcine model of endotoxicosis demonstrated that ischemic gut is not a source of cytokines [31]. However, activation of Kupffer cells in the liver by endotoxin is another possible source of cytokines. In this study ischemia was confined to the lower limb which indicates that lower limb ischemia reperfusion injury per se is the stimulus for cytokine production. Furthermore, the fact that IL-6 concentrations were higher in the systemic circulation than those in the portal circulation excludes the gut as the main source of cytokines. Several studies have suggested that IL-6 is released directly from the endothelium of injured tissue within hours of injury or surgery [19]. It is conceivable that inflammatory mediators produced locally in the limb circulate on reperfusion and mediate the increase in intestinal permeability that leads to endotoxemia. The subsequent activation of Kupffer cells in the liver by endotoxin would explain the higher concentrations of cytokines in the systemic blood than in the portal blood and the development of remote organ dysfunction.

The continuous rise in plasma concentration of IL-6 throughout the period of reperfusion suggests that reperfusion injury, rather than ischemia, is the stimulus for the cytokine production. Further, it implies that IL-6 production is proportional to the extent of reperfusion injury. In support of this concept is the observation that plasma cytokines concentrations were significantly higher in patients following major surgery or severe trauma than those in patients following minor surgery or mild trauma [17, 19].

The development of remote organ dysfunction was observed only following reperfusion, which implies that humoral and/or cellular mediators produced locally in the limb were responsible for mediating remote organ injury. The failure to identify histological evidence of injury in the liver or the kidney may indicate that injury in these organs was not severe enough to cause structural damage. Since functional derangement normally precedes evidence of structural organ injury, it is possible that the experimental period was not long enough to allow for the structural changes to develop. Although histological evidence of injury was demonstrated only in the lung, the biochemical evidence of simultaneous hepatic and renal dysfunction indicates that multiple organ dysfunction following lower limb ischemia reperfusion occurs as a central systemic event rather than sequential failure of individual organs [5, 32, 33].

The findings of hepatic and renal dysfunction in this study are supported by recent reports that patients undergoing abdominal aortic aneurysm surgery have elevated serum levels of transaminases and an increase in urinary protein excretion (microproteinuria) [34–37]. Our findings are in agreement with previous reports that a varying degree of pulmonary dysfunction, characterized by increased microvascular permeability and leukocytic sequestration within the lung and ranging from self-limiting mild pulmonary edema to adult respiratory distress syndrome (ARDS), has been reported within hours of declamping of the aorta in patients undergoing abdominal aortic aneurysm repair [38–40].

It is not clear why the lung is particularly vulnerable to remote ischemia reperfusion compared with other organs. It has been suggested that humoral mediators generated in the reperfused tissue attract and activate leukocytes which then accumulate in the lung and cause pulmonary damage by adhering to vascular endothelium, plugging the capillaries and releasing their lysosomal enzymes [32, 41, 42]. It is possible that inflammatory cells resident locally in the lung (e.g., macrophage and mast cells) amplify the reperfusion injury by producing inflammatory mediators and leukocyte chemoattractant substances. It has been shown that lower limb ischemia reperfusion leads to the production of leukotriene B_4, a potent neutrophil chemoattractant, by pulmonary mast cells [43]. In addition, mast cells can produce other inflammatory mediators such as histamine, serotonin, and platelet activating factor [44, 45]. In this study an increase in neutrophil infiltration within the liver or the kidney was not observed. In a previous study, intestinal mucosal injury, following lower limb ischaemia reperfusion, was demonstrated without evidence of an increase in neutrophil infiltration within the bowel wall [6, 46]. Several studies have demonstrated pulmonary ischemia reperfusion injury in neutrophil-depleted animals [47, 48]. It is conceivable that neutrophil accumulation may represent a response to reperfusion injury rather than be the primary cause of it. Nevertheless, neutrophil accumulation is likely to significantly amplify reperfusion-induced microvascular injury.

In conclusion, the data from this study provide evidence that acute ischemia reperfusion of the lower limb leads to cytokine activation and simultaneous multiple organ dysfunction. The systemic complications observed in patients undergoing abdominal aortic aneurysm surgery may be interrelated to a systemic inflammatory response triggered by reperfusion of the acutely ischemic extremities.

Résumé. La restauration du débit sanguin après ischémie aiguë du membre inférieur peut, paradoxalement, déclencher des complications systémiques et augmenter la mortalité de faon inopinée. Nous avons exploré l'effet de l'ischémie-reperfusion aiguë du membre inférieur sur la production de cytokines et la fonction viscérale. On a déterminé les concentrations plasmatiques des facteurs de nécrose tissulaire-alpha (TNF-α) et l'interleukine-6 (IL-6) dans cinq groupes de rats Wistar mles: groupe contrle, après 3 heures d'ischémie de membre inférieur bilatérale, et trois heures d'ischémie de membre inférieur, suivie, respectivement, de 1 heure, de 2 heures et de trois heures de reperfusion. Dans une deuxième séries d'expérimentations, on a évalué, du point de vue biochimique et histologique, les effets de l'ischémie-reperfusion sur les organes à distance (poumon, foie et rein). Les concentrations plasmatiques de TNF-α chez les animaux soumis à trois heures d'ischémie de membre inférieur, suivies d'une heure de reperfusion (40.1 ± 4.4 pg/ml), étaient significativement plus élevées par rapport aux controles, 22.6 ± 4.4 pg/ml, ou par rapport au groupe d'animaux en ischémie seule, 16.3 ± 5.2 ($p < 0.05$). La concentration plasmatique en IL-6 a augmenté progressivement et significativement chez les animaux soumis à l'ischémie de membre inférieur bilatérale suivie d'une heure de reperfusion, 720 ± 107 pg/ml, de 2 h de reperfusion, 1987 ± 489 pg/ml, ou de 3 h de reperfusion, 6284 ± 1244 pg/ml ($p < 0.0001$), comparée aux contrles, 104 ± 43 pg/ml, ou les animaux dans le groupe d'ischémie seule, 140 ± 55 pg/ml. Dans l'étude comparant les concentrations portales et systémiqes d'IL-6, la concentration systémique d'IL-6, 967 ± 184 pg/ml, était significativement plus élevée que celles du système porte 577 ± 127 pg/ml ($p < 0.05$). La concentration plasmatique en urée, en créatinine, en transaminases aspartiques et en deshydrogénase lactique était augmentée chez les animaux en reperfusion par rapport aux animaux de contrle ($p < 0.001$). La morbidité et la mortalité après reperfusion d'un membre inférieur en ischémie aiguë peuvent tre des manifestations de dysfonctionnement multiviscéral en rapport avec une réponse inflammatoire systémique déclenchée par la reperfusion des extrémités ischémiques.

Resumen. La restauración del flujo sanguíneo a un miembro en estado de isquemia aguda puede, en forma paradójica, resultar en complicaciones sistémicas y mortalidad inesperada. Nos propusimos investigar el efecto de la isquemia-reperfusión aguda sobre la producción de citocinas y sobre la función orgánica. Las concentraciones plasmáticas de factor de necrosis tumoral-alpha (TNF-α) y de Interlucina-6 (IL-6) fueron determinadas en cinco grupos de ratas: control, 3 horas de isquemia bilateral de las extremidades posteriores, y 3 horas de isquemia bilateral de las extremidades inferiores seguida de reperfusión a 1, 2 y 3 horas, respectivamente. En un segundo experimento se efectuó la evaluación bioquímica e histológica de los efectos de la isquemia-reperfusión de las extremidades inferiores sobre órganos remotos (pulmón, hígado y riñón). Se observó un incremento significativo en la concentración de TNF-α en el plasma de los animales sometidos a tres horas de isquemia bilateral de las extremidades posteriores seguida de 1 hora de reperfusión, 40.1 ± 4.4 pg/ml, en comparación con los controles, 22.6 ± 4.4 pg/ml, o con los animales sometidos a isquemia solamente, 16.3 ± 5.2 ($p < 0.05$). La concentración plasmática de IL-6 ascendió en forma progresiva y significativa en los animales sometidos a isquemia bilateral de las extremidades posteriores seguida de 1 hora de reperfusión, 720 ± 107 pg/ml, de 2 horas de reperfusión, 1987 ± 489 pg/ml, o de 3 horas de reperfusión, 6284 ± 1244 ($p < 0.0001$), en comparación con los controles, 104 ± 43 pg/ml, o los animales de isquemia solamente, 140 ± 55 pg/ml. En el estudio para comparar las concentraciones portales y sistémicas de IL-6, la sistémica, 967 ± 184 pg/ml apareció significativamente más alta que la portal, 577 ± 127 pg/ml ($p < 0.05$). Se observó un incremento significativo en las concentraciones plasmáticas de úrea, creatinina, aspartatotransaminasa, alaninotransaminasa y deshidrogenasa láctica en los animales reperfundidos en comparación con los controles ($p < 0.001$). La morbilidad y la mortalidad luego de la reperfusión de un miembro en estado de isquemia aguda pueden ser una manifestación de disfunción orgánica múltiple causada por una respuesta inflamatoria sistémica generada por la reperfusión de extremidades isquémicas.

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