Transpl Int (1994) 7 [Suppl 1]: S139–S143

Jean Gugenheim
Francesco Crafa
Giovanni Dammerota
Alberto Evangelista
Marie-Christine Saint-Paul
Chantal Cavanel
Françoise Lapalus
Jean Mouiel

Role of non-parenchymal liver cells in ischaemia-reperfusion liver injury: protective effects of muramyl dipeptide

J. Gugenheim (✉)
Hôpital Saint-Roch,
Service de Transplantation Hépatique,
5 rue Pierre Dévoluy,
F-06006 Nice, France

F. Crafa · G. Dammerota
A. Evangelista · M.-C. Saint-Paul
C. Cavanel · F. Lapalus · J. Mouiel
Laboratoire de Recherche Chirurgicale,
Universite de Nice-Sophia Antipolis,
Hôpital Saint-Roch,
5 rue Pierre Devoluy,
F-06006 Nice, France

Abstract It has been suggested that non-parenchymal liver cells play a central role after ischaemia and reperfusion of the liver. Male Lewis rats were subjected to 90 min of warm liver ischaemia. Four groups were constituted: group 1, no treatment; group 2, muramyl dipeptide treatment, activation of Kupffer cells; group 3, dextran sulphate injection, Kupffer cell blockade; and group 4, gadolinium chloride administration, Kupffer cell blockade. Dextran sulphate (4 mg/100 g) and gadolinium chloride ($GdCl_2$, 0.7 mg/100 g) were given intravenously on day 2. MDP was injected intravenously (500 mg/250 g) 24 h before and 10 min after the intervention. Mortality rates were assessed and serum transaminases, histology of the liver and Kupffer cell phagocytic activity were evaluated 6 h after the end of ischaemia. MDP treatment significantly ($P < 0.001$) reduced mortality (30%) in comparison with the non-treated group (60%). The mortality rate was significantly higher in the dextran sulphate-treated (80%) and gadolinium chloride-treated (90%) groups in comparison with group 1. A significant reduction in transaminase levels was observed after MDP treatment, while blockade of Kupffer cells resulted in higher serum transaminase levels. The extent of necrosis and congestion was improved by MDP administration, while disruption of the vascular and sinusoidal integrity of the liver and extensive areas of necrosis were observed in dextran sulphate and gadolinium chloride-treated rats. Sheep red blood cell ^{51}Cr liver uptake was deeply depressed 6 h after the end of ischaemia in group 1 (10 ± 1.2 %/g tissue). MDP injection restored the Kupffer cell activity (30.6 ± 3.22 %/g tissue) while dextran sulphate and gadolinium chloride administration markedly decreased SRBC ^{51}Cr liver uptake. Our findings demonstrate that MDP in able to protect the liver from ischaemic insult while blockade of Kupffer cells was deleterious in rats subjected to liver ischaemia.

Key words Normothermic ischemia
Kupffer cell function · Muramyl dipeptide · Gadolinium chloride
Dextran sulphate

Introduction

Organ injury after ischaemia and reperfusion (I/R) remains one of the most important limiting factors in the field of transplantation [1]. After liver I/R, both hepatocytes and non-parenchymal liver cells (NPLC) are injured. Among NPLC, Kupffer cells (KC) may play a major role in the lesions of I/R. Modifications in two of their important functions, phagocytic activity and monokine release, may have important consequences for liver viability. It has been shown in baboons [2] and in dogs [3] that hepatic ischaemia may lead to reticuloendothelial system (RES) phagocytic dysfunction which may contribute to systemic endotoxinaemia and death. We have suggested that PgE_1 may act in ischaemia-induced hepatic injury through their action on hepatic macrophages [4]. Several experimental studies have demonstrated that the immunoadjuvant muramyl dipeptide (MDP) has stimulatory effects on the monocyte/macrophage axis [5–7] while dextran sulphate (DS) and gadolinium chloride (Gd) have a depressing effect on Kupffer cell function [8]. The aim of this study was to assess the role of fixed hepatic macrophages during a 90-min segmental normothermic liver ischaemia in rats.

Animals and methods

Animals

Male Lewis rats (LEW RT1[1]), weighing 250–300 g, were purchased from the CNRS-CNSEAL (Orléans La Source, France).

Experimental protocol

Rats were divided into four experimental groups of 30 animals: group 1, hepatic ischaemia and ringer lactate administration; group 2, hepatic ischaemia and MDP treatment; group 3, hepatic ischaemia and DS administration; group 4, liver ischaemia and Gd administration.

Surgical procedures

A segmental normothermic ischaemia of the liver was induced as follows. A clean but not sterile procedure was used. The abdomen was entered through a midline incision under ether anaesthesia. The liver was dissected from its peritoneal connections and the accessory hepatic arteries were divided if present. The hilum of the median and left lateral lobes was exposed and ischaemia induced by occluding the blood vessels including the bile duct to the lobes with an atraumatic vascular clamp, and then the abdomen was closed. This technique enabled portal stasis, which may lead to fatal haemodynamic instability [9], to be avoided. After 90 min of warm ischaemia the vascular clamp was released and the non-ischaemic (right lateral and caudate) lobes resected, leaving only the ischaemic anterior lobes. In group 1, 1 ml of Ringer lactate was administrated i.v. 2 min before clamping and 2 min before unclamping. The abdominal incision was closed. After surgery the animals were kept in individual cages and allowed food and water ad libitum. Mortality and survival time were recorded every 6 h. Animals alive 7 days after surgery were considered as permanent survivors. Necropsy was performed on all animals to verify the absence of surgery-related complications. Blood samples weere taken 6 h after the end of ischaemia from ten rats to assay the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum levels in each experimental group by routine methods.

Kupffer cell blockade

DS (4 mg/100 g; Sigma and Gd (0.7 mg/100 g; Aldrich), a lanthanoid known to inhibit the attachment phase of KC phagocytosis, were dissolved in saline solution and given intravenously on day 2 to treated rats [8–10].

Activation of Kupffer cells

MDP (*N*-acetylmuramyl-D-alanyl-D-isoglutamine, Sigma) was injected intravenously (500 µg/250 g) 24 h before and 10 min after the intervention.

Assessment of Kupffer cell phagocytic activity

The reticuloendothelial system (RES) phagocytic activity was tested as previously described [11] measuring the hepatic uptake of ^{51}Cr labelled erythrocytes, in five normal rats and in groups 1, 2, 3 and 4 (ten rats for each group). Rats were intravenously injected 6 h after the end of ischaemia with 5×10^8 sheep red blood cells (SBRC) prepared as follows. SRBC (5 ml) were washed twice with Hank's solution using centrifugation at 2500 r.p.m. for 10 min. The globular pellet was resuspended in 5 ml Hank's solution and 200 µCi of ^{51}Cr was added to the suspension. The mixture was incubated at 37 °C for 45 min. The pellet was washed three times with Hank's solution to remove unfixed radioactivity. The pellet was diluted with Hank's solution to obtain a cellular suspension containing 10^9 ^{51}Cr-erythrocytes/ml. Each rat received 500 µl of this suspension. The rats were sacrificed 3 h later and the liver and spleen removed and weighed. Radioactivity of each organ was measured using a gamma counter. Control measurement of the radioactivity injected was made by counting the radioactivity of 500 µl of ^{51}Cr-erythrocytes. Results are expressed as percentage of control for 1 g of the organ.

Histology

Liver tissue was taken 6 h after the end of ischaemia from ten rats from each group. Specimens were fixed in 10% formalin and stained with haematoxylin and eosin for light microscopic examination and were evaluated blindly. Congestion and necrosis were considered as parameters. Histological scoring for each specimen was done according to the following criteria: no evidence (0); low intensity (1), mild intensity (2), high intensity (3).

Statistical analysis

Results were expressed as means ± SEM. Comparisons for statistical significance were performed using Student's *t*-test for ALT and AST serum activities, percentage of ^{51}Cr-labelled SRBC and histological parameters. The generalized Wilcoxon's test was used for comparing survival rates.

Results

Survival time and mortality rate

The mortality rates of the four experimental groups are shown in Fig. 1. MDP treatment led to a significant ($P < 0.001$) decrease in the mortality rate (30%) while DS and Gd administration significantly ($P < 0.001$) increased the mortality rate (80% in group 3, 90% in group 4) in comparison with non-treated animals (60%). Rats still alive 7 days after the end of liver ischaemia were considered as survivors.

Serum biochemistry

In group 1 the mean AST level was 5882 ± 656 UI/l and the mean ALT level 5964 ± 693 UI/l 6 h after the end of the ischaemic period. In group 2 the mean AST level was 1515 ± 298 UI/l and the mean ALT level was 1921 ± 684 UI/l. The differences between group 1 and group 2 for both ALT and AST were significant ($P < 0.005$) (Fig. 2). In groups 3 and 4 the mean AST levels were, respectively, 8963 ± 307 UI/l and 9835 ± 167 UI/l, and the mean ALT levels were 9176 ± 255 UI/l and 12283 ± 1048 UI/l. The differences between these two groups and group 1 were significant ($P < 0.005$).

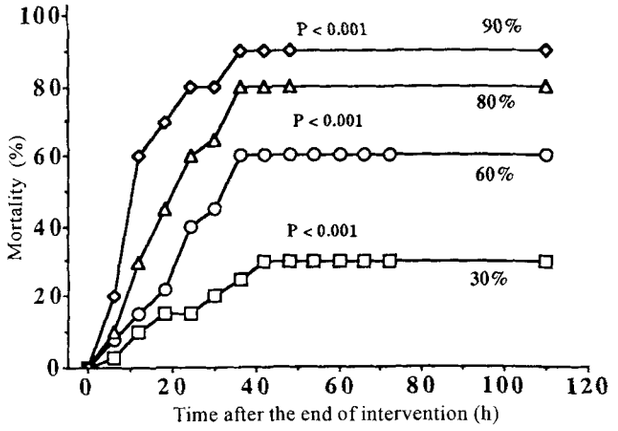

Fig. 1 Cumulative mortality rate after liver ischaemia in rats. Blockade of Kupffer cells (groups 3 and 4) significantly increases the mortality rate while MDP administration (group 2) significantly increases the survival rate (○ group 1, □ group 2, △ group 3, ◇ group 4)

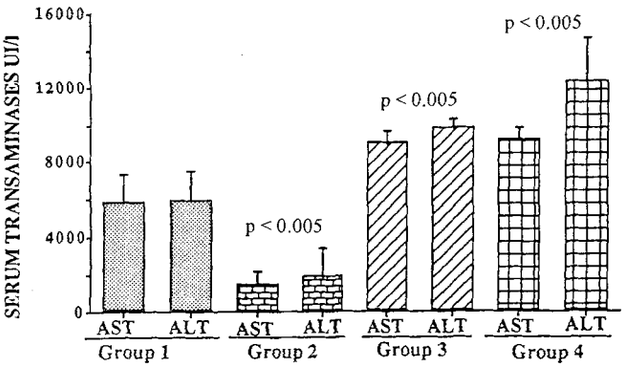

Fig. 2 Changes in serum activities of transaminases (ALT and AST) 6 h after the end of liver ischaemia in non-treated rats (group 1), stimulated Kupffer cell rats (group 2) and previously blockaded Kupffer cell rats (groups 3 and 4)

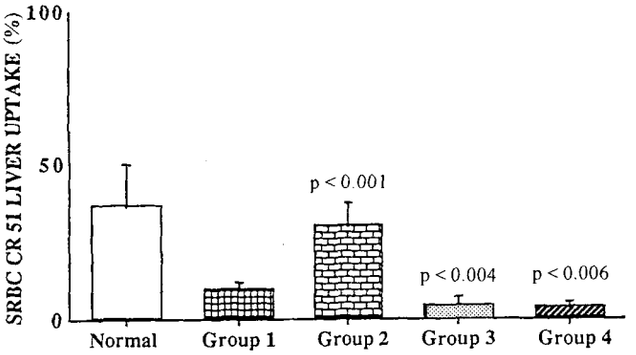

Fig. 3 Percentage of SRBC ^{51}Cr liver uptake 6 h after the end of ischaemia in normal rats (group 1, 90 min liver ischemia), in MDP-treated rats (group 2) and previously blockaded Kupffer cell rats (groups 3 and 4).

^{51}Cr-SRBC uptake (Fig. 3)

^{51}Cr-SRBC uptake decreased significantly 6 h after the end of liver ischaemia: 10 ± 1.2%/g tissue in group 1 versus 40 ± 4%/g tissue in normal rats ($P < 0.001$). In group 2, MDP treatment partially restored KC activity, the ^{51}Cr-SRBC uptake was 30.6 ± 3.22%/g tissue (group 1 vs group 2, $P < 0.0001$; Fig. 3). In groups 3 and 4, DS and Gd administration decreased activity, and the ^{51}Cr-SBRC uptake was respectively 4.6 ± 5.8%/g tissue and 3.6 ± 0.81%/g tissue (group 3 vs group 1, $P < 0.004$; group 4 vs group 1, $P < 0.006$). The splenic uptake of SRBC was 6 ± 0.32%/g tissue in normal rats, 7.2 ± 0.37%/g tissue in group 1, 8.17 ± 0.48%/g tissue in the KC-activated group, and 3.33 ± 0.42%/g tissue and 2.5 ± 0.22%/g tissue in the DS- and Gd-treated groups.

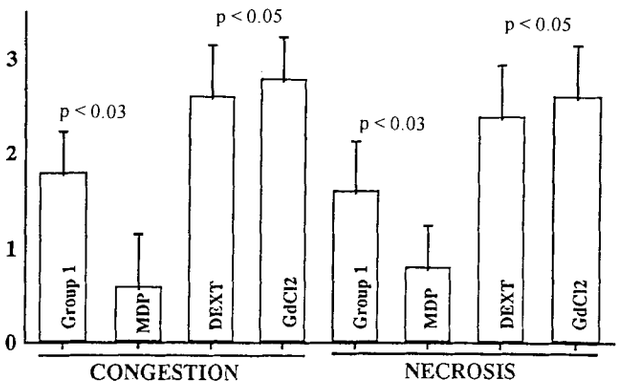

Fig. 4 Light microscopic scoring for congestion and hepatocellular necorosis. 6 hours after the end of the ischemia, congestion and hepatocellular necrosis were significantly lower in MDP-treated rates (group 2) than in non-treated rats (group 1), dextran sulphate-treated rats (group 3) and gadolinium chloride-treated rats (group 4)

Macroscopic appearance and histology (Fig. 4)

Immediately after occlusion of the hepatic vessels the anterior lobes became pale. After releasing the clamp the liver turned dark and quickly recovered its normal colour. In group 1 6 h after the end of ischaemia histological examination showed confluent areas of necrosis (1.6 ± 0.24) and fresh haemorrhages (1.8 ± 0.2). Histological examination of hepatic biopsy specimens showed minimal pathological changes in group 2 (MDP-treated rats) with less congestion (0.6 ± 0.24; group 1 vs group 2, $P < 0.03$) and necrosis (0.8 ± 0.2; group 1 vs group 2, $P < 0.05$). In contrast, DS and Gd administration increased the incidence of congestion (2.6 ± 0.2 and 2.8 ± 0.2; group 1 vs groups 3 and 4, $P < 0.05$) and necrosis (2.4 ± 0.2 and 2.6 ± 0.2; group 1 vs groups 3 and 4, $P < 0.05$).

Discussion

Recent studies have emphasized the role of NPLC in hepatic injury after I/R. The following sequence of events in reperfusion injury has been suggested. On re-entry of blood into the organ, overproduction of toxic oxygen radicals [12] followed by endothelial cell damage [13] and KC activation [14] occurs and causes disturbances in the microcirculation of the liver. Activation of KC after I/R of the liver may have deleterious effects. Activated KC may release a variety of toxic metabolites including oxygen radicals, eicosanoids and various monokines (IL-1, IL-6, TNFα, TNFβ) [15] inducing local injuries to endothelial cells and to hepatocytes [16] with expression of adhesion molecules and subsequent infiltration with the recipient's monocytes and neutrophils. Activation of KC is also associated with a reduced phagocytic activity which may have important consequences. The inability of KC to clear endotoxin from the portal venous blood may lead to systemic endotoxinaemia [17] which has been implicated in the pathogenesis of renal failure, intravascular coagulation and gastric mucosal haemorrhages in patients with hepatic failure [18, 19].

In this experimental study in inbred rats, we demonstrated that treatment with MDP improved the survival rate of animals after normothermic ischaemia of the liver, whereas administration of DS and Gd reduced the survival rate. These effects on survival were well correlated with the extent of hepatic injury and necrosis as demonstrated by the biological and histological findings. Moreover, the marked decrease in RES phagocytic function observed after normothermic ischaemia of the liver was partially corrected by MDP, whereas KC blockade by DS and Gd worsened the decrease in the KC phagocytic capacity after normothermic liver ischaemia.

MDP, which has been demonstrated to have a non-specific immunostimulating effect in various pathological conditions such as septic shock and bacterial challenge [20, 21], is a known activator of macrophages. In our study, MDP restored the phagocytic capacity of KC after I/R of the liver. This may reduce the systemic endotoxinemia observed after liver ischaemia [22], and the subsequent activation of KC with an overwhelming release of biologically active mediators. In contrast, previous blockade of KC (by DS or Gd) increased the mortality rate observed after I/R of the liver. These results are in line with previous studies showing that blockade of KC induces severe liver damage in rats receiving galactosamine [23], and increases mortality in an experimental model of peritonitis [24].

Our results confirm those of previous studies showing a reduced hepatic RES phagocytic activity after liver I/R in monkeys [2], dogs [3] and rats [4]. The precise mechanisms of this reduction are unknown, but reduced hepatic blood flow is probably not responsible [2]. A decrease in serum opsonins (fibronectin, complement) and/or uptake of hepatic cell debris by KC are probably involved. Whatever the mechanism, our study demonstrates that improvement in RES phagocytic activity by MDP is associated with an increased survival, whereas KC blockade increases mortality after I/R of the liver. Control of macrophage activity may offer a new strategy for reducing ischaemic injury to the liver.

References

1. Van Thiel DH, Schade RR, Hakala TR, et al (1984) Liver procurement for orthotopic transplantation, an analysis of the Pittsburgh experience. Hepatology [Suppl] 4:66–71
2. Holper K, Olcay I, Kitahama A, et al (1974) Effect of ischemia on hepatic and reticuloendothelial function in the baboon. Surgery 73:423–432
3. Yuji U, Kenechi M, Takashi K, et al (1989) Protective effect of prostaglandin E1 (PgE1) on energy metabolism and reticuloendothelial function in the ischemically damaged canine liver. Liver 9:6–13
4. Crafa F, Gugenheim J, Saint-Paul MC, et al (1991) Protective effect of prostaglandin E1 on normothermic liver ischemia. Eur Surg Res 23:278–284
5. Ingoldby CJH, Skinner C, Ausobsky JR, Giles GR (1988) The effect of muramyl peptide on endotoxin handling in normal and jaundiced rats. Surg Res Commun 3:15–20
6. Pain JA, Collier DSTJ, Ritson A (1987) Reticuloendothelial system function in obstructive jaundice and its modification by muramyl dipeptide analogue. Eur Surg Res 19:16–22
7. Phillips NC, Rioux J, Tsao MS (1988) Activation of murine Kupffer cell tumoricidal activity by liposomes containing lipophilic muramyl dipeptide. Hepatology 8:1046–1050
8. Gugenheim J, Charpentier B, Gigou M, et al (1988) Delayed rejection of heart allografts after extracorporeal donor-specific liver hemoperfusion. Role of Kupffer cells. Transplantation 45:628–632
9. Van der Meer C, Van der Kley GA, Valkenburg PW (1976) Studies on the cause of death after permanent and temporary occlusion of the portal vein in rats. Circ Shock 3:191–202
10. Husztik E, Lasar G, Parducz A (1980) Electron microscopic study of Kupffer cell phagocytosis blockade induced by gadolinium chloride. Br J Pathol 61:624–630
11. Capron-Laudereau M, Gugenheim J, Gigou M, et al (1987) Decreased reticuloendothelial phagocytic capacity in cirrhotic and portocaval shunt rats. Eur Surg Res 19:388–394
12. McCord JM (1985) Oxygen derived free radicals in post ischemic tissue injury. N Engl J Med 312:159–163
13. Caldwell-Kenkel JC, Carrin RT, Tanaka Y, Thurman RG, Lemasters JJ (1989) Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology 10:292–299
14. Caldwell-Kenkel JC, Carrin RT, Tanaka Y, Thurman RG, Lemasters JJ (1991) Kupffer cell activation and endothelial cell damage after storage of rat livers: effect of reperfusion. Hepatology 13:83–95
15. Clavien PA, Harvey PRC, Strasberg SM (1992) Preservation and reperfusion injuries in liver allografts. Transplantation 53:957–978
16. West MA, Billiar TR, Curran RD, Hyland BJ, Simmons RL (1989) Evidence that rat Kupffer cells stimulate and inhibit hepatocyte protein synthesis in vitro by different mechanisms. Gastroenterology 26:1572–1582
17. Bradfield JWB (1974) Control of spillover: the importance of Kupffer cell function in clinical medicine. Lancet 11:883–886
18. Grun M, Liehr H, Grun W, et al (1974) Influence of liver RES on toxic cell damage due to galactosamine. Acta Hepatogastroenterol 21:515–521
19. Wilkinson SP, Gazzard BG, Arroyo V, et al (1974) Relation of renal impairment and hemorrhagic diathesis to endotoxinemia in fulminant hepatic failure. Lancet 1:521–524
20. Chedid C, Parant M, Parant F, et al (1977) Enhancement of non-specific immunity to K. pneumoniae infection by MDP and several analogs. Proc Natl Acad Sci USA 74:2089–2093
21. Ausobky JR, Cheadle WG, Brosky BG, Polk HC (1984) Muramyl dipeptide increases tolerance to shock and bacterial challenge in mice. Br J Surg 71:151–153
22. Di Luzio NR, Olcay I, Holper K, et al (1975) The relationship of the reticuloendothelial dysfunction and endotoxinemia to survival after hepatic ischemia injury in the baboon. Circ Shock 2:77–89
23. Grun M, Liehr H, Grun W, Rosenack M, Brunsnig D (1974) Influence of liver RES on toxic liver cell damage due to galactosamine. Acta Hepatogastroenterol 21:515–526
24. Callery MP, Kamei T, Flye MW (1990) Kupffer cell blockade increases mortality during intraabdominal sepsis despite improving systemic immunity. Arch Surg 125:36–42