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Intramucosal pH and liver endotoxin clearance during experimental liver transplantation

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Abstract The study was designed to assess the gastrointestinal ischaemia and the influence of the specific Kupffer cell toxin gadolinium chloride ($GdCl_3$) on the hepatic and extrahepatic endotoxin [lipopolysaccharide (LPS)] clearance during experimental orthotopic liver transplantation (OLT) in pigs. In eight pig liver transplantations, the donors received 20 mg/kg of $GdCl_3$ 24 h before explantation, while controls ($n = 8$) received normal saline. Gastric and sigmoid intramucosal pH (pHi), LPS and endotoxin-neutralising capacity (ENC) levels were measured in the portal vein and superior vena cava after laparatomy, at the end of the anhepatic phase and 1 h after reperfusion. During the anhepatic phase, the sigmoid pHi decreased significantly from 7.32 ± 0.02 to 7.29 ± 0.03 ($P < 0.001$) and was associated with a substantial increase of portal LPS. Following reperfusion, the systemic LPS concentrations were significantly lower in the pretreated group [39 ± 23 pg/ml (Control); 14 ± 7 ($GdCl_3$); $P < 0.05$] suggesting an improved liver LPS clearance [86% ($GdCl_3$); 58.2% (Control); $P < 0.05$]. This corresponded to an increased ENC in the pretreated group [118 ± 52 ENU/ml ($GdCl_3$) vs 81 ± 45 ENU/ml (Control); $P < 0.05$]. The anhepatic phase induced splanchnic ischaemia which correlated with portal endotoxaemia. Donor preconditioning with $GdCl_3$ leads to lower systemic LPS concentrations in the recipient and increases ENC values in the early phase after OLT. An improved hepatocellular LPS extraction and/or an activation of the extrahepatic reticulo-endothelial system as a result of $GdCl_3$ treatment is discussed.

Key words pHi · Donor preconditioning · Kupffer cells · Liver transplantation

Introduction

During liver transplantation, inadequate tissue oxygenation occurs in the intestinal mucosa, which is highly susceptibel to a reduction in blood flow. The countercurrent exchange system within the intestinal mucosa renders the mucosa particularly vulnerable to ischaemia [11]. Gastrointestinal tissue acidosis, as determined by tonometry, has been found to be an early indicator of reduced oxygen delivery, metabolic imbalance and tissue oxygenation in both intensive care unit and postoperative patients [8]. Hypoxia results in superficial mucosal disruption [18] which promotes the translocation of bacteria and lipopolysaccharide (LPS) into the systemic and lymph circulations [2]. This might be more relevant in the anhepatic phase, when clamping of the portal vein is required for the recipient hepatectomy [15]. The highest LPS concentrations were measured at the end of the anhepatic phase before reperfusion of the graft, and were found to correlate with postoperative patient outcome [23]. The gastric and sigmoid intramucosal pH (pHi) were assessed in order to test the hypothesis that

a low pHi during the anhepatic phase is related to the occurence of endotoxaemia and that low pHi values after reperfusion are an indicator of poor graft function [20]. Treatment with gadolinium chloride ($GdCl_3$) of the rat liver graft reduces the production of reactive oxygen species, the liberation of inflammatory mediators and the expression of adhesion molecules [21]. Preconditioning of the donor liver with $GdCl_3$ in experimental pig liver transplantation was designed to assess the influence on the LPS extraction after reperfusion.

Materials and methods

Experimental groups

We performed a standard orthotopic liver transplantation (OLT) procedure in 16 pairs of German Landrace pigs (10–16 weeks, 24.7 ± 2.5 kg). Twenty-four hours before transplantation, the donor animals were randomly divided into two groups: eight pigs received $GdCl_3$ (20 mg/kg i.v.) and eight control pigs received saline only. Gastric and sigmoid pHi, portal and systemic LPS measurements were performed in the recipient before and at the end of the anhepatic phase and 1 h following transplantation. The experiments followed the principles of laboratory animal care and the German Law on the protection of animals.

Transplantation

Conventional gas and i.v. anaesthesia was performed during the operation and vital parameters were monitored continuously. Explantation of donor organs was carried out with UW perfusion following the cross-clamping technique. OLT was performed after 288 ± 74 min cold ischaemia in UW solution. A passive porto-caval shunt (VVBP) was used during the anhepatic phase. Following completion of the experimental protocol (24 h) the survivor animals were killed in narcosis by intravenous administration of standard potassium infusion.

Biochemical parameters

Blood count, liver enzymes and blood coagulation parameters were evaluated in the clinical laboratory.

Gastric and sigmoid pHi

Tonometers were inserted in the gastric lumen or per anus into the sigmoid colon after laparotomy. After equilibration of pCO_2 between the saline (tonometer) and the lumen (mucosa), a 1.5-ml sample was drawn simultaneously from the tonometer and the arterial blood. The pHi at 37 °C was calculated using a modified Henderson-Hasselbalch equation.

Endotoxin (LPS) and endotoxin-neutralising capacity (ENC)

LPS and ENC were measured using the automated kinetic turbimetric Limulus Amebocyte Lysate (LAL) microtitre test [7] with intraindividual internal standardisation Pyroquant (Associates of Cape Cod, Woods Hole) [8].

Statistics

The data are reported in the text as mean ± SD and are represented in graphics as mean ± SEM. The differences between groups were assessed using the non-parametric comparison tests for dependent data (Wilcoxon test), for independent data (Mann-Whitney *U*-test) and Fischer's exact test for contingency tables. Bivariant correlation was performed by Spearman's rank non-parametric correlation test. Overall significance was determined at $P < 0.05$.

Results

Gastric and sigmoid pHi

The gastric [7.32 ± 0.04 (control), 7.33 ± 0.05 ($GdCl_3$)] and sigmoid pHi [7.33 ± 0.03 (control), 7.33 ± 0.04 ($GdCl_3$)] levels decreased significantly at the end of the anhepatic phase in both groups [stomach: 7.28 ± 0.03 (control), 7.27 ± 0.03 ($GdCl_3$), $P < 0.001$; sigmoid pHi: 7.28 ± 0.04 (control), 7.29 ± 0.02 ($GdCl_3$), $P < 0.001$; Fig. 1]. One hour after reperfusion the pHi levels recovered only partialy but did not reached the initial values. The difference between both groups was not significant in all the three phases.

LPS and ENC levels

While portal LPS levels were not different at any point in time before and after transplantation, systemic LPS levels 1 h after reperfusion in recipients of $GdCl_3$-treated grafts were significantly lower than levels in recipients of untreated controls. Systemic LPS levels in recipients of control livers were significantly lower than in Kupffer cell (KC)-depleted livers [47 ± 14 pg/ml (control), 7.3 ± 5 pg/ml ($GdCl_3$), $P < 0.05$; Fig. 2] and consequently the transhepatic LPS gradient was higher [38 ± 10% (control) vs 78 ± 11% ($GdCl_3$), $P < 0.05$; Fig. 3]. At the end of the anhepatic phase the LPS concentrations correlate with the sigmoid pHi levels ($r_s = -0.59$, $P < 0.001$; Fig. 4) ENC almost disappeared at the end of the anhepatic phase and 1 h after reperfusion the systemic ENC in the $GdCl_3$ group (139 ± 41 ENU/ml) was substantially higher than in the control group (82 ± 37 ENU/ml, $P < 0.05$).

GOT levels

GOT levels [33 ± 13 U/l (control), 31 ± 11 U/l ($GdCl_3$)] increased 1 h after reperfusion and were significantly higher in the control group [112 ± 46 U/l ($GdCl_3$), 201 ± 96 U/l (control), $P < 0.05$]. Twenty-four hours after transplantation, the GOT levels were still higher in the control (694 ± 650 U/l) compared to the $GdCl_3$ group (358 ± 164 U/l, $P < 0.05$).

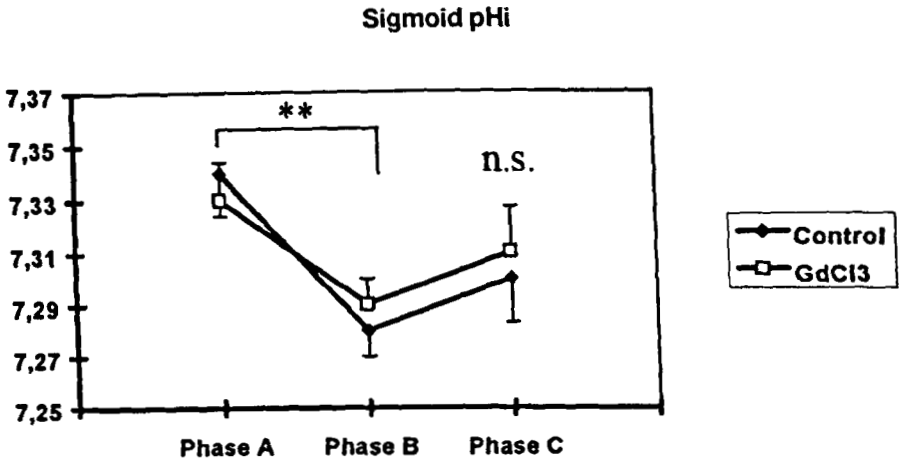

Fig. 1 Mean sigmoid intramucosal pH (*pHi*) ± SEM levels during experimental orthotopic liver transplantation (OLT) in control group vs $GdCl_3$-treated group. *Phase A* Before anhepatic phase, *Phase B* at end of anhepatic phase, *Phase C* 1 h following transplantation, * $P < 0.001$ (Mann-Whitney Wilcoxon test, comparison in the same group between phase A and B), *n.s.* not significant (Mann-Whitney *U*-test, comparison between groups in phase C)

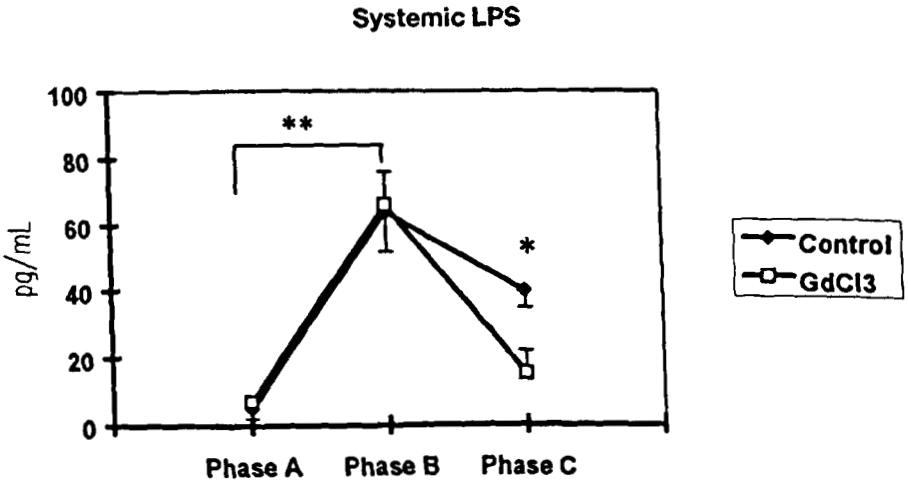

Fig. 2 Mean systemic lipopolysaccharide (LPS) concentrations ± SD in control group vs $GdCl_3$ group during experimental OLT. * $P < 0.001$ (Mann-Whitney Wilcoxon test, comparison in the same group between phases A and B), ** $P < 0.05$ (Mann-Whitney *U*-test, comparison between groups in phase C)

Discussion

During the anheptic phase of OLT gut blood flow is reduced following the clamping of the portal vein and will eventually lead to gastrointestinal ischaemia [22]. Due to the vascular architecture of the villi, which allows oxygen to be shunted from the central artery to the venules thus leading to ischaemia at the tips of the villi [11], there is an uneven distribution of oxygen within the bowel wall. Microscopic studies of the gut damaged by ischaemia show that the first detectable changes are found at the top of the villi [18], although there are also functional changes, such as increased capillary and mucosal permeability, which are likely to occur before any histological signs of ischaemic injury are seen.

The pHi is a diagnostic tool to assess whether oxygen delivery is sufficient for the need at any time [3]. Despite veno-venous bypass, the reduction of intestinal venous outflow during the anhepatic phase was reflected by a significant decrease in gastric and sigmoid pHi. Contrary to a study of partial bowel ischaemia [3], the gastric and sigmoid pHi were still increased 1 h after reperfusion indicating a persistent intramural hypoxia of the gastrointestinal mucosa, despite maintained portal blood by VVBP. Since perioperative pHi changes were essentially similar at both locations (stomach and sigmoid colon), the reduction in gastric pHi cannot be attributed to alterations in gastric mucosal perfusion as induced by surgical manipulation or falsely low pHi values due to reflux of alkaline duodenal fluid [8]. The low pHi 35–45 min into the anhepatic phase suggests the short period of complete portal clamping necessary to allow insertion of the VVBP cannula into the portal vein may be sufficient to induce a prolonged impairment of microvascular perfusion. Although we could not exclude the possibility that pHi may have decreased to even lower values without the use of VVB, the finding of impaired mucosal oxygenation while portal blood flow was (at least partially) maintained suggests that additional mechanisms must account for the preservation of mucosal perfusion during OLT.

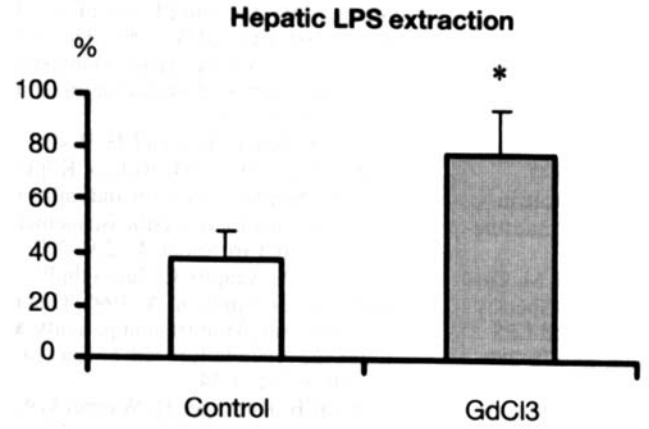

Fig. 3 The transhepatic LPS gradient (%) in control group vs $GdCl_3$ group, 1 h after reperfusion. * $P < 0.05$ (Mann-Whitney *U*-test)

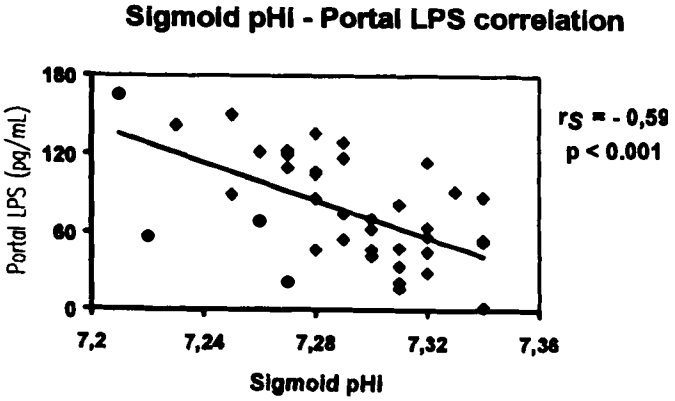

Fig. 4 Spearman's rank correlation between sigmoid pHi and portal LPS concentrations. $r_s = -0.59$; $P < 0.001$

Even though the anatomic integrity of the mucosa is not modified, the insufficient blood supply of the intestinal mucosa leads to the alteration of the barrier function [12] and allows for the translocation of endogenous bacteria into the portal blood. In several animal models it was shown that an increased mucosal permeability occurs after 30 min of ischaemia [16] and reaches a maximum at 12–24 h [4]. Electron microscopy studies demonstrated that bacteria and LPS are translocated either through [1] or between the mucosal epithelial cells [5, 13]. We found significantly higher portal and systemic LPS concentration which imply the occurrence of mucosal hypoxia, allowing for translocation of bacteria and LPS. The removal of the liver, the main organ for LPS clearance, leads to almost complete disappearance of ENC in the portal and systemic blood and corresponds to the rise in serum LPS concentrations.

Treatment with $GdCl_3$ has been shown to destroy 80% of the KC after 24 h, reduce the hepatocellular injury and improve the survival rate after hepatic ischaemia/reperfusion injury in rats [19]. Gadolinium crystals are taken up by macrophages and intracellular dissociation of the crystals is followed by a pH burst of the macrophages. Following reperfusion, the serum GOT levels were significantly higher in the control than in the $GdCl_3$ group. It would be expected that the selective blocking of KC or phagocytic dysfunction would impede the host defence and thus lower survival following LPS exposure [6]; other results demonstrate an improved survival after liver ischaemia/reperfusion and KC blocking [19, 21].

In our study, LPS extraction in the preconditioned livers is better than in the control group. So far we know of two independent LPS clearance systems in the liver, KC and a hepatocyte microtubule dependent system (HMDS) [9, 14]. While the KC clearance system is selectively blocked by $GdCl_3$, the HMDS is selectively blocked by colchicine [15]. The LPS is taken up by the KC through the CD14 receptor and then redistributed to the hepatocytes and transferred from the vascular to the biliary pole [15]. The HMDS on the other hand has LPS receptors on the hepatocyte which rapidly transport LPS from the hepatic sinusoids to the biliary canaliculi [17]. In our study, blocking of KC reduced the hepatic reperfusion injury (GOT) in the early phase of reperfusion. The HMDS or an activated extrahepatic reticulo-endothelial system (spleen, lung) in the $GdCl_3$ group could serve as a possible explanation for the improved LPS clearance [10].

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