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Tauroursodeoxycholate ameliorates reperfusion injury after pig liver transplantation

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Abstract Reperfusion injury is a serious problem after clinical liver transplantation, often leading to dys- or even non-function of grafts. The present study was designed to determine whether the hydrophilic bile salt tauroursodeoxycholate (TUDC), known to be hepatoprotective in cholestatic liver disease, mitigates reperfusion injury in an in vivo pig liver transplantation model. Liver transplantation was performed in 12 pigs after a preservation time of 8 h. TUDC was administered to donor and recipient animals, and saline to controls. Blood was drawn at different time points for determination of liver enzymes. Bile samples were collected, and bile flow (BF), and bile salt secretion rate (BSSR) determined. Samples of liver tissue and bile ducts were taken for assessment by light and electron microscopy. Liver enzymes were significantly lower in the TUDC group. BF and BSSR were significantly higher. Microscopy revealed better preservation of bile duct architecture of the TUDC-infused animals. We can conclude that infusions of TUDC in pig livers ameliorate reperfusion injury in vivo. The molecular basis for this finding may be the membrane stabilizing effect of TUDC. Further studies are warranted to clarify its effect.

Key words Pig · Liver transplantation · Tauroursodeoxycholate · Reperfusion injury · Liver enzymes

Introduction

Preservation and reperfusion injuries are well described, serious problems after clinical liver transplantation. Many studies have demonstrated a correlation between high enzyme release in the early postoperative phase, and primary dysfunction or even non-function [8, 28, 36]. Howard et al. also found an association with the rate of rejection [20].

Administration of the hydrophilic bile salt, tauroursodeoxycholate (TUDC), reduces serum liver enzymes in patients with a variety of chronic liver diseases [13, 29]. We have recently shown that TUDC infusion ameliorates reperfusion-injury in a rat IPRL-model, while administration in the donor decreased AST release after reperfusion [14, 15]. Previously we found that TUDC protects pig bile-duct architecture for up to 20 h of cold preservation [16]. The present study was designed to determine whether TUDC infusion before donor liver procurement and during reperfusion mitigates reperfusion injury in an in vivo pig liver transplantation model after 8 h of preservation, which is a rather long preservation time for the pig, despite the fact that preservation times of 12 h have been reported [6]. However, the one day survival in this one study was only 83 %. In our own preliminary studies we were not able to exceed these results and felt, that in order to be able obtain comparable data for several days post-transplant, we could not store the livers longer than for 8 h.

Materials and methods

Twenty-four male Landrace pigs weighing from 25–34 kg were acclimated for at least one week prior to surgery in our animal facility where they received standard pig chow once daily and water *ad libitum*. Donor and recipient animals were food-fasted overnight, but allowed water up to the procedure. Principles of laboratory animal care (NIH publication No. 86–23, revised 1985) were followed. The experimental protocol was approved by the animal ethics committee of the University of Hamburg in accordance with the current version of the German Law on the Protection of Animals.

Experimental design

In the control group, 0.9% saline was intravenously infused at a rate of 1.25 ml/min over 20 min into the donor animal. In the experimental group, donors received TUDC of the same volume at a rate of 2 μmol/kg body weight/min. In recipient animals, infusion started at the time of reperfusion. Saline was infused at a rate of 15 ml/h per hour for 6 h in the control group. In the experimental group 0.2 μmol/kg body weight/min was administered over the same duration.

Donor operation

The animals were premedicated with a single intramuscular injection of ketamine, acepromazine and midazolam. After endotracheal intubation, anesthesia was maintained with intravenous ketamine and midazolam. After opening the abdomen through a transverse incision extended to the xyphoid (Chevron incision), the liver was exposed and the hepatic artery, portal vein, infrahepatic vena cava, and abdominal aorta distal to the inferior mesenteric artery were isolated. The cystic duct was ligated close to the gallbladder, and a cannula was introduced into the distal common bile duct and secured. Bile was collected for 5 min and bile flow recorded. A 20-min intravenous infusion of TUDC or 0.9% saline was then begun. At 5-min intervals, after the start of the infusion, further bile samples were collected. Aortic puncture was performed and two units of whole blood (total around 900 ml) were collected in transfusion bags for later use during the recipient procedure. Normal saline solution was infused during the phlebotomy procedure to maintain stable hemodynamics. After the abdominal aorta was cannulated with an 18 Ch. perfusion catheter, the intrathoracic aorta was cross-clamped, and the liver flushed with one liter of ice-cold UW solution. Topical application of saline ice slush was also used to rapidly cool the organ. The liver was immediately harvested and placed on ice on the back table. The portal vein was flushed with 200 ml ice-cold UW solution and the vessels prepared for transplantation. The diaphragmatic veins were oversewn and a cuff applied to the infrahepatic vena cava. Briefly, the cut end of the vessel was everted over a 1 cm segment from a 5 ml syringe. As in standard clinical practice, the common bile duct was flushed retrograde with 30 ml of University of Wisconsin solution, despite the fact that we have shown, in a previous study, that the bile ducts do not become resistant against preservation injury after this treatment [16]. A cholecystectomy was performed and the liver stored in double sterile Lahey bags surrounded by UW solution and ice slush. Total preservation time (cold and warm ischemic time) was kept constant at 8 h in both groups.

Recipient operation

The recipient animals were premedicated, intubated and anesthetized as described for the donor. Catheters were inserted in the right external carotid artery and jugular vein for pressure monitoring and fluid administration. The left jugular vein was exposed in preparation for veno-veno-bypass. Again, a laparotomy via an extended chevron incision was performed and retractors placed for adequate exposure of the liver. The recipient hepatic artery, portal vein, and supra- and infrahepatic vena cava were prepared in close proximity to the liver, to obtain long segments to facilitate end-to-end anastomoses. The animals were then anticoagulated with 3000 IU of heparin, and veno-veno bypass was performed. The infrahepatic vena cava and portal vein were cross-clamped and divided, then cannulated for connection via a Y-connector to the left jugular vein. The suprahepatic vena cava was cross-clamped, the hepatic artery divided, and the liver removed. All intravenous anesthetics were discontinued at this point until reperfusion. The donor organ was taken out of the ice and placed in the right upper quadrant for anastomosis to the recipient vessels. All anastomoses were performed with absorbable monofilament sutures in the following order: suprahepatic vena cava (4–0 PDS), hepatic artery (7–0 PDS), and finally portal vein (5–0 PDS). Prior to completing the portal vein anastomosis, a 6 Fr. flush catheter was placed in the portal vein and 500 ml of cold lactated Ringer's solution were infused. The portal vein anastomosis was then completed and the liver was reperfused in the following order: the suprahepatic vena cava clamp was removed followed by portal vein, then hepatic artery reperfusion. After careful hemostasis was achieved, the infrahepatic vena cava was anastomosed using a cuff technique. The common bile duct was repaired with 5–0 Vicryl sutures utilizing an end-to-end technique. A T-tube (2.5 mm outer diameter) was introduced distal to the repair site and externalized to the right flank. Hourly bile samples were collected for 7 h. Hemostasis was confirmed, and the abdomen closed in three layers, using interrupted absorbable sutures for deep – and nylon for skin closure. The animals were extubated when alert with spontaneous respirations.

Immunosuppressive regimen

One day before surgery, recipient pigs received 5 mg/kg cyclosporin (Optoral, Sandoz) orally. During surgery, 100 mg dexamethasone were administered in the anhepatic phase. After transplantation, the animals received a daily oral CyA dose of 5 mg/kg. This dose was reduced to 3 mg/kg on the tenth postoperative day. The blood level of CyA was aimed at to be between 50 and 150 μg/l.

Liver core temperature measurement

Temperature was measured after the 8-hour preservation period on removing the liver from the cold UW solution and again, immediately prior to reperfusion. A needle probe (model J-150, Yellow Springs Intrument Co, Yellow Springs, USA) was centrally inserted in the right lobe and the temperature measured by a compatible tele-thermometer (YSI Precision 4000 Thermometer, Yellow Springs Instruments Co.).

Laboratory investigations

Blood was drawn at time zero (control) and 4 and 12 h after reperfusion as well as on postoperative days 1 to 7 and 14. AST, ALT,

GLDH, AP, GGT, bilirubin, LDH and HBDH were analyzed using a BM/Hitachi 747 analyzer. GST was determined using porcine alpha-GST EIA immunoassay (Biotrin, Dublin). Monoclonal CyA was determined with an fluorescence polarization immunoassay using monoclonal mouse antibody and fluorescein tracer (analyzer FLX, Abbott). Red blood cell, white blood cell and platelet count were determined with a Bayer H^3 analyzer (Munich, FRG) The prothrombin time was determined using a Dade CA 600 analyzer (Munich, FRG).

Bacteriologic examinations

The recipient and donor common bile ducts were swabbed just prior to anastomosis and sent for culture to the clinical laboratory.

Bile analyses

The samles of the bile collected from the donors before and during infusion of TUDC or saline, and during the reperfusion phase in the recipient, were was frozen at -80 °C until further analysis. Total bile salt concentration from each sample was quantified in triplicate using an enzymatic assay (Sigma, St. Louis, MO, [34]). The total bile salt concentration was standardized per 100 g liver tissue. Bile salt secretion rate (BSSR) was also calculated.

Histology

Liver and common bile duct samples for light and electron microscopy were taken from all animals immediately after flush of the donor liver, after taking the liver out of the Lahey bags at the end of cold preservation, common bile duct samples were taken at the time of bile duct anastomosis. Samples were processed for light and scanning electron microscopy as follows:

Light microscopy
Bile ducts and liver specimen were fixed in Bouin's solution for 2 weeks, then dehydrated with alcohol and embedded in parrafin blocks. Six µm slices were stained with hematoxylin and eosin.

Scanning electron microscopy
Bile ducts were fixed in 2.5 % glutaraldehyde solution for 24 h, followed by postfixation with Bouin's solution. After alcohol dehydration and critical temperature drying, specimen were sputtered with gold and examined using a Leitz DSM 940 scanning electron microscope (Jena, Germany). The histologic grading of the scanning electron microscopic specimen was done by one of the coauthors, who is an experienced expert in the field (D. K.), in a blinded fashion.

Statistical analyses

Comparisons between experimental groups were performed using analysis of variance (ANOVA). If ANOVA detected significant differences, then multiple comparisons testing was performed (Scheffe's procedure). An unpaired *t*-test was used to compare single data within groups. Significance was determined at a p-value $P < 0.05$. Measured values are reported as mean ± SD.

Results

Comparing the two groups, there were no significant differences in the treatment-independent parameters. The mean body weight was 29.5 ± 3 kg (28.3 ± 3.4 kg TUDC vs. 30.7 ± 2.3 kg saline; NS). Mean liver weight was 657 ± 39 g in the TUDC group and 648 ± 29 g in the control group (NS). Preservation (cold ischemia) and "warm" ischemia times too, were not significantly different between the experimental- and the control group. Mean "warm" ischemia time, the time required to sew-in the liver, was 61.8 ± 3.7 min in the TUDC group versus 65.3 ± 4.9 min in the saline group (NS). Mean total ischemic time, from start of cold preservation until reperfusion in the recipient, was 481 ± 19 min for the TUDC- infused animals compared to 463 ± 21 min for controls (NS). The temperature of the livers was 1.2 ± 0.9 0 °C on removal from the cold UW solution. At the end of the warm ischemia period, shortly before reperfusion, temperature had risen to 28.9 ± 2.0 °C. There was no difference between groups. In most cases, culture results from the donor and recipient common bile ducts were negative. Rarely, contamination with "few" staphylococcal or streptococcal species was found among animals in both groups. This contamination was not associated with the subsequent development of infectious complications.

Postoperative survival

Six of the 12 animals included in this series died between postoperative days 4 and 72, while the other six are alive and healthy. Seven day survival was 92 % (11/12) while 14-day survival was 67 % (8/12). Split into the two experimental groups, 14-day survival was 4/6 (67 %) for the TUDC-group, and 5/6 (83 %) for the NaCl-group (NS). The only death during the first week occurred on POD 4 in a TUDC-infused animal, and was the result of acute respiratory arrest, secondary to an unrecognized unilateral pleural effusion. At the time of death, liver function was excellent. Three pigs died as a result of massive hemorrhage from stress erosions despite omeprazole therapy (POD 10 NaCl, POD 14 TUDC, and POD 30 NaCl). The fifth pig (TUDC) developed septic shock necessitating reoperation. A small bowel perforation was found and repaired, but it died intraoperatively. Finally, one animal in the TUDC group developed icterus during the third postoperative week, but was otherwise asymptomatic to its last days. In the final 3 weeks it developed progressive liver failure and died on POD 72. An autopsy revealed that the hepatic artery was occluded, due to an anastomotic stricture, and that the common duct had stenosed over a 2 cm segment proximal to its anastomosis.

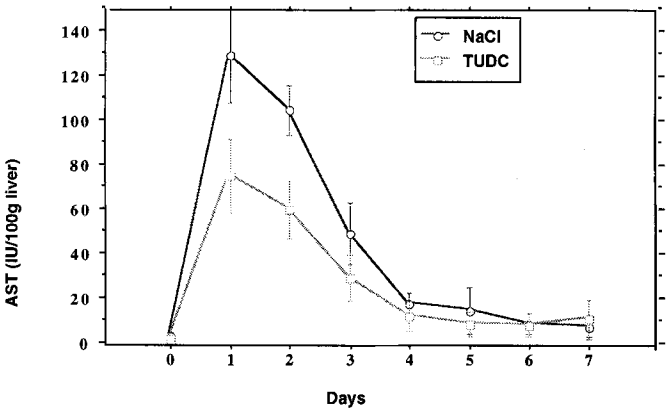

Fig. 1 AST release in the first 7 days after transplantation. Note the rapid increase of AST with a peak at POD 1. Then the curve slopes down slowly, but AST values are still elevated after 7 days. Starting with POD 4 curves are similar. *NaCl* Saline infused group, *TUDC* TUDC infused group. Data are graphed as IU/100 g liver, mean ± SD

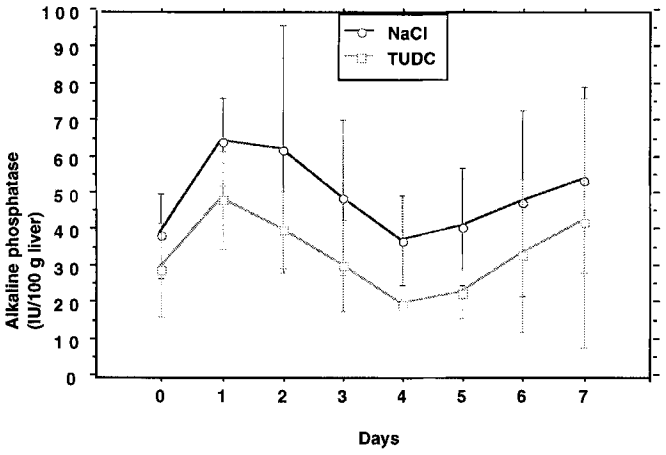

Fig. 3 The AP showed a first peak on POD 1, followed by a second increase starting POD 4. A reason for this finding could not be found. The values of the saline group are higher than in the TUDC group. *Abbreviations* see Fig. 1

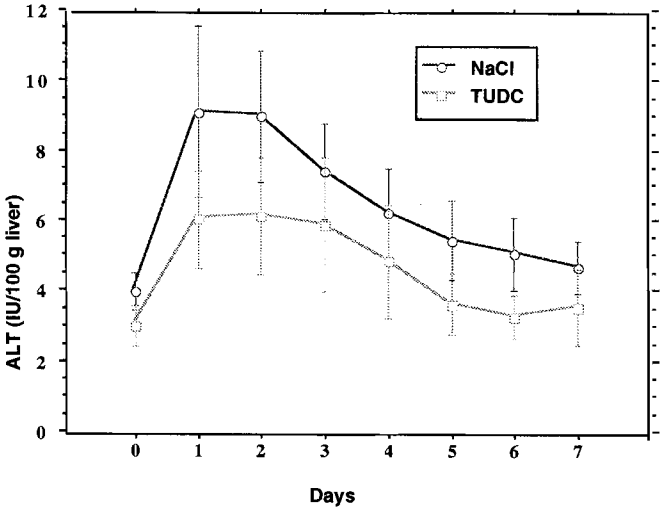

Fig. 2 ALT release. The slope of the curve is similar to the one of AST in Figure 2. However, ALT values in the saline group never came down as much as the ones of the TUDC group. *Abbreviations* see Figure 1

Enzyme release

Impressive differences were found comparing postoperative AST and ALT enzyme release (Fig. 1). Peak AST values were noted on POD 1 in both groups, however enzyme release in the saline group was significantly higher than for TUDC-treated animals (115 ± 39 vs. 72 ± 24 IU/100 g liver, $P < 0.005$). Comparing the first 3 days, the mean AST value was significantly higher in the control animals compared to the TUDC-treated animals (76 ± 51 vs. 46 ± 30 IU/100 g liver; $P < 0.005$). ALT release in the control group did not peak until POD 2, while the TUDC group peak was noted by 12 h, and declined thereafter (12 h values not shown in Fig. 2). Comparing peak values at 12 h, ALT-release was significantly higher in the control group, compared to experimental controls (12 ± 9.5 vs. 6.2 ± 1.1 IU/100 g liver; $P < 0.005$). The mean values for the first 3 days after transplantation were 7.8 ± 4.8 saline vs. 5.2 ± 1.8 TUDC, $P < 0.005$).

Alkaline phosphatase, located in hepatocytes and biliary epithelium, is an indicator of cholestasis. It was also significantly higher in the saline group than in the TUDC group ($P < 0.05$), and peaked on POD 1 with 65 ± 15 IU/100 g liver (50 ± 12 IU/100 g liver in the TUDC group). Then the values decreased until POD 4, when they rose again in both groups for unknown reason (Fig. 3). On POD 3, GGT was lower in the TUDC group (2.4 ± 0.3 vs. 6.4 ± 2.9, $P < 0.05$). However, ANOVA showed no significance comparing the first 3 postoperative days. LDH and HBDH were not significantly different, but the ratio LDH/HBDH as an indicator of parenchymal liver damage was 1.65 ± 0.2 in the TUDC-group compared to 1.79 ± 0.2 in the saline group ($P < 0.05$).

GST is an enzyme located in the hepatocyte, and its superiority compared to commonly used enzymes in the diagnosis of conditions affecting the hepatocyte has only recently been recognized [30, 37]. There was a rapid rise after reperfusion (Fig. 4), peaking by 12 h to 1.8 ± 1.5 µg/l/100 g liver in the saline group, versus 0.3 ± 0.2 µg/l/100 g liver in the TUDC-infused animals ($P < 0.05$). By POD 1, values had dropped to 0.4 ± 0.1 and 0.2 ± 0.03 µg/l/100 g liver, for saline and TUDC groups, respectively.

GLDH, the release of which reflects severe structural damage in the hepatocyte is an enzyme found in the

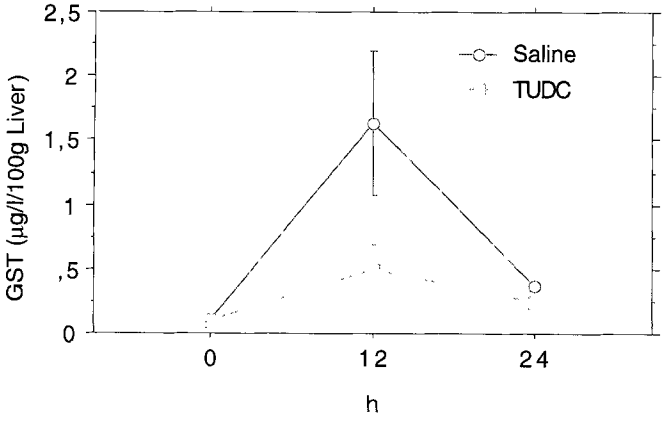

Fig. 4 The GST was the enzyme with the biggest difference between groups. The first 24 h after reperfusion are depicted. The peak was at 12 h post transplantation, and by 24 h it was almost back to baseline values in both groups

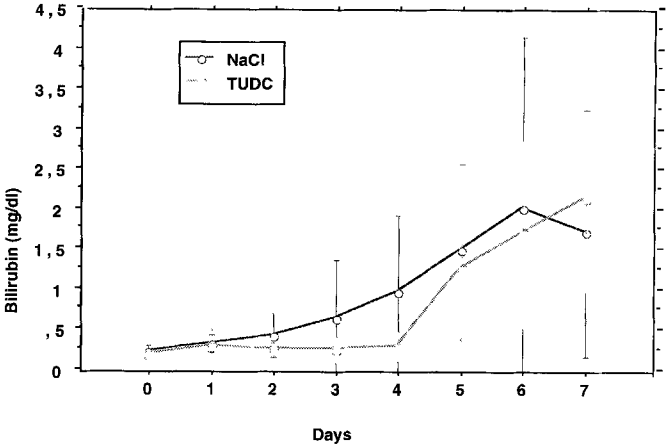

Fig. 6 Total bilirubin during the first seven days postoperatively. Note the increase in the saline group starting day 1, in the TUDC group the values stay low up to POD 4

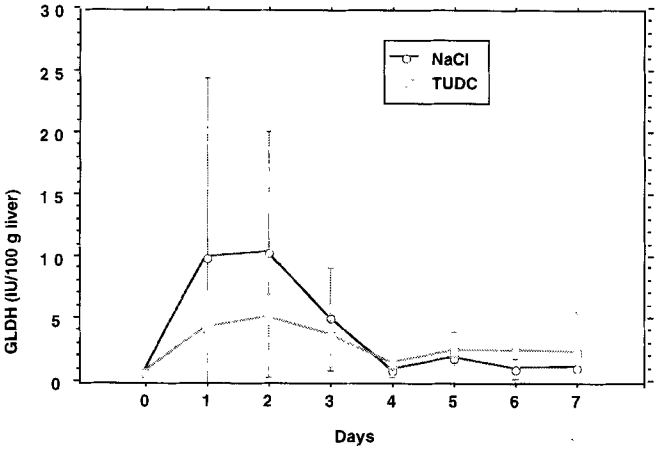

Fig. 5 GLDH seemed to be higher in the saline group. However, analyses did not show significance due to high SD's (omitted). *Abbreviations* see Figure 1

mitochondria. While this value tended to be lower in the TUDC group, the difference was not significant. Two peaks in GLDH-release were seen in the TUDC-group; after 12 h (6.1 ± 6.6 IU/1 g liver), and again on POD 2 (5.3 ± 2.2 IU/100 g liver, Fig. 5). Only a single peak was noted on POD 2 in the saline group (12.2 ± 11 IU/100 g liver). For the first 3 days, GLDH release was 3.7 ± 3.7 IU/100 g liver in the TUDC group compared to 6.3 ± 9.7 IU/100 g liver in the control group.

Other laboratory assessments

Total protein, albumin, bilirubin, prothrombin time, Platelet and WBC count were not significantly different between groups. Interestingly, the pattern of bilirubin release was different. Total bilirubin in the TUDC group was less than 0.2 mg/dl until POD 5, when it rose (Fig. 6) to values in the control group.

Bile flow and bile salt secretion rate

Prior to TUDC-infusion in the donor animals, there were no differences in bile flow (BF) between groups (TUDC 2 ± 0.4 ml/5 min vs. NaCl 1.9 ± 0.3 ml/5 min). Bile salt secretion rate (BSSR) was 14.4 ± 5.4 µmol/min for TUDC-treated animals and 13.3 ± 7.1 µmol/min in controls. After infusion of TUDC, there was a marked increase in BF (2.6 ± 0.5 ml/5 min vs. 1.8 ± 0.4 ml/5 min, $P < 0.05$) and BSSR (24.6 ± 3.3 µmol/min vs. 10.5 ± 5.3 µmol/min, $P = 0.001$, Fig. 7). The same results were achieved in the recipients after reperfusion (Fig 8). In the TUDC-group BF and BSSR were significantly higher compared to saline group. BF was 14.4 ± 3.9 ml/h over the first 7 h while in the NaCl group it was 9 ± 4.9 ml/h ($P < 0.0001$). BSSR for the experimental animals was 5.9 ± 3.5 µmol/min versus 2.1 ± 2.4 µmol/min in the control group ($P < 0.0005$).

Histology

Light-microscopy of the liver parenchyma did not show any noticable difference among the two groups. The number of vacuoles were few and did not differ between the groups either. However, light microscopy of the bile ducts showed well preserved epithelium in the TUDC-infused animals, while control animals showed a variation of preservation from normal to complete destruction (not shown, see Ref. [16]). Scanning electron microscopy of the common bile duct revealed an obvious differ-

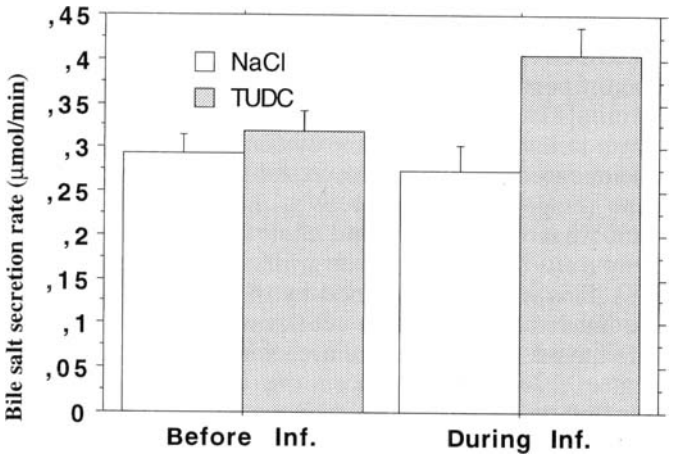

Fig. 7 Bile salt secretion rate before and after infusion of TUDC or saline in the donor animal. Note the steep increase in the TUDC group

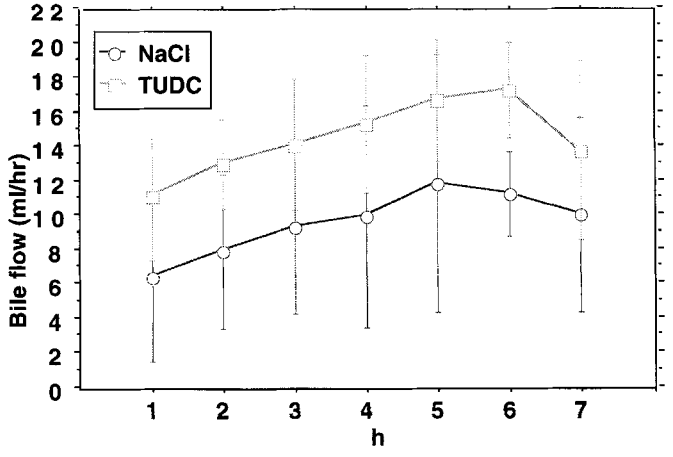

Fig. 8 The bile flow was significantly higher even immediately after reperfusion. Whether that was due to the infusion of TUDC in the donor animal or the immediate effect of TUDC infusion at the time of reperfusion is not clear

ence between the TUDC infused animals and the control. As in light microscopy, saline-infused animals had severe damage to the biliary epithelium, which was either absent or damaged as shown in Fig. 9. Epithelial cells had lost their membrane integrity or their micrivilli, bleb formation was seen on others. All of the TUDC-treated animals had well-preserved epithelium with close cell-to-cell contact, well-preserved microvilli and, rarely, discrete bleb formation at the time of reperfusion (Fig. 10).

Discussion

The principle finding of this study is the fact that infusion of TUDC in pig livers protects against reperfusion injury *in vivo*. This was demonstrated by lower ALT, AST, and alpha-GST release after transplantation, increased bile flow, increased bile salt secretion rate in TUDC-treated animals, and better preserved biliary epithelium, as shown by light and electron microscopy.

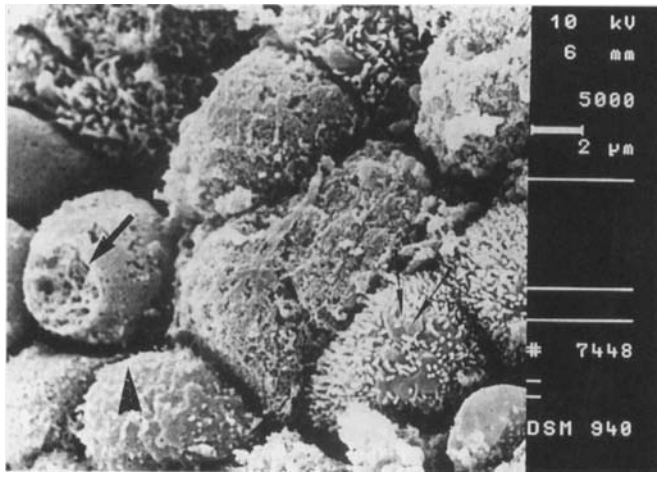

Fig. 9 Scanning electron microscopy of an eight hours preserved common bile duct of a saline infused animal. This picture was taken from a bile duct of an animal with medium grade injury. Several stages of the injury are visible: The cell on the *right* is best preserved with microvilli and only little blebbing (*small arrows*). The cell on the left, however, is lacking microvilli and part of the cell membrane is broken off, uncovering the cytosceleton (*arrow*). Cell-cell-contacts are lost (*arrowhead*). The cells in the center are partially covered with mucus. Original magnification × 5000

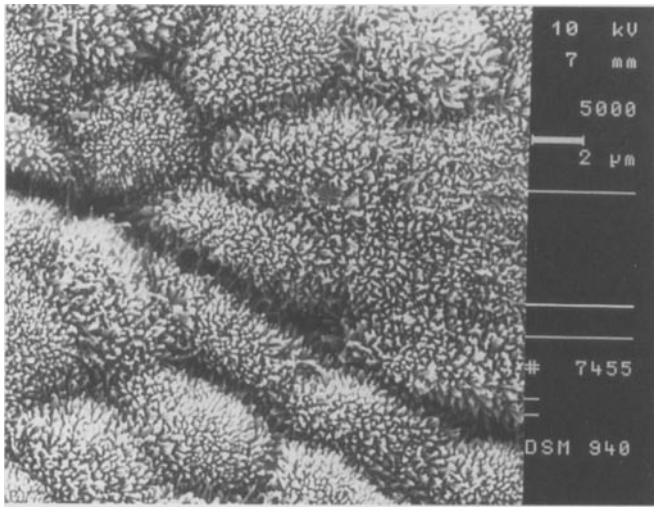

Fig. 10 Scanning electron microscopy of an eight hours preserved common bile duct of an animal of the experimental group. TUDC infusion led to good preservation of the epithelium, comparable to the unpreserved control. Cell-cell-contacts are relatively close, the crack in the center is a fixation artefact. Microvilli are abundant on all cells. Original magnification × 5000

Numerous clinical studies have found a positive correlation between postoperative enzyme release and the rate of primary dys- or nonfunctioning livers after transplantation [2, 11, 20, 26, 28]. Primary nonfunction occurs after liver transplantation procedures in 6–8%, and requires immediate retransplantation to save the patient's life. By infusing TUDC in both the donor and the recipient, postoperative enzyme release decreased from 30% (ALT) to 80% (GST). The molecular basis for this finding is not known, but may be secondary to a membrane stabilizing effect of TUDC [12, 19]. Additionally, increased uptake of hydrophilic bile salts like TUDC may competitively inhibit the uptake of hydrophobic bile salts and stimulate total bile salt secretion [29].

Bile flow is regarded by many authors as a reliable index of postoperative liver function [9, 23, 25, 27, 33]. This is not suprising, since bile production and secretion is very complex, requiring the proper function of many interdependent cellular processes. The calculation of the BSSR factors in bile flow and bile salt production and secretion, is therefore also a very important parameter. Using this index, one can discern bile salt-independent bile flow, which results from stimulation of the secretory component of the biliary epithelium, but does not depend on hepatocyte function. Both BSSR and bile flow were significantly higher in our study, reflecting improved postoperative hepatocyte function in TUDC-treated animals.

In humans, the introduction of UW storage solution allows preservation times of up to 34 h. However, preservation times > 12 h have been associated with an increased incidence of primary dysfunction and other complications and are not recommended [1]. In comparison, the pig liver is much more susceptible to cold ischemia than the human liver [32]. Ploeg et. al. defined postoperative dysfunction at AST values > 2000 IU and a prothrombin time > 16 s [28]. In our study, such an enzyme release was not reached, the peak enzyme release in the control group being about 1300 IU. Therefore one might say that the preservation time applied in our study was not long enough to allow comparison with the clinical situation. However, in our preliminary experiments, only 5/15 animals survived one week after liver transplantation with organs that were cold-preserved for 12–16 h. Three of those 5 survivors died between POD 7 and 10. There were two long-term survivors (> 3 months), one of whom had severe primary dysfunction for two weeks after a preservation time of 16 h. These results are in accordance with the study of Boudjema et al. [6], who achieved a one-day survival of 83% after preserving pig livers for 12 h. For our purpose, this short survival would not have been long enough to collect sufficient data to compare both groups. On the other hand, clinical liver transplantation is currently done after preservation times not longer than 12–15 h, fearing ischemic bile duct injury and postoperative biliary strictures. Interestingly, warm ischemia is much better tolerated in the pig. Survival after 6 h has been reported [24], while in humans, the limit is about 90 min [21, 22]. A relationship between the duration of warm ischemia and primary dysfunction has been well-documented. While sewing in the pig liver grafts, liver core temperature increased to 29 °C. This is higher than what we found in the clinical setting, where the temperature in human liver grafts rose only to 17.2 °C [17]. This is easily explained by the fact that pig livers are much smaller than an adult human liver and therefore have a greater surface area which facilitates better heat exchange. Furthermore, our warm ischemia time was slightly longer; 60 min compared to 45 in the clinical study [17].

The idea of administering bile salt to protect hepatocytes was derived from studies that showed the protective effect of hydrophilic bile acids. Ursodeoxycholate is a naturally occurring bile salt in humans. It is synthesized in low amounts from chenodeoxycholate. In isolated hepatocytes TUDC ameliorates the toxic effects of glycochenodeoxycholate [10]. TUDC also protects *in vitro* against toxic effects of taurodeoxycholate and taurochenodeoxycholate in rat livers [19]. Pigs have mainly hyo-bile salts, having an alpha hydroxy-group in the C_6 instead of the C_{12} position. These bile slats are quite hydrophilic, and it is therefore somewhat surprising that TUDC still exerted such a significant hepatoprotective effect.

There are a number of chronic liver disease states in which TUDC- and UDC (the unconjugated form) administration have been shown to be effective. The mechanism by which hydrophilic bile salts exert their effect is not precisely known. In cirrhosis patients, TUDC therapy improved liver function parameters (AST, ALT, bilirubin). The concentration of hydrophobic bile salts in bile of those patients remained unchanged, while adminstration of TUDC in control people led to decrease of the endogenous deoxycholate pool [5]. In patients with primary sclerosing cholangitis, TUDC administration decreased liver enzyme release, particularly alkaline phosphatase, and serum bilirubin levels [4]. TUDC is also effective in primary biliary cirrhosis by reducing liver enzymes in the serum [13], the concentration of hydrophobic bile salts in the serum [29], and aberrant HLA-Class-II-expression on the hepatocyte [7]. TUDC exerts an initial positive effect in children with biliary atresia, unfortunately, liver function parameters ultimately return to pretreatment values [38]. UDC, the unconjugated form of TUDC, is an effective therapeutic agent in cases of mild to medium grade cholestasis [18]. The hepatoprotective effect was also demonstrated by Bellentani et. al., who treated patients with elevated liver function tests of unknown etiology with TUDC. After 3 months of therapy, liver enzymes were significanlty lower [3].

Histology supported results from our previously performed study [16]. In that study, we were able to show protection of the biliary epithelium for up to 20 h by infusions of hydrophilic bile salts into the donor animal. However, scanning electron microscopy was not performed, so that the ultrastructural changes were not visualized. In the literature we did not find any study dealing with preservation injury of bile ducts on a electron microscopic level. Sakato et al. looked at the common bile duct of golden hamsters fed a lithogenic diet [31]. An abundance of microvilli were seen on the epithelia of the control animals, while in treated hamsters the number of mucus producing goblet cells had increased significantly. We did not find any goblet cells in the specimens at the scanning electron microscopy level, despite the fact that in the former study [16], goblet cells were present, so that non-appearance in the present study is not due to non-existence. TUDC, while increasing biliary secretion, may not induce increased mucus secretion. Microvilli are structurally not different among species like pig, hamster and dog [35]. Infusion of TUDC did not chance number or appearance of microvilli, but preservation led to clubbing and reduction in number and density.

In pig liver transplantation, postoperative immunosuppression is of great importance. There are no studies of liver transplantation using CyA in the pig, so we orientated ourselves by the clinical situation. There are no studies comparing the survival rates of pigs with or without immunosupression. On one hand, we found in our study no indication for rejection, on the other hand we encountered no infections or fungal disease that could have been contributed to over-immunosuppression. Occasionally, wound separations and infections occurred which healed spontaneously despite CyA therapy. Furthermore, our pigs were able to control fairly big wound infections resulting from the naturally bad sanitary situation in a pig pen.

In humans, an infusion of TUDC would be easy to perform in the liver donor. In the pig model we were limited to 6 h after reperfusion, because we could not guarantee safe, continuous infusion in an awake and moving animal. Because of variability in appetite in our animals in the first postoperative days, we could not regulate oral intake of medications. This was not attempted because of the uncertainty and variability it would have introduced to the study. The introduction of a gastrostomy was no option because of the high risk of infection. It is well likely, that continued infusions of bile salts for longer than the initial hours would be helpful in the human setting. Also, bile salts could be given to the recipient via the stomach tube or via i.v. route initially. In our study, bilirubin was low in the TUDC-treated group for the first 4 days after surgery, compared to the controls. We do not know if this positive finding was related to a persistent positive effect of the TUDC. If it was, continued administration of TUDC in the postoperative period for days to weeks may be beneficial. Furthermore, the dose we chose was relatively low, and increasing the concentration of TUDC may further mitigate enzyme release. Such an approach seems justifiable in light of the paucity of side-effects related to TUDC administration.

In summary, this study shows the following:

- Adminstration of TUDC in donor and recipient leads to protection of the transplanted pig liver as shown by lower enzyme release and increased biliary secretion.
- Light and scanning electron microscopy shows that TUDC-infusions lead to better protection of the common bile duct.

Whether these findings will lead to better preservation of the hepatic function, and possibly lead to the use of marginal donor livers in the clinical setting, needs to be investigated further.

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