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Influence of liver transplantation and cyclosporin on bile secretion – an experimental study in the rat

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Abstract Bile secretion is reduced after liver transplantation. It has been suggested that this is due either to the effect of cyclosporin or to the damage to the liver graft during preservation and reperfusion. The aim of this study was to explore the influence of cyclosporin as well as of liver transplantation on bile secretion. Bile flow was studied in an experimental model in the rat. In syngeneic liver-transplanted animals, the bile flow was increased compared to the bile flow in the control group (1.29 ± 0.09 ml/h vs 0.66 ± 0.03 ml/h; $P < 0.01$), mainly due to an increased bile acid-independent flow (0.76 ml/h vs 0.50 ml/h; $P < 0.01$). The findings in the liver-transplanted rats contrasted with those in a group of nontransplanted animals treated with cyclosporin. Cyclosporin treatment resulted in a reduced bile acid-independent fraction (0.37 ml/h vs 0.50 ml/h, $P < 0.05$) of the bile flow, although no biochemical signs of hepatotoxicity were present. This reduction in the bile acid-independent fraction could, however, not be demonstrated when cyclosporin was given to a group of liver-transplanted rats, although a reduced total bile flow was recorded in the 1st hour measurements. In contrast to previous studies, we found that the cyclosporin vehicle (Cremophor EL), when administered chronically, induced a higher bile flow than that in the control rats. This effect was not seen in the transplanted rats. Our findings in this experimental rat model indicate that cyclosporin will influence and reduce bile secretion and bile acid secretion even if no other signs of liver dysfunction are present. On the other hand, the preservation and reperfusion in this model resulted in an increased bile flow, while bile acid secretion remained constant.

Key words Bile secretion, liver transplantation, rat · Cyclosporin A, bile secretion, rat · Liver transplantation, bile secretion, rat

Introduction

Bile secretion is an important parameter to follow after liver transplantation. A reduction in the bile flow can be an early sign of graft dysfunction. In 1971, Javitt and coworkers first reported on bile secretion after liver transplantation [19]. The active transport of bile acids into the bile canaliculi is the driving force behind bile secretion [28]. Bile thus formed is considered to be the bile acid-dependent flow (BADF). An excellent correlation exists between bile flow and the bile salt secretion rate in all mammals tested, and the different fractions of bile flow can, thus, be schematically shown, as in Fig. 1. The estimated bile production at a zero bile acid secretion rate is then considered to be the bile acid-independent flow (BAIF) [35]. The driving force

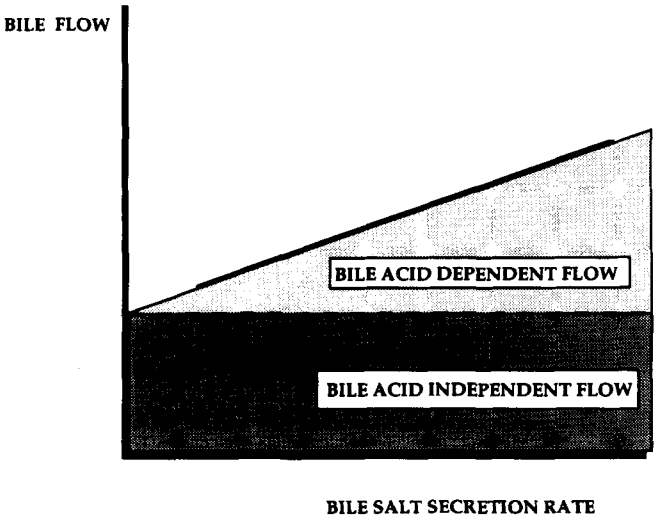

Fig. 1 Diagrammatic presentation of the relationship between bile flow and the bile salt secretion rate

behind the formation of the BAIF is probably the active secretion of other osmotically active anions, such as $HCO3^{-1}$ ions.

Bile flow is initially depressed after transplantation, but the bile volumes and the bile acid concentration soon increase and reach stable levels after 2–3 weeks [3, 8, 17, 27]. Studies of bile secretion after liver transplantation have shown that the bile acid-independent fraction of the bile flow is reduced, although liver enzymes are normal [14]. It has been suggested that this is an effect of cyclosporin or a result of preservation and reperfusion damage to the liver graft, or a combination of both [14, 36].

Cyclosporin is metabolized and excreted by the liver [36]. This immunosuppressive drug has a narrow therapeutic range and has a major side effect in the form of hepatotoxicity. Cholestasis has been reported after transplantation of different organs [5], and although this effect of cyclosporin has been less prominent in latter years, an effect on bile secretion after liver transplantation is probable, even if no other evident signs of hepatotoxicity can be seen. The vehicle for cyclosporin (Cremophor EL) has been shown to have an acute cholestatic effect in the rat, although the cholestatic effect of cyclosporin remained when the vehicle was removed [24]. The effect of chronic administration of the vehicle has previously not been tested.

The aim of the present study was to further explore the influence of cyclosporin and of orthotopic liver transplantation on the bile formation process using an experimental model in the rat.

Materials and methods

Chemicals and drugs

Sodium taurocholate (grade I, 99 % pure) was obtained from Sigma Chemicals (St. Louis, Mo., USA). Cyclosporin (Sandimmun) and the vehicle (Cremophor EL), were obtained from Sandoz (Basel, Switzerland). Belzer University of Wisconsin (UW) solution was purchased from Dupont Pharma (The Netherlands) and buprenorphine from Reckitt & Colman, (Hull, UK).

Animals

Female Wistar-Furth rats weighing 201 ± 2.4 g (range 140–248 g) were used. The weights were recorded on the day of the bile secretion experiment. The liver-transplanted rats' average body weight was slightly higher (209 ± 4.0 g) than that of the nontransplanted rats (195.5 ± 2.9 g). Animals were kept under controlled conditions at a constant temperature and were maintained on a normal day and night cycle. All rats had free access to tap water and pellets ad libitum. Female Wistar-Furth rats weighing around 200 g were used as donors.

Liver transplantation procedure

Ether was used for induction and maintenance of anesthesia in donor and recipient animals. Apart from atropine (0.1 mg/100 g body weight) for premedication and buprenorphine, given as a postoperative analgesic, no other drugs were used. No fluid, heparin, or antibiotics were administered.

The technique of orthotopic liver transplantation in the rat without rearterialization used in this study is described in detail in a previous paper [31]. Briefly, the vascular anastomosis was performed with a running suture technique. The donor liver was perfused in situ under low pressure through the portal vein with 5 ml UW solution and then excised. The preserved livers were stored in UW solution at 4 °C and the preservation time did not exceed 45 min. The donor liver was positioned orthotopically in the abdominal cavity. The suprahepatic caval vein and the portal vein were anastomosed with a running suture. Blood flow was restored. The anhepatic time was less than 20 min in all cases. The infrahepatic caval vein was then anastomosed with a running suture. Finally, the bile duct anastomosis was performed with a polyethylene tube (PE 25) as a splint in the bile duct. All transplantations were syngeneic in this study.

This model has proved reliable and scientifically acceptable for many types of biochemical and immunological studies of orthotopic liver transplantation [10, 20, 38].

Experimental design

Thirty syngeneic rat liver transplantations (LTx) were included in this study. They were assigned to three groups:

1. The LTx group (n = 14) did not receive any drugs and the effect of preservation and reperfusion on bile formation was studied.
2. The LTx CyA group (n = 8) was treated with cyclosporin after the transplantation.
3. The LTx vehicle group (n = 8) was treated with the cyclosporin vehicle (Cremophor EL) after the transplanation.

Another set of 41 nontransplanted rats were divided into four groups:

1. The control group (n = 11) did not receive any drugs and served as a reference group.

Table 1 Liver enzymes and bilirubin on the day of the bile secretion studies

Group	S-bilirubin (μmol/l)	S-AST (μcat/l)	S-ALT (μcat/l)
Control	9.3 ± 0.8	3.2 ± 0.8	1.7 ± 0.4
LTx	12.2 ± 1.8	2.6 ± 0.7	1.4 ± 0.2
CyA	10.6 ± 0.8	1.5 ± 0.2	0.7 ± 0.1
LTx + CyA	18.1 ± 2.7	5.7 ± 2.3	2.4 ± 1.0
Vehicle	13.5 ± 1.6	1.0 ± 0.3	0.7 ± 0.1
Vehicle + LTx	9.9 ± 0.9	4.9 ± 1.3	1.7 ± 0.2
HAL	16.4 ± 1.6	3.3 ± 1.7	1.7 ± 0.5

2. The CyA group (n = 18) was treated with cyclosporin.
3. The vehicle group (n = 5) was treated with the cyclosporin vehicle (Cremophor EL) only.
4. The HAL group (n = 7) was the group in which the hepatic artery was ligated 3 weeks prior to the bile secretion studies.

Altogether, 71 rats were included in the bile secretion studies. In the first experiment, bile flow and bile acid secretion were studied during continuous depletion of the bile acid pool for 5 h, after which the same parameters were studied during intravenous bile acid infusion for 2 h.

Cyclosporin and vehicle administration

Animals receiving cyclosporin received daily intraperitoneal injections at a dose of 10 mg/kg body weight for 3 weeks. The vehicle group received Cremophor EL in a corresponding dose (13 mg/kg body weight per day) over the same period of time.

Experimental procedure

The bile secretion studies were performed 3 weeks after the start of cyclosporin or vehicle treatment or 3 weeks after the transplantation procedure. The animals receiving cyclosporin or vehicle treatment were given their last dose 24 h before the acute experiments. The rats were sacrificed at the end of the study.

The rats were anesthetized with pentobarbitone and the jugular vein was catheterized. A laparotomy was performed and the bile duct was divided and catheterized with a polyethylene tube in the proximal direction. Bile samples were collected in preweighed vials that were changed every hour. Bile was collected continuously for 5 h, after which sodium taurocholate was infused intravenously at a rate of 2 μmol/min per kilogram body weight for 1 h and then 4 μmol/min per kilogram for the following hour. During the bile acid infusion period, bile collected during the last 40 min of every hour was used in the calculations.

Analysis

On the day of the bile secretion studies, venous blood samples were taken from the tail veins and analyzed for liver enzymes and bilirubin (Reflotron, Boehringer-Mannheim, FRG). At the end of the experiment and after the bile flow studies, blood samples were taken from the jugular vein and serum cyclosporin levels were measured by whole blood radioimmunoassay (Cyclo-Trac, Incstar, Stillwater, Minn., USA). This method uses a specific monoclonal antibody and thus only measures cyclosporin and not its metabolites.

Bile acid assays

The total bile salt concentration in bile was determined with an enzymatic method using 3α-hydroxysteroid dehydrogenase (Sterognost-3α, Nyegaard, Oslo, Norway). Sodium-chenodeoxycholate provided by Nyegaard was used as a standard. The intra-assay coefficient of variation for determination of sodium-chenodeoxycholate was 2.8 % at a concentration of 25 mmol/l and 1.9 % at a concentration of 50 mmol/l [9].

Calculations and statistics

Results are presented as means ± SE. An ANOVA was used to analyze the liver enzymes and bilirubin as well as bile flow and bile salt secretion rate (BSSR). When the analyses indicated the presence of a significant difference between the groups, the Fisher test was used to compare means. The relationships between bile flow and BSSR were analyzed by least square linear regression analysis. The calculated intercepts were considered to represent the bile acid-independent flow (BAIF) and the slopes were termed the bile acid-dependent flow (BADF). Multiple regression analysis with dummy variables was used to compare slopes and intercepts between groups.

Results

Liver biochemistry

No significant change in liver enzymes or bilirubin was registered among the different groups at the time of the bile secretion studies (Table 1).

Cyclosporin Concentrations

The concentrations were measured after the bile secretion studies and at least 24 h since the last given dose. The cyclosporin concentrations were 1462 ± 98 μg/l in the CyA group and 1533 ± 122 μg/l in the LTx-CyA group. No difference between the two groups given cyclosporin treatment was noted, and biologically significant concentrations were registered in all animals.

Liver weights

After the bile secretion studies, the livers were weighed. The liver wet weights were similar in the control group (6.0 ± 0.2 g), the CyA group (6.1 ± 0.3 g), the HAL group (5.8 ± 0.4 g) and the vehicle group (6.8 ± 0.3 g), whereas in the three liver-transplanted groups (LTx; LTx + vehicle; LTx + CyA) a weight increase was noted (8.9 ± 0.4 g, 8.3 ± 0.6 g and 9.1 ± 0.5 g, respectively).

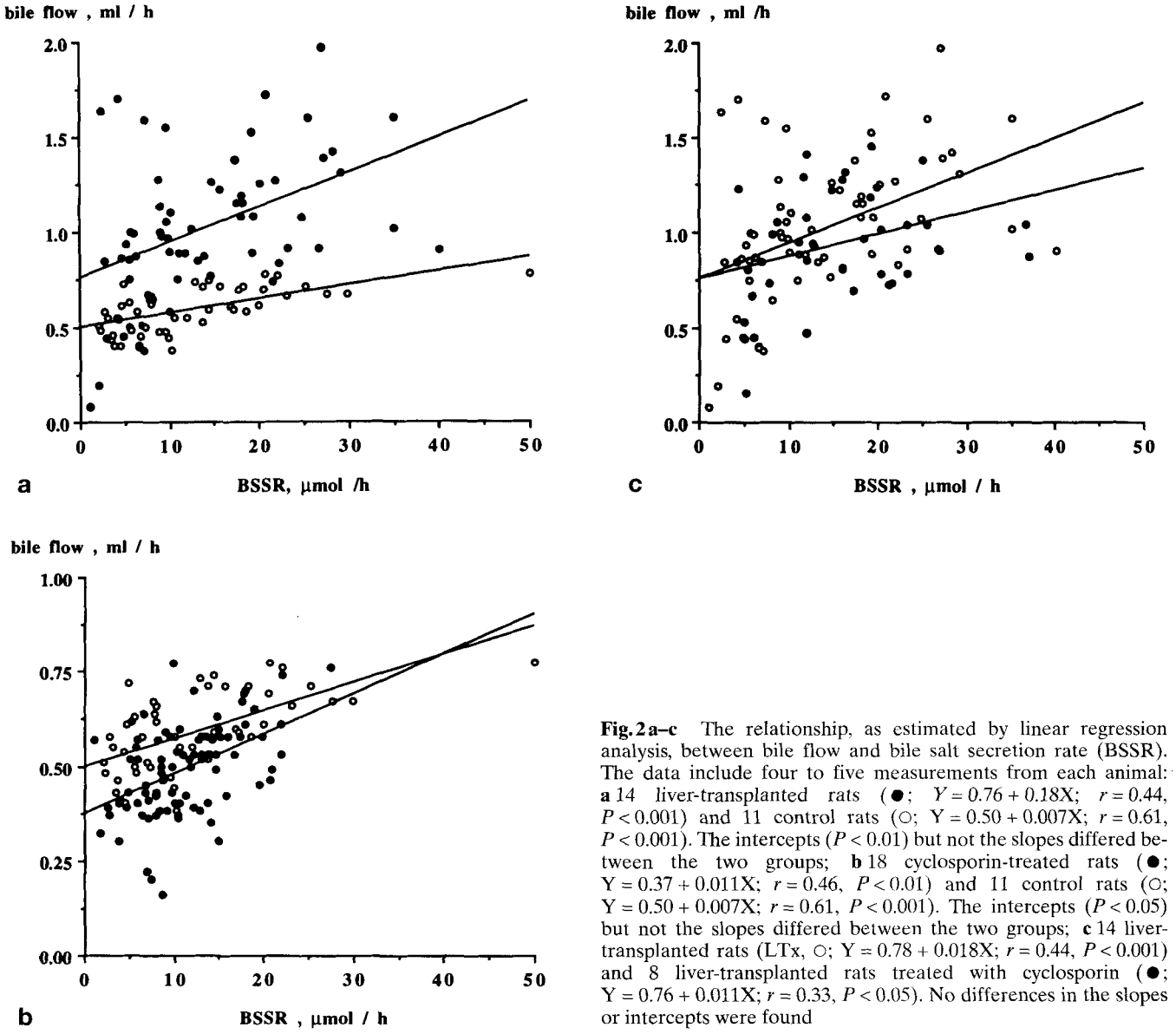

Fig. 2a–c The relationship, as estimated by linear regression analysis, between bile flow and bile salt secretion rate (BSSR). The data include four to five measurements from each animal: **a** 14 liver-transplanted rats (●; $Y = 0.76 + 0.18X$; $r = 0.44$, $P < 0.001$) and 11 control rats (○; $Y = 0.50 + 0.007X$; $r = 0.61$, $P < 0.001$). The intercepts ($P < 0.01$) but not the slopes differed between the two groups; **b** 18 cyclosporin-treated rats (●; $Y = 0.37 + 0.011X$; $r = 0.46$, $P < 0.01$) and 11 control rats (○; $Y = 0.50 + 0.007X$; $r = 0.61$, $P < 0.001$). The intercepts ($P < 0.05$) but not the slopes differed between the two groups; **c** 14 liver-transplanted rats (LTx, ○; $Y = 0.78 + 0.018X$; $r = 0.44$, $P < 0.001$) and 8 liver-transplanted rats treated with cyclosporin (●; $Y = 0.76 + 0.011X$; $r = 0.33$, $P < 0.05$). No differences in the slopes or intercepts were found

Bile secretion studies during bile acid depletion

In the first experimental set-up during the acute bile secretion studies, bile flow was registered during depletion of the bile acid pool for 5 h. Using multiple regression analysis, all groups showed a significant correlation between bile flow and BSSR.

LTx vs control

The regression line calculated for the liver-transplanted animals had a higher intercept and thus an increased BAIF when compared with the control animals. No significant change of the slope of the regression line and, thus, of the BADF occurred when the two groups were compared (Fig. 2 A). When the initial bile secretion was studied during the first measurement hour, the liver-transplanted animals had an increased bile flow compared with the controls, but no change in the BSSR was seen. When HAL rats were used as controls, an increased bile secretion was still noted in the liver-transplanted rats (Table 2 A).

Table 2 Bile flow and bile secretion rate during the 1st hour of registration

A Liver-transplanted animals (LTx) compared with animals with ligated hepatic artery (HAL) and controls

	Bile flow (ml/h)	BSSR (μmol/h)
Control	0.66 ± 0.03	22.9 ± 3.8
HAL	0.96 ± 0.06*	18.9 ± 1.4
LTx	1.29 ± 0.09*, **	21.7 ± 2.1

* $P < 0.05$ compared with control; ** $P < 0.05$ compared with HAL

B Nontransplanted cyclosporin-treated animals (CyA) compared with controls

	bile flow (ml/h)	BSSR (μmol/h)
Control	0.66 ± 0.03	22.9 ± 3.4
CyA	0.51 ± 0.03*	15.7 ± 1.4*

* $P < 0.05$ compared with control

C Liver-transplanted cyclosporin-treated animals compared with liver-transplanted animals without cyclosporin

	Bile flow (ml/h)	BSSR (μmol/h)
LTx	1.29 ± 0.09	21.7 ± 2.1
LTx + CyA	1.01 ± 0.08*	20.3 ± 1.9

* $P < 0.05$ compared with LTX

D Vehicle-treated animals compared with controls

	Bile flow (ml/h)	BSSR (μmol/h)
Control	0.66 ± 0.03	22.9 ± 3.4
Vehicle	1.10 ± 0.04*	20.6 ± 1.6
LTx	1.29 ± 0.09*	21.6 ± 2.1
Vehicle + LTx	0.95 ± 0.13*, **	20.8 ± 3.1

* $P < 0.05$ compared with control; ** $P < 0.05$ compared with LTX

CyA vs control

The cyclosporin-treated animals, on the other hand, had a reduced BAIF when compared with the control animals (Fig. 2B). A reduction in bile flow in the CyA group was also noted during the 1st hour of registration. In the cyclosporin-treated animals, a reduction in the BSSR was also seen (Table 2B).

LTx + CyA vs LTx

No significant effect of cyclosporin on bile flow or BSSR could be detected in the liver-transplanted rats when the regression lines between bile flow and BSSR were compared (Fig. 2C). However, in conformity with the non-transplanted cyclosporin-treated rats, we found a reduced bile flow during the first hours of measurement (Table 2C).

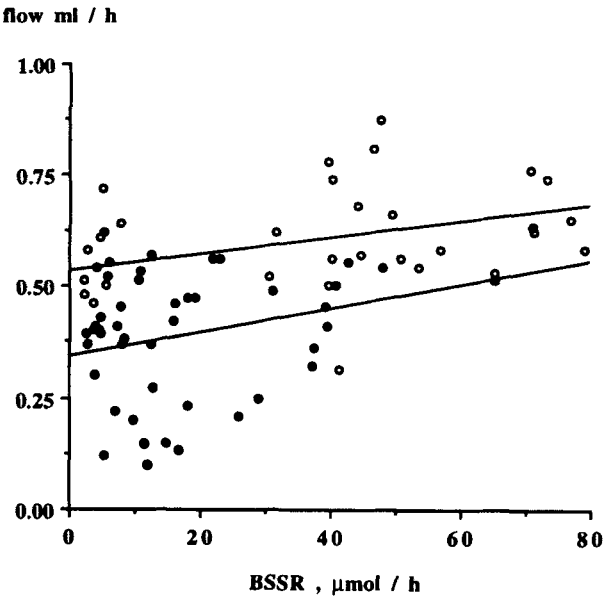

Fig. 3 The relationship, as estimated by linear regression analysis, between bile flow and bile salt secretion rate (BSSR) when bile flow was stimulated by intravenous bile acid infusion in 18 cyclosporin-treated rats (●; $Y = 0.34 + 0.003X$; $r = 0.31$, $P < 0.05$) and in 11 control rats (○; $Y = 0.53 + 0.002X$; $r = 0.39$, $P < 0.05$). The data include three measurements from each animal. The intercepts ($P < 0.05$) but not the slopes differed between the two groups

Vehicle vs control

The vehicle-treated animals had a significantly increased bile flow compared with the control animals. This effect was not registered in the liver-transplanted rats treated with the vehicle; on the contrary, they responded with a reduction in the bile flow (Table 2D).

Bile secretion studies during bile acid infusion

When taurocholic acid was infused, the control animals responded as expected and the bile flow correlated well ($P < 0.01$) to the BSSR. The cyclosporin-treated animals had a lower intercept and, thus, a reduced BAIF, also in this set-up (Fig. 3).

Stimulation of bile secretion by bile acid infusion resulted in a lower response of BSSR in the cyclosporin-treated group compared to the control group. In the LTx group, the response was low, and in these groups no significant regression lines could be drawn. A reduction in bile flow was, however, only seen in the CyA group when compared with the control group (0.38 ± 0.03 ml/h vs 0.63 ± 0.03 ml/h).

Discussion

In this experimental study we have shown that cyclosporin-treated rats have a reduced bile flow, mainly caused by a reduction in the BAIF, but also a reduction in the BSSR. Cyclosporin-treated rats also responded with a lower BSSR and bile flow compared with controls when bile acids were infused. Liver transplantation, on the other hand, resulted in an increased volume of bile, due to an increased BAIF, but no change in the BSSR. However, when bile acids were given intravenously, the liver-transplanted animals did not respond with the same high BSSR rate as did the control animals.

The model of bile secretion studies in the rat is well validated [2]. The dose of cyclosporin used in the present study has been tested by Stone and coworkers [29]. The transplantation model has previously been tested in other kinds of studies but thus far not in bile secretion studies [20, 21, 31, 38].

In a number of experimental studies, mainly in rodents, cyclosporin has been shown to reduce bile flow and the bile acid secretion rate [23, 26, 29, 34]. This observation is further supported by our present data. In order to be able to separate the BAIF from the BADF, bile secretion has to be observed during a dynamic change. This is accomplished either by depletion of the bile acids by drainage, thus reducing bile secretion with time, or by enhancing bile secretion by infusion of bile acids intravenously. In the regression lines thus calculated, the slope will give an estimate of the BADF and the intercept an estimate of the BAIF [2, 35]. Using the depletion model, one will get a steeper slope than in the infusion model [35], and it is important to have that in mind when comparing data obtained with the two different set-ups.

Two studies of the effect of chronic administration of cyclosporin have been performed previously. Stone and coworkers found a reduction in the BAIF in rats treated with cyclosporin in a similar manner as in the present study, using the infusion model [29]. On the other hand, Thai and coworkers, also using a bile acid infusion model, did not see this reduction. When bile acids were infused in our study, we saw not only a reduction in the BAIF but also a lower BSSR in the cyclosporin-treated animals. The reduction in the BAIF was also registered when the bile acid depletion was used in the present study.

In studies with acute infusions of cyclosporin, a reduction in BSSR and bile flow has been demonstrated in the perfused rat liver [26] as well as in rats in vivo [6, 25]. Moseley and coworkers, using basolateral and canalicular rat liver plasma membrane vesicles, were recently able to show that cyclosporin induced a competitive inhibition of taurocholate transport [23]. Roman and coworkers have even shown that the acute cholestatic effect of the vehicle does not influence the acute effect of cyclosporin, since bile flow was reduced even when the vehicle was removed [24]. When the vehicle Cremophor EL was chronically administered in our study, we even found a significantly elevated bile flow and, thus, a choleretic effect of this vehicle. This was, however, not seen in the liver-transplanted rats where, in fact, a reduction in bile secretion of the same magnitude as in the cyclosporin-treated animals occurred. The finding implies that one must be cautions when using this agent in studies of bile formation, either as a control or together with cyclosporin. Cyclosporin-treated rats seem to respond with a reduction in bile secretion, mainly the BAIF, whereas another species tested, dogs, seems to differ in this respect [4, 30].

One aim of this study was to separate the effect of cyclosporin from that of preservation and reperfusion damage to a grafted liver. Surprisingly, we have, in this experimental set-up, found that syngeneic rat liver grafts have an increased bile flow. This is the first time this phenomenon is reported and we can only speculate as to the mechanisms behind these observations. Sperber, in 1959, postulated that osmotic forces following the active secretion of substances like bile acids generated water flow into the bile canaliculi, and numerous studies have since confirmed this assumption [28]. Bile formation is thus initiated at the level of the hepatocytes and is modified by absorption or secretion more distally along the biliary tree. The canalicular water influx and distal absorption is probably quite high [10, 12, 18], and it is possible that minor damage to the biliary epithelium could cause a reduced water absorption and, thus, a higher bile flow rate. Our data do not rule out an enhanced canalicular influx of water, but that is a more improbable explanation since the water flux is osmotically dependent on the active secretion of bile acids and the bile acid secretion rate was not changed in the liver-transplanted rats.

Liver transplantation causes denervation of the liver, and it has been demonstrated that liver transplantation is an effective technique to suppress adrenergic responses in the liver [7]. The role of sympathetic and parasympathetic innervation in bile secretion has received little attention. It has been suggested that adrenergic stimulation reduces bile flow and, thus, denervation could influence the volume of bile [13, 33]. In fact, it has been suggested that adrenergic stimuli enhance water absorption from the gallbladder and the bile ducts [13].

The transplanted livers increased in weight, and this could be due to an increased density of portal tracts [31] or maybe to a bile duct proliferation [37]. Some authors have claimed that these findings can be attributed to an insufficient arterial supply [37]. We have, in a previous study, found a slight portal fibrosis with an enhanced density of the portal tracts in the liver-transplanted rats compared to controls, but in that study we did not find any morphological difference if an arterial

anastomosis had been performed or not [31]. This has also been confirmed by Kamada and coworkers in a recent publication [22]. In a recent study it was shown that, in this model, an arterial revascularization occurs very rapidly, and after 3 weeks the arterial blood supply is even higher than in control rats with intact arterial supply [32].

In this study, we have not expressed our data in relation to liver weight, since this morphological change occurs in the transplanted livers and the changes in bile secretion seemed more relevant to the transplanted organ. When interpreting our data, these findings should, however, be kept in mind since, in some respects, they mimic the morphological changes seen in bile duct-ligated animals, where an enhanced bile formation was also registered [1]. Liver cirrhosis and/or bile duct obstruction have been associated with an increased choleresis in humans, although the explanation for this phenomenon is thus far speculative [11, 16]. In the present series, the bile duct anastomosis was intact in all rats included in our study, and they showed no change in their liver enzymes that would indicate bile duct obstruction. Histology in the transplanted rats showed bile duct proliferation but no signs of cirrhosis or bile duct obstruction. The hepatocytes were normal in appearance.

In this experimental model, the liver transplantation procedure-induced preservation and/or reperfusion injury did not result in an impaired bile flow, although one may speculate that the higher bile volume in the transplanted animals could be explained by a reduction in the water absorption in a damaged biliary system. The present study also shows that cyclosporin is a potent cholestatic drug that will influence the bile formation process even if no other signs of liver dysfunction are present. An important observation was that bile acid infusion did not enhance bile flow and BSSR as expected in the cyclosporin-treated rats.

Our results indicate that cyclosporin may be responsible for the reduction in bile flow seen after liver transplantation in humans, although we could only show a significant cholestatic effect in the nontransplanted animals since the reduction seen in the transplanted rats might have been caused by the vehicle. The increased bile flow in the transplanted rats seemed to dominate over the cholestatic effect of cyclosporin in this rat model. Since this is a syngeneic transplantation model, the effect on bile flow of the constant immunological challenge to the allogeneic liver graft has not been tested. Bile acid feeding can reduce the cholestatic effect of cyclosporin in rats [6] and has also been tried in humans [15]. Caution is, however, warranted when transposing current, as well as previous, rat model data to the effects on bile secretion seen in liver-transplanted patients. The effects of liver transplantation, immunosuppression, and bile acid treatment on bile secretion in humans have to be investigated further.

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References

1. Alpini G, Lenzi R, Sarkozi L, Tavoloni N (1988) Biliary physiology in rats with bile ductular cell hyperplasia. Evidence for a secretory function of proliferated bile ductules. J Clin Invest 81: 569–578
2. Balabaud C, Kron KA, Gumucio JJ (1977) The assessment of the bile salt-nondependent fraction of canalicular bile water in the rat. J Lab Clin Med 89: 393–399
3. Bowers BA, Rotolo FS, Watters CR, Cucciaro G, Branum GD, Meyers WC (1989) Regulation of bile secretion following liver transplantation. Transplant Proc 21: 3354
4. Brems JJ, Reese J, Kane R III, Kaminski DL (1991) Effect of cyclosporin and steroids on canine bile flow. Hepatology 14: 523–527
5. Calne RY, White DJG, Thiru S, McMaster P, Craddock GN, Evans DB, Dunn DC, Pentlow BD, Rolles KV (1978) Cyclosporin A in patients receiving renal allografts from cadaver donors. Lancet II: 1323–1327
6. Chanussot F, Botta-Fridlund D, Lechene de la Porte P, Sbarra V, Portugal H, Pauli A-M, Hauton J, Gauthier A, Lafont H (1992) Effects of cyclosporin and corticosteroids on bile secretion in the rat. Transplantation 54: 226–231
7. Cucchiaro G, Yamaguchi Y, Mills E, Kuhn CM, Anthony DC, Branum GD, Epstein R, Meyers WC (1990) Evaluation of selective liver denervation methods. Am J Physiol 259: G 781–G 785
8. Ericzon B-G, Eusufzai S, Kubota K, Einarsson K, Angelin B (1990) Biliary lipid secretion early after liver transplantation. Transplant Proc 22: 1537–1538
9. Fausa O, Skålhegg BA (1974) Quantitative determination of bile acids and their conjugates using thin-layer chromatography and a purified 3a-hydroxysteroid dehydrogenase. Scand J Gastroenterol 9: 249–254
10. Friman S, Rådberg G, Svanvik J (1988) Hepatic clearance of PEG-900 and mannitol in the pig. Digestion 39: 172–180
11. Friman S, Filipsson S, Svanvik J (1989) Hypercholeresis after release of protracted extrahepatic cholestasis. Acta Chir Scand 155: 355–356
12. Friman S, Leandersson P, Tagesson C, Svanvik J (1990) Biliary excretion of different sized polyethylene-glycols in the cat. J Hepatol 11: 215–220
13. Friman S, Rådberg G, Svanvik J (1990) Adrenergic influence on bile secretion – an experimental study in the cat. Acta Physiol Scand 140: 287–293

14. Friman S, Persson H, Karlberg I, Svanvik J (1992) The bile acid independent flow is reduced in the transplanted liver. Transpl Int 5 [Suppl]: S163–S167
15. Friman S, Persson H, Scherstén T, Svanvik J, Karlberg I (1992). Adjuvant treatment with ursodeoxycholic acid reduces acute rejection after liver transplantation. Transpl Int 5 [Suppl]: S187–S189
16. Friman S, Wingren U, Aszetély M, Persson H, Karlberg I (1992) Extreme hypercholeresis in a patient with a transplanted liver. Transplant Proc 24: 394–395
17. Haagsma EB, Huizenga JR, Vonk RJ, Albers CJEM, Grond J, Krom RAF, Gips CH (1987) Composition of bile after orthotopic liver transplantation. Scand J Gastroenterol 22: 1049–1055
18. Javitt NB (1982) Hepatic bile formation: assessment of water flow using mannitol and polyethyleneglycol MW 900. In: Bradley SE, Purcell (eds) The paracellular pathway. Macy Foundation, New York, pp 234–241
19. Javitt NB, Shiu MH, Fortner JG (1971) Bile salt synthesis in the transplanted human liver. Gastroenterology 60: 405–408
20. Kamada N, Calne RY (1983) A surgical experience with five hundred thirty liver transplant in the rat. Surgery 93: 64–69
21. Kamada N, Davies HFFS, Wight D, Culank L, Roser B (1983) Liver transplantation in the rat. Transplantation 35: 304–311
22. Kamada N, Sumunito R, Kaneda K (1992) Hepatic artery reconstruction and rat liver transplantation. Surgery 111: 195–200
23. Moseley R, Johnson TR, Morrisette JM (1990) Inhibition of bile acid transport by cyclosporine A in rat liver plasma membrane vesicles. J Pharmacol Exp Ther 253: 974–980
24. Roman ID, Monte MJ, Esteller A, Jiménez R (1989) Cholestasis in rats by means of intravenous administration of cyclosporin vehicle, Cremophor EL. Transplantation 48: 554–558
25. Roman ID, Monte MJ, Gonzalez-Buttargo JM, Esteller A, Jimenez R (1990) Inhibition of hepatocytory vesicular transport by cyclosporin A in the rat: relationship with cholestasis and hyperbilirubinemia. Hepatology 12: 83–91
26. Rotolo FS, Branum GD, Bowers BA, Meyers WC (1985) Effect of cyclosporin on bile secretion in rats. Am J Surg 151: 35–40
27. Shiffman ML, Carithers RL Jr, Posner M, Moore EW (1991) Recovery of bile secretion following orthotopic liver transplantation. J Hepatol 12: 351–361
28. Sperber I (1959) Secretion of organic anion in the formation of urine and bile. Pharmacol Rev 11: 109–134
29. Stone BG, Udani M, Sanghvi A, Warty V, Plocki K, Bedetti C, Thiel DH van (1987) Cyclosporin A – induced cholestasis. Gastroenterology 93: 344–351
30. Sutherland FR, Preshaw RM, Shaffer EA (1990) The effect of cyclosporine A on bile secretion in dogs. Can J Physiol Pharmacol 68: 136–138
31. Svensson G, Karlberg I (1991) Effect of rearterialization on short-term graft function in orthotopic rat liver transplantation. Eur Surg Res 23: 269–277
32. Svensson G, Naredi P, Hafström LO, Tufveson G (1994) Quantitative measurements of collateral arterial blood flow in nonarterialized rat liver grafts. Transpl Int 7: 136–139
33. Tanturi CA, Ivy AC (1938) A study of the effect of vascular changes in the liver and the excitation of its nerve supply on the formation of bile. Am J Physiol 121: 61
34. Thai B Le, Dumont M, Michel A, Erlinger S, Houssin D (1988) Cholestatic effect of cyclosporin in the rat: an inhibition of bile acid secretion. Transplantation 46: 510–512
35. Wheeler HO, Ross ED, Bradley SE (1968) Canalicular bile production in dogs. Am J Physiol 214: 866–875
36. Wood AJ, Maurer G, Niederberger W, Beveridge T (1983) Cyclosporin: pharmackinetics, metabolism and drug interactions. Transplant Proc 15: 2409–2418
37. Zhao D, Zimmermann A, Wheatley AM (1992) Importance of an intact arterial supply in rat liver transplantation: a time related study. J Hepatol 16: S46
38. Zimmerman FA, Buther GW, Davies HS, Brons G, Kamada N, Turel O (1979) Techniques for orthotopic liver transplantation in the rat and some studies of the immunologic responses to fully allogenetic liver grafts. Transplant Proc 11: 571–577