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The bile acid independent flow is reduced in the transplanted liver

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Abstract. Bile secretion is an important indicator of liver graft function. Reports on bile formation by the transplanted liver with stable function some months after operation are scarce. In this study bile flow, bile salt secretion rate (BSSR) and biliary clearance of polyethylene glycol (PEG) 900, a marker of canalicular bile flow, were studied in a group of liver-transplanted (LTX) patients ($n = 8$) 3–6 months after transplantation. A group of cholecystectomized patients with indwelling T-tubes ($n = 6$) served as a control group. Both groups were treated with oral ursodeoxycholic acid (500 mg/day). On the day of the study bile was drained for 6 h by gravity and four-hourly samples were used in the calculations. The relation between bile flow and BSSR analysed with linear regression showed a reduced bile acid independent flow in the liver-transplanted group (0.11 ml/min) compared with the control group (0.20 ml/min). The relation between biliary clearance of PEG 900 and BSSR showed a significantly steeper slope for the cholecystectomized control patients (1.40 ml/μmol) compared with the liver-transplanted patients (0.30 ml/μmol). We conclude, that in spite of stable graft function with normal liver enzmyes, the transplanted liver has a reduced bile acid independent bile flow. The transplanted liver also has a reduced biliary clearance of PEG 900 indicating a reduced canalicular bile flow. The cause of this impaired bile formation could be due to the influence of the immunosuppressive drug cyclosporin, the result of damage to the liver during preservation and reperfusion or the continuous immunological challenge to the graft.

Key words: Bile – Bile secretion – Liver transplantation – Ursodeoxycholic acid – Polyethylene glycol – Cyclosporin

Bile secretion is an important indicator of liver graft function. Bile flow is initially profoundly depressed after orthotopic liver transplantation (OLT), but over the following few days the bile volumes and bile acid secretion increase and reach stable levels after 10–14 days, provided that there is a well-functioning liver graft [5, 10, 12, 21].

Bile is an aqueous solution of electrolytes and organic compounds. Bile acids, bilirubin, cholesterol and phospholipds are the major components. Hepatic bile consists of 90% water and the composition is regulated by the canalicular and ductular water fluxes. 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 assertion [22]. Bile formation is thus initiated at the level of the hepatocytes and is modified by absorption or secretion more distally along the biliary tree. To separate the canalicular from the ductular fraction of the hepatic bile formation, biliary clearances of carbohydrates like erythritol and mannitol have been used for the last 20 years [7, 24]. The validity of these markers has been questioned, and recent studies have shown a higher biliary clearance of polyethylene glycol (PEG) 900, a marker used in studies of kidney physiology, indicating a considerably higher canalicular water influx than previously estimated [8, 9, 11].

Although initial bile formation is an important indicator of graft function, especially during the first postoperative weeks, the reports of bile formation after a few months following liver transplantation when liver graft function is stable, are scarce [1].

The aim of the present study was to investigate the different fractions of the bile formation process in well-functioning liver allografts with no signs of graft dysfunction.

Materials and methods

Drugs, chemicals and solutions

[1,2-^{3}H]-PEG (6.75 mCi/g, range 800–1000 Da with an average molecular weight of 900 Da) and [24-^{14}C]-taurocholate (46.7 mCi/mmol) were purchased from NEN Boston, Mass., USA.

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Table 1. Basal patient data and liver function tests at the time of bile sampling in the liver-transplanted group

Diagnosis	Age	Sex	Time post-transplantation (months)	S-bil (μmol/l)	S-ALP (μcat/l)	S-AST (μcat/l)	S-ALT (μcat/l)
PBC	51	female	3	23	3.7	0.35	0.19
Scl.chol.	26	male	3	48	7.2	0.87	0.93
CAH	43	female	6	45	3.3	0.53	0.56
α-1 anti tryp. def	47	male	4	6	1.9	0.11	0.44
CAH	62	male	3	14	6.9	0.34	0.63
Morbus Osler	40	female	4	9	5.4	0.65	1.00
ALCI	52	male	3	15	5.2	0.16	0.31
Amyloidosis	29	male	3	24	1.9	0.40	0.30
Mean ± SEM	45 ± 5			23 ± 8	4.7 ± 0.9	0.48 ± 0.11	0.63 ± 0.12

ALP, alkaline phosphatase; AST, asparate aminotransferase; ALT, alanine aminotransferase; PBC, primary biliary cirrhosis; CAH, chronic active hepatitis; Scl. chol, sclerosing cholangitis; ALCI, alcohol cirrhosis

Table 2. Basal patient data and liver function tests at the time of bile sampling in the cholecystectomy group

	Age	Sex	Time post-operation (weeks)	S-bil (μmol/l)	S-ALP (μcat/l)	S-AST (μcat/l)	S-ALT (μcat/l)
	58	female	3	18	4.3	0.57	0.62
	44	female	3	20	4.2	0.63	0.68
	38	female	10	17	3.7	0.32	0.53
	73	female	4	14	4.7	0.18	0.34
	50	female	4	7	3.0	0.64	0.63
	79	male	4	17	14	0.67	0.81
Mean ± SEM	57 ± 7			16.3 ± 1.9	5.6 ± 1.7	0.50 ± 0.08	0.65 ± 0.05

ALP, alkaline phosphatase; AST, asparate aminotransferase; ALT, alanine aminotransferase

Sodium glycocholate (grade I, 99% pure) was obtained from Sigma Chemicals, St. Louis, Mo., USA. Ursodeoxycholic acid (Ursofalk) was manufactured by Falk Co, FRG; Prednisolon by KabiVitrum AB, Stockholm, Sweden; azathioprine (Imural) by Wellcome, London, UK; and cyclosporin (Sandimmun) by Sandoz AG, Basel, Switzerland.

Patients

A group of liver transplanted (LTX) patients ($n = 8$) was studied 3–6 months postoperatively (Table 1). The patients all had stable graft function with normal or only slightly elevated liver enzymes. A choledocho-choledochostomy with insertion of a T-tube had been performed in all cases. The enterohepatic circulation was re-established by clamping the T-tubes within 10 days after transplantation. These patients followed an immunosuppressive protocol of sequential quadruple drug therapy. At the time of investigation their immunosuppression had been reduced to corticosteroids (Prednisolon) 10 mg/day, azathioprine (Imurel) 1–2 mg/kg per day and oral cyclosporin (Sandimmun) 4 mg/kg per day (whole blood levels 150–200 ng/ml). These patients were also given adjuvant treatment with ursodeoxycholic acid (UDCA) starting the first postoperative week at a dose of 500 mg/day.

A group of cholecystectomized patients ($n = 6$), in whom the common duct had been explored at a T-tube inserted, served as a control group. These patients were investigated 3–6 weeks following a cholecystectomy. UDCA at a dose of 500 mg/day was given to these control patients for 14–15 days prior to investigation. All patients had normal or only slightly elevated plasma levels of aminotransferases and bilirubin at the time of the study (Table 2). In this group the T-tubes had been clamped at least 10 days prior to the bile secretion studies.

In a separate group of liver transplanted patients ($n = 8$) the initial bile secretion was followed during the first 7–14 days as long as the T-tube was opened.

All of the patients gave informed consent, and the study was approved by the Ethical Committee of University of Gothenburg for investigations involving human subjects.

Study protocol

Studies of bile formation with stable graft function. The patients fasted during the 12-h period prior to and throughout the test. Fluid loss was compensated by intravenous infusions of Ringer's solution. At the start of the study the T-tube was opened. ^{3}H-labelled PEG 900 (5 μCi) was given intravenously as single bolus injections followed by constant infusions of the marker molecule in saline at a rate of 1.5 μCi/h throughout the study. A period of 2 h was allowed to achieve steady-state plasma concentrations of the two markers. Bile and plasma samples from the following 4 h were used in the calculations. The bile was drained by gravity and collected in pre-weighed vials that were changed every hour. Plasma samples were also collected hourly.

Studies of initial bile formation. In eight patients the initial bile formation was followed. The total bile volume over a 24-h period was collected and from this a sample of bile was analysed as to the bile acid content.

Control experiments. Previous studies have shown that bile volumes drained by gravity correspond well to those obtained when the distal part of the common bile duct is occluded with a balloon [2, 19].

In order to test whether all bile was drained in the described manner, ^{14}C-labelled taurocholic acid was injected intravenously and the recovery in bile over time was measured in one cholecystectomized and one liver-transplanted patient. If bile was lost into the intestines the recovery of the labelled bile acid would be low and the radioactivity would later reappear in bile due to its enterohepatic circulation.

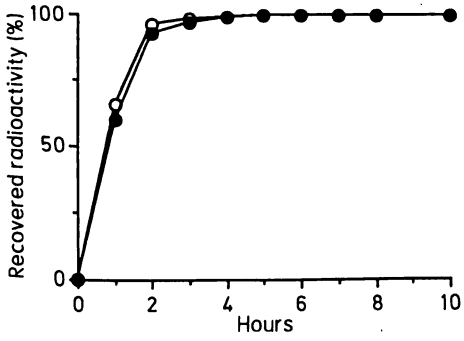

Fig. 1. Time course of recovery of intravenously infused ^{14}C-labelled taurocholic acid in two patients. ●, liver transplanted patient; ○, cholecystectomized patient

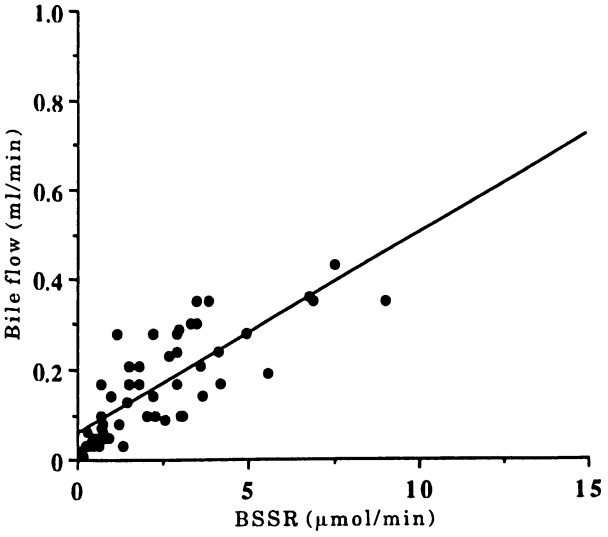

Fig. 2. The relation between bile flow and bile salt secreting rate (BSSR) as expressed with linear regression analysis in eight liver-transplanted patients collected during the first 14 days postoperatively. $Y = 0.06 + 0.044x$; $r = 0.78$; $P < 0.001$. The intercept does not significantly differ from zero

Bile salt assays

The total bile salt concentration in bile was determined by an enzymatic method using 3a-hydroxysteroid dehydrogenase (Sterognost-3a, Nyegaard and Co, Oslo, Norway). Sodium-chenodeoxycholate provided by Nyegaard and Co, 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. The intra-assay coefficient of variation for determination of sodium-glycocholate was 3.8% at a concentration of 25 mmol/l and 2.9% at a concentration of 50 mmol/l [6].

Measurement of radioactivity

To reduce quenching, bile samples were bleached with 10% trichloroacetic acid. To samples of plasma and bile (200 μl) were added 10 ml of Opti-Fluor (Packard Inst., Dovners Grove, Ill., USA). The radioactivity was counted in a TRI-CARB 1500 scintillation counter (Packard). Correction for sample quenching was performed by the spectral index method [17].

Calculations and statistics

Results are presented as means ± SEM. The relationships between bile flow and bile salt secretion rate were analysed by means of linear regression. The calculated intercepts 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.

The clearance of PEG 900 and mannitol was calculated as the product of bile flow and the ratio between bile and plasma concentrations of the tracers at steady-state conditions.

$$\text{Biliary clearance} = \frac{dpm\ bile}{dpm\ plasma} \times \text{bile flow}$$

The relationships between biliary clearance of PEG 900 and bile salt secretion rate were analysed by means of linear regression. Four-hourly data obtained from each patient were used in these calculations.

Results

Tracer concentrations in plasma

The ^{3}H activity in plasma was stable from the beginning of the first sampling period and throughout the studies.

Recovery in bile of an IV bolus injection of ^{14}C-taurocholic acid

In two separate control experiments the efficiency of the bile drainage procedure was tested by studying the early recovery in bile of an IV bolus injection of a labelled bile acid. During the first 2 h after the injection the majority of the radioactivity was recovered in the hepatic bile outflow (Fig. 1). There was no evident recirculation of labelled bile acids detected by an increased radioactivity in the bile outflow in the following 8 h studied.

Initial bile formation

Bile flow correlated well with the BSSR during the first 7–14 days ($P < 0.01$) (Fig. 2). When the data from the first postoperative days were explored in this manner, the bile acid independent flow of 0.06 ml/min did not differ significantly from zero.

Bile formation with stable graft function

The bile flow, the bile acid concentration and the BSSR were measured during the first hour following the opening of the T tubes. No differences between the control and LTX group were found (Table 3).

Bile flow vs BSSR

Bile flow correlated well with BSSR in both groups of patients (Fig. 3). The slopes of the regression lines did not differ, but the intercept was lower for the LTX group indicating a reduced BAIF (0.11 ml/min) compared with the control group (0.20 ml/min) ($P < 0.05$).

Biliary clearance of PEG 900 vs BSSR

The biliary clearance of PEG 900 correlated well with the BSSR in both groups of patients (Fig. 4). The biliary clearance of PEG 900 was reduced in the LTX group (0.30 ml/μmol) compared with the control group (1.40 ml/μmol) ($P < 0.05$).

Table 3. Bile flow, bile acid concentration and BSSR during the first hour of bile sampling

	Bile flow (ml/min)	Bile acid concentration (mmol/l)	BSSR (μmol/min)
Control ($n = 6$)	0.49 ± 0.09	39.2 ± 3.6	19.1 ± 3.6
LTX ($n = 8$)	0.55 ± 0.07	45.8 ± 2.0	25.1 ± 3.2

None of the differences between the control and LTX groups was significant

Discussion

In this study the bile formation in liver-transplanted patients with stable graft function was compared with that of a group of cholecystectomized patients. We registered a reduced BAIF as well as reduced biliary clearance of PEG 900 in the liver-transplanted patients.

Previous studies of bile secretion following OLT have mainly been performed during the first 2–3 postoperative weeks and with an interrupted enterohepatic circulation. These studies showed extremely low bile salt secretion rates partly due to continuous drainage of bile and bile acids in combination with low initial bile acid synthesis [5, 10, 12, 21]. The conclusion that can be drawn from these studies, is that bile flow as well as the secretion of bile acids recover gradually during the first 10–14 days. These data are confirmed by our own data (Fig. 2). Our patients received UDCA from the first postoperative day which could account for a slightly higher, but still very low BSSR in our patients. By analysing such initial collections of bile it has been suggested that the BAIF is very low and even absent in the transplanted liver. We feel that this is not a conclusion that should be drawn on bile secretion data from the first postoperative days, since it is obvious that the transplanted liver starts out with no bile secretion at all and that both the BAIF and the BADF increase and stabilize after 2–3 weeks provided that there is no graft dysfunction and that the enterohepatic circulation is restored [1].

The driving force of bile secretion is the active secretion of bile acids into the bile canaliculi [22]. The fraction of hepatic bile generated by the active secretion of bile acids is the BADF. The bile flow is usually linearly related to the BSSR, and the extrapolated bile flow at zero BSSR measures the BAIF [24]. The BADF in our control group was slightly higher (16 μl/μmol) than in previous studies of reference populations [1, 14, 16], probably due to the influence of UDCA. UDCA is known to have high choleretic potency [4, 20] and patients on treatment with UDCA can be expected to have a bile acid pool that consists of 50% UDCA [15]. Our liver-transplanted patients also had a comparable BADF (18 μl/μmol).

The average bile outflow of 0.49 ml/min during the first hour of measurement in the control patients corresponds well to the figure obtained by Prandi et al. [16] and slightly exceeds that reported by Lindblad et al. [14]. Since the present data correspond well to those obtained in earlier studies, where complete biliary drainage was secured by occlusion of the distal common bile duct by a balloon catheter, it is reasonable to assume that all bile was collected also in the present study where drainage by gravity was

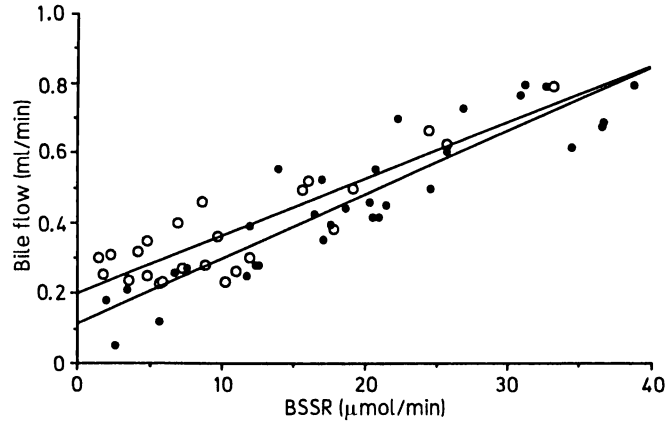

Fig. 3. The relation between bile flow and bile salt secreting rate (BSSR) as expressed with linear regression analysis. LTX *(filled symbols):* $Y = 0.11 + 0.018x$; $r = 0.92$; $P < 0.001$. Control *(unfilled symobols):* $Y = 0.20 + 0.016x$; $r = 0.88$; $P < 0.001$. The intercepts are significantly different ($P < 0.05$)

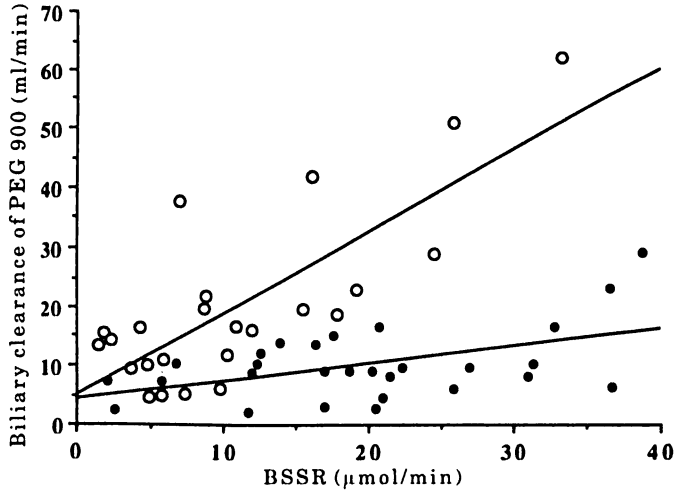

Fig. 4. The relation between biliary clearance of PEG 900 and BSSR as expressed with linear regression analysis. LTX *(filled symbols):* $Y = 4.8 + 0.30x$; $r = 0.55$; $P < 0.01$. Control *(unfilled symbols):* $Y = 4.96 + 1.40x$; $r = 0.78$; $P < 0.001$. The slopes are significantly different ($P < 0.01$)

used. This is further supported by the high biliary recovery within 2 h of intravenously-injected labelled bile acids in the present set-up.

The slightly higher values for bile flow and BSSR seen both in our control patients and even more in our LTX patients are probably due to the bile acid treatment given to these patients. It should be noted that in these observations during the first hour of bile collection no significant differences between the LTX and control patients were found.

The bile acid independent bile flow in man has previously been estimated to be 0.20–0.25 ml/min [1, 14, 16], which is in coherence with our present results in the control group. Our liver-transplanted patients, however, seem to have a reduced bile acid independent bile flow (0.11 ml/min).

Since no method of direct measurement of the canalicular fraction of bile exists, marker molecules like

PEG 900 are used [8, 9, 11]. We found, as a further indication of impaired bile formation in the transplanted liver, a reduced biliary clearance of PEG 900 indicating a reduced canalicular bile flow.

It is well established that cyclosporin in high doses has cholestatic side effects [3], and from animal studies we know that cyclosporin reduces bile formation although no deterioration of liver biochemistry occurs [13, 18, 23]. Our patients were on a low-dose regimen of cyclosporin, but a side effect of this drug must be considered as the cause of the impaired bile formation together with factors like the continuous immunological challenge to the graft and possible permanent injury during preservation and reperfusion.

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