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Influence of tacrolimus on bile acid and lipid composition in continuously drained bile using a rat model

Comparative study with cyclosporine

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Abstract Cholestatic effects have been reported for cyclosporine (CsA), but information is still limited for tacrolimus (TCR). The purpose of this study was to investigate the influence of TCR on biliary bile acid and lipid composition as compared with CsA, using a continuously bile-drained rat model. Adult male Wistar rats received TCR (0.4 mg/kg, 1 mg/kg, and 4 mg/kg) or CsA (2.5 mg/kg, 10 mg/kg, and 25 mg/kg) by intramuscular injection (i.m.) daily for 10 days. On day 7, the common bile duct of all rats was cannulated, then bile was continuously collected for the following 3 days. Bile flow, bile acid secretion rate (BASR), and biliary lipids secretion were measured for each of the groups. TCR increased bile acid-dependent flow (BADF) but with no statistical significance. However, this agent did not influence total bile flow and biliary lipids secretion, while bile acid-independent flow (BAIF) was significantly reduced and bile acid synthesis (mainly cholic acid, CA, synthesis) was increased. In contrast, CsA was cholestatic, showing a tendency to reduce both BADF and BAIF. BASR was dose-dependently suppressed, especially in chenodeoxycholic acid (CDCA). Furthermore, biliary lipids secretions were also significantly decreased under a higher dose of CsA. TCR increased BADF with no influence on total bile flow, having a stimulating effect on CA production, although CsA dose-dependently diminishes CDCA production and consequently reduced bile secretion. Our results suggest that TCR is a less effective agent on cholestasis as compared to CsA.

Key words Cyclosporine (CsA) – Tacrolimus (TCR) – Bile acids and salts – Lipids – Cholic acid (CA) – Chenodeoxycholic acid (CDCA)

Introduction

Cyclosporine (CsA) has been widely used for organ transplantation. However, cholestasis caused by a decrease in the bile flow and bile acids secretion is a concern in liver-transplant patients who receive CsA for a long-term period [7]. There are several studies showing that CsA does have an influence on bile secretion in vitro [1, 23] and in short-term in vivo studies [3, 12]. Mechanisms for the cholestasis induced by CsA have been considered to inhibit canalicular transport of bile acids [1, 11]. CsA has been observed to inhibit chenodeoxycholic acid (CDCA) synthesis in cultured hepatocytes [19]. In a preceding report, we have also shown that CsA reduces the secretion of newly synthesized bile acids dose-dependently, especially that of CDCA, using a rat model with continuous bile drainage [17].

On the other hand, tacrolimus (TCR) has become one of the key drugs for immunosuppressive therapy. Although TCR is chemically different from CsA, its mechanism of action is very similar. Like CsA, TCR inhibits such early T-cell activation gene transcription as

interleukin-2 (IL-2), interferon-gamma, and granulocyte-macrophage colony-stimulating factor (CSF). Its immunosuppressive potency in vitro is 10–100 times higher than CsA [31]. Information regarding the effect of TCR on bile formation is still limited except for a few clinical analyses in liver transplant patients [5, 16]. The purpose of this study was to study the influence of TCR on bile and lipid compositions in vivo, compared with those of CsA. We have refined a rat model with continuous bile drainage in order to study the effects of immunosuppressants on bile composition over a long period of time [18]. Here, we studied the bile secretory change without enterohepatic circulation under the repeated administration of immunosuppressive drugs.

Materials and methods

Rats

Adult male Wistar rats (Japan, Shizuoka, Japan) weighing 300–340 g were maintained under a constant 12-h light-dark cycle. Rats had free access to standard rat chow and tap water.

Chemicals

TCR and its vehicle were supplied by Fujisawa Pharmaceutical Company, Osaka, Japan. The original drug containing 37% of TCR in mannitol and polyoxyethylene hydrogenated castor oil 60 (HCO60) was suspended in saline for i. m. use. Vehicle (only mannitol and HCO60) was also suspended in saline in the control group. CsA (Sandimmun; Sandoz, Basel, Switzerland) was dissolved in olive oil (Kanto Chemical, Tokyo, Japan) for i. m. injection. The vehicle group received equivalent olive oil (1 ml/kg per day) as a control.

Experimental design

Twenty-two rats were randomly assigned to the following 4 groups:

1. TCR 0.4 mg/kg per day ($n = 5$)
2. TCR 1.0 mg/kg per day ($n = 5$)
3. TCR 4.0 mg/kg per day ($n = 5$)
4. Vehicle ($n = 7$)

(TCR dosage is expressed by the final concentration instead of original TCR dosage.)

The other 27 rats were divided into the following-four groups:

1. CsA 2.5 mg/kg per day ($n = 6$)
2. CsA 10 mg/kg per day ($n = 6$)
3. CsA 25 mg/kg per day ($n = 8$)
4. Vehicle (olive oil; $n = 7$)

Experimental procedure

Animals in the TCR study received TCR at a dose of 0.4 mg/kg per day, 1.0 mg/kg per day, or 4.0 mg/kg per day or vehicle of TCR i. m. for 10 days. Animals in the CsA study received CsA at a dose of 2.5 mg/kg per day, 10 mg/kg per day, or 25 mg/kg per day or vehicle (olive oil) i. m. injection for 10 days. On day 7, bile from the animals was completely drained by a modified method reported originally by Vonk et al. [32].

Prior to this experiment, more than 50 rats were kept continuously drained of bile in a bile circadian study. To keep the experimental rats healthy after complete bile drainage, oral intake of normal saline was essential[18]. The circulating bile pool was washed out 12–18 h after drainage, and the circadian variation in newly synthesized bile flow was recovered from 2 days after continuous bile drainage. Therefore, the analysis of bile acid synthesis and biliary lipids secretion under a steady state were performed on the 3rd day after continuous bile drainage (day 10). The methods will be described in detail elsewhere [18].

Briefly, the rats were anesthetized with ether (Sigma Chemical, St. Louis, USA). At laparotomy, the common bile duct was ligated approx. 1 cm below the juncture of the hepatic ducts. A small incision was made in the bile duct and a polyethylene catheter PE 50 (Clay Adams, Parsippany, N.J.) was inserted 5 mm into the duct. The bile duct was dissected after positioning the catheter. The free end of the catheter was then pulled out of the dorsal skin of the rats subcutaneously from the abdominal wall. After surgery, the rats were left unrestrained in cages and allowed to move freely. Bile was continuously collected at 6-h or 12-h intervals using an automatic fraction collector (LKB, Sweden). Twenty-four hours after the final dosing, the rats were killed for blood sampling.

Bile analysis

Biliary bile acids were analyzed by a 3-alpha-hydroxysteroid dehydrogenase assay [15]. Bile acid composition was determined using a HPLC method [14]. Cholesterol and phospholipid in bile were determined by enzymatic methods [13, 10].

Blood analysis

Serum bile acids were measured using a 3-alpha-hydroxysteroid dehydrogenase method [15]. Serum cholesterol was assayed using an automatic enzymatic technique [22]. Serum phospholipid levels were analyzed by enzymatic methods [28]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by automatic ultraviolet (UV) methods [20]. Plasma TCR levels 24 h after the final dose was administered were measured using enzyme immunoassay (EIA) methods [30]. The concentration of CsA in whole blood was measured at the same time using a fluorescence polarization immunoassay kit (Dainabot, Tokyo, Japan).

Statistical analysis

Results are presented as means ± SEM. The values of bile flow (milliliters per hour), bile acid secretion rate (BASR; millimoles per hour), which represents bile acid synthesis rate in this study, and the secretion rates of cholesterol (millimoles per hour) and phospholipid (millimoles per hour) into bile were normalized for body weight. Statistical analysis was performed using an analysis of variance test (one-way ANOVA) or unpaired Student's t-test as appropriate. A P-value of 0.05 or less was considered as significant. The relationships between bile flow and BASR were analyzed by linear regression analysis. The calculated intercepts and slopes of the regression line were considered to represent the bile acid-independent flow (BAIF) and the bile acid-dependent flow (BADF), respectively [33]. Multiple regression analysis with dummy variables was used to compare intercepts and slopes between groups.

Table 1 Serum biochemistry and blood concentrations of tacrolimus (*TCR*) or cyclosporine (*CsA*) in rats treated for 10 days with TCR or CsA. Results are expressed as mean ± SEM (*ALT* alanine aminotransferase, *AST* aspartate aminotransferase)

	AST (IU/l)	ALT (IU/l)	Bile acid (mmol/ml)	Cholesterol (mg/dl)	Phospholipid (mg/dl)	TCR or CsA level (ng/ml)
tacrolimus						
0.4 mg/kg (n = 5)	169 ± 12	62 ± 8	0.7 ± 0.3	72.8 ± 2.1	134.6 ± 4.8	9.0 ± 0.7
1.0 mg/kg (n = 5)	124 ± 21	54 ± 5	0.8 ± 0.4	80.2 ± 2.9	147.0 ± 2.9	15.8 ± 1.8
4.0 mg/kg (n = 5)	158 ± 31	54 ± 11	0.8 ± 0.3	69.6 ± 1.5	140.8 ± 5.3	61.3 ± 6.7[ab]
vehicle (n = 7)	116 ± 18	48 ± 7	0.6 ± 0.2	66.3 ± 3.7	137.4 ± 5.8	–
cyclosporine						
2.5 mg/kg (n = 6)	178 ± 16	57 ± 37	1.2 ± 0.1	63.6 ± 7.3	131.3 ± 17.8	535 ± 38
10 mg/kg (n = 6)	98 ± 8	36 ± 47	1.3 ± 0.3	72.0 ± 9.3	135.4 ± 12.1	2707 ± 281[c]
25 mg/kg (n = 8)	115 ± 7	38 ± 57	2.9 ± 0.9	55.3 ± 2.9	129.8 ± 6.7	5556 ± 229[cd]
vehicle (n = 7)	98 ± 13	36 ± 67	2.0 ± 0.9	61.4 ± 3.8	131.5 ± 11.1	–

[a] $P < 0.05$ compared with the TCR 0.4 mg group
[b] $P < 0.05$ compared with the TCR 1.0 mg group
[c] $P < 0.05$ compared with the CsA 2.5 mg group
[d] $P < 0.05$ compared with the CsA 10 mg group
The data of blood concentration of CsA were partly reported in reference [17].

Table 2 Bile flow, bile acid secretion rate (*BASR*), and biliary lipids secretion rate on day 10 in TCR and CsA groups. Results are expressed as mean ± SEM

	Bile flow (ml/kg/hr)	BASR (mmol/kg/hr)	Cholesterol (mmol/kg/hr)	Phospholipid (mmol/kg/hr)
tacrolimus				
0.4 mg/kg (n = 5)	1.96 ± 0.13	22.03 ± 3.74	0.96 ± 0.05	8.83 ± 0.56
1.0 mg/kg (n = 5)	1.78 ± 0.07	19.63 ± 2.19	0.84 ± 0.04	8.34 ± 0.46
4.0 mg/kg (n =5)	1.94 ± 0.11	22.24 ± 1.81	0.88 ± 0.06	9.01 ± 0.84
vehicle (n = 7)	1.94 ± 0.08	15.75 ± 0.42	0.90 ± 0.10	8.41 ± 0.78
cyclosporine				
2.5 mg/kg (n = 6)	1.61 ± 0.09[a]	15.59 ± 1.07	0.69 ± 0.09	5.44 ± 1.07
10 mg/kg (n = 6)	1.52 ± 0.06[a]	12.26 ± 1.02	0.62 ± 0.06	5.31 ± 1.06
25 mg/kg (n = 8)	1.49 ± 0.12[a]	9.37 ± 0.62[ab]	0.43 ± 0.03[a]	2.84 ± 0.54[a]
vehicle (n = 7)	2.03 ± 0.09	15.19 ± 0.90	0.91 ± 0.17	8.71 ± 0.60

[a] $P < 0.05$ compared with the vehicle (olive oil) group
[b] $P < 0.05$ compared with the CsA 2.5 mg group

Results

Serum biochemistry and blood concentration after administration of TCR or CsA are summarized in Table 1. No significant difference in serum AST, ALT, bile acid, cholesterol, and phospholipid levels was observed between the TCR-treated groups and the vehicle group. There was also no significant change in serum liver enzymes, bile acid, and lipids levels among all three CsA-treated groups and the vehicle group. The TCR concentrations in plasma and CsA concentrations in whole blood dose-dependently increased.

Bile flow, BASR, and biliary lipids secretion rate under a repeated administration of TCR or CsA are shown in Table 2. Bile flow in all three TCR-treated groups was similar to that of the vehicle rats; however, BASR was increased by TCR administration. In contrast, these parameters in the CsA groups significantly decreased in a dose-dependent manner. In the biliary lipids secretion rate, there was no significant difference between the TCR-treated and the vehicle groups. However, the biliary secretion rate of cholesterol and phospholipid in the CsA groups decreased dose-dependently. Especially, those of the 25-mg CsA-treated group were significantly diminished (53% in cholesterol, 66% in phospholipid, respectively) as compared with the control group.

Changes in biliary acid composition are shown in Fig. 1. Primary bile acids [i.e., cholic acid (CA) and CDCA] were dominant in the secreted bile in all groups, and secondary bile acids (i.e., deoxycholic acid and lithocholic acid) were not detected in the present study. In all TCR-treated groups, the synthesis rate of CA increased compared with the vehicle group but did not reach significant levels (Fig. 1A). The synthesis rate of CDCA in the 0.4-mg TCR-treated group tended to increase, but that of other TCR groups showed a similar value to the vehicle group (Fig. 1B). In the CsA study, the synthesis rate of CA did not significantly change except for a decrease found in the 25-mg CsA group (Fig. 1C). However, the synthesis of CDCA significantly decreased in a dose-dependent manner. Especially, those of the 10-mg and 25-mg CsA-treated groups were suppressed by 58% and 88%, respectively, when compared with the vehicle group (Fig. 1D).

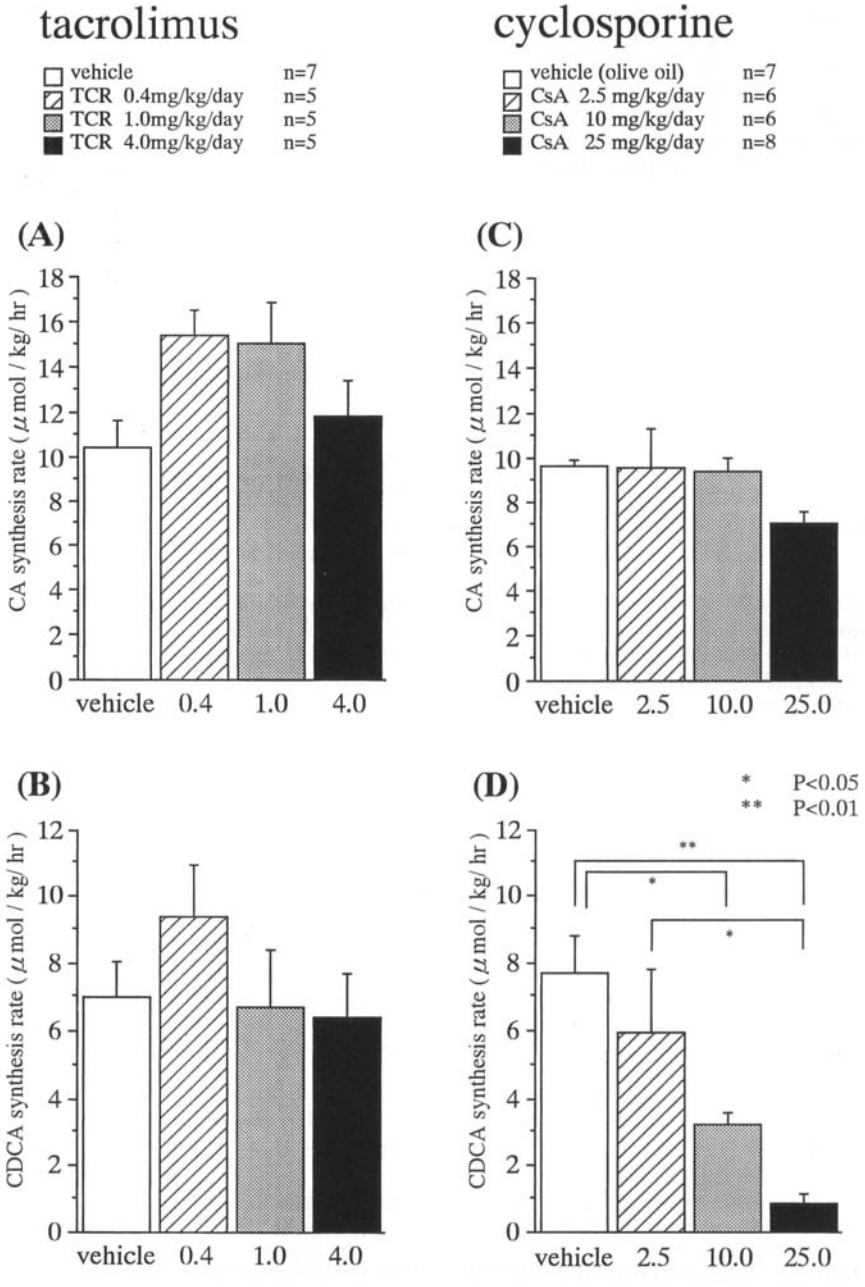

Fig. 1 A–D The synthesis rate (millimoles per kilograms per hour) of primary bile acids [cholic acid (*CA*)and chenodeoxycholic acid (*CDCA*)] on day 10 in tacrolimus (*TCR*) (**A,B**) and cyclosporine (*CsA*) (**C,D**) groups. Results are expressed as means ± SEM. Some of the data in **C** and **D** were reported in reference 17

The individual plots for each rat revealed a positive correlation between BASR and bile flow (Fig. 2). In the TCR-treated rats, the intercept of the regression line (i.e., BAIF) was significantly lower (41%) than that of the vehicle group. However, the slope of the regression line (i.e., BADF) conversely increased (38%) with TCR, although the difference was not statistically significant (Fig. 2A). On the other hand, CsA treatment had a tendency to decrease both the BADF (18%) and BAIF (17%) compared with the vehicle group (Fig. 2B).

Discussion

There have been a few reports showing the influence of TCR on bile flow and bile secretion in vivo [21] and in vitro [11]. Our study showed that TCR did not have a cholestatic effect and that BADF was increased by this agent, while BAIF was reduced. In contrast, CsA was cholestatic, reducing both BADF and BAIF, although the difference were not statistically significant. TCR has a similar action on immunosuppression to CsA; however, the effects of TCR on cholestasis and the ex-

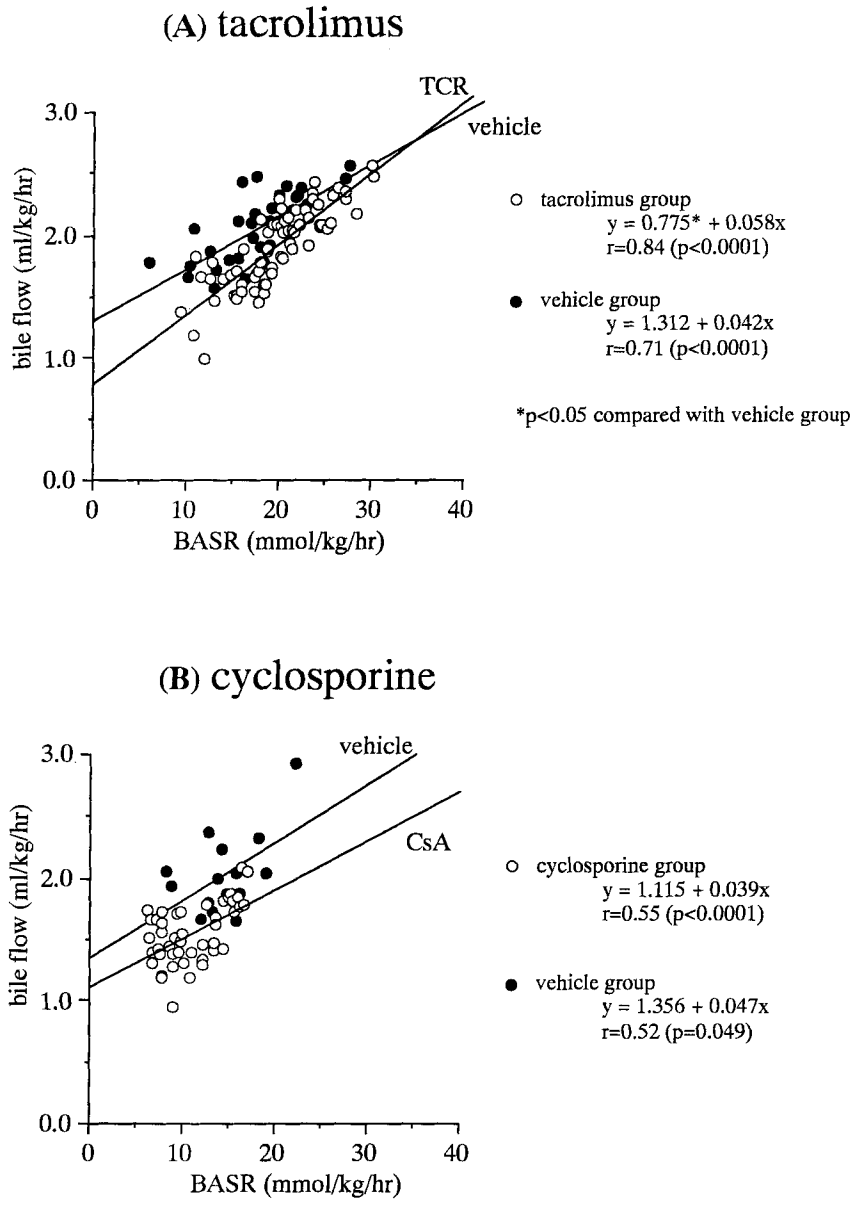

Fig. 2 The relationship between bile acid secretion rate (*BASR*) and bile flow on day 10 in TCR-treated rats (**A**) and CsA-treated rats (**B**). Regression lines show the mean line observed from individual rats in each group

cretory liver function are not fully understood. Reese et al. [21] showed that intravenous administration of TCR increased hepatic bile flow in a dose-related manner in dogs, but that oral administration of TCR did not increase bile flow.

Regarding change in bile acid component after administration of TCR, little clinical evidence has been reported [5, 16, 24]. Our study showed that TCR increased bile acid synthesis rate, mainly CA synthesis. On the other hand, CsA reduced the bile acid synthesis rate in a dose-dependent manner, caused by the suppression of CDCA synthesis. An increase in CA to CDCA ratio in bile composition after liver transplantation might be dependent on elevation of CA and/or decrease in CDCA [5, 16, 24]. Sauer et al. [24] demonstrated that the secretion rate of CDCA in TCR-treated patients was not different from healthy non-treated controls, whereas that of CA significantly increased. On the other hand, McCashland et al. [16] found that bile acid secretion and bile flow were decreased in patients receiving either CsA or TCR and that a reduction in CDCA biosynthesis was related only to patients receiving CsA. Our in vivo rat model of continuous bile drainage demonstrated a positive effect of TCR on CA synthesis and a negative effect of CsA on CDCA synthesis. Ericzon et al. [5] speculated that the rapid recovery of bile synthesis in TCR-treated patients after transplantation was due to a greater hepatotrophic effect of TCR. The hepato-

trophic effect of TCR has been shown in in vitro or in vivo studies [8, 26]. Princen et al. [19] found that CsA blocked specifically the synthesis of CDCA by inhibition of 27-hydroxylase activity in in vitro-cultured hepatocytes. Our preceding study using continuously bile-drained rats also showed that secretion of newly synthesized CDCA was markedly reduced by CsA administration in a dose-dependent manner [17].

Although it is well known that CsA administration affects biliary lipid secretion, information about TCR is strictly limited except for a few clinical analyses [5, 24]. Our experimental study showed that CsA administration reduced biliary lipids secretion dose-dependently, while serum phospholipid and cholesterol did not change. However, there was no significant difference between these lipid parameters in TCR and vehicle. Changes of biliary lipid secretion by CsA have been reported to inhibit biliary output of both cholesterol and phospholipid [9], cholesterol alone [3], and phospholipid alone [2, 27]. Although the exact mechanism of biliary lipids secretion remains unclear, it has been shown that CsA inhibits the activity of the multidrug efflux pump (P-glycoproteins) dose-dependently, but TCR is less effective in the isolated hepatocyte couplets [29]. P-glycoproteins are active transporters that effluxe a variety of structurally diverse xenobiotics. One type of P-glycoprotein is therefore considered to play an essential role in the secretion of phospholipid into bile [4, 25]. In our animal experiment, the CsA-related decrease in biliary phospholipid secretion might be reflected by the more powerful inhibition of P-glycoproteins compared with TCR.

In summary, CsA reduced bile flow and BASR in a dose-dependent manner, caused by inhibition of CDCA synthesis. CsA further disturbed biliary lipids secretion. On the other hand, TCR did not influence total bile flow and biliary lipids secretion, changing BAIF and BADF. In addition, TCR might have a positive effect on CA synthesis in a rat model with continuous bile drainage. These data suggest that TCR has a less cholestatic effect than CsA under the experimentally high concentration of drug levels. In a clinical setting, this information would be considered as representing adverse effects of TCR and CsA [6].

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References

1. Bohme M, Muller M, Leier I, Jedlitschky G, Keppler D (1994) Cholestasis caused by inhibition of the adenosine triphosphate-dependent bile salt transport in rat liver. Gastroenterology 107: 255–265
2. Chan FK, Shaffer EA (1997) Cholestatic effects of cyclosporine in the rat. Transplantation 63: 1574–1578
3. Chanussot F, Botta-Fridlund D, Lechene de la Porte P, Sbarra V, Portugal H, Pauli AM, Hauton J, Gauthier A, Lafont H (1992) Effects of cyclosporine and corticosteroids on bile secretion in the rat. Transplantation 54: 226–231
4. Elferink RP, Tytgat GN, Groen AK (1997) Hepatic canalicular membrane. 1. The role of mdr2 P-glycoprotein in hepatobiliary lipid transport. FASEB J 11: 19–28
5. Ericzon BG, Eusufzai S, Soderdahl G, Duraj F, Einarsson K, Angelin B (1997) Secretion and composition of bile after human liver transplantation: studies on the effects of cyclosporine and tacrolimus. Transplantation 63: 74–80
6. Ericzon BG, Eusufzai S, Soderdahl G, Duraj F, Einarsson K, Angelin B (1998) Dose-dependent reduction of bile secretion in cyclosporine-treated rats. Transplantation 65: 759
7. Farouk M, Branum GD, Watters CR, Cucchiaro G, Helms M, McCann R, Bollinger R, Meyers WC (1991) Bile compositional changes and cholesterol stone formation following orthotopic liver transplantation. Transplantation 52: 727–730
8. Francavilla A, Starzl TE, Carr B, Azzarone A, Carrieri G, Zeng QH, Porter KA (1991) The effects of FK 506, cyclosporine, and rapamycin on liver growth in vitro and in vivo. Transplant Proc 23: 2817–2820
9. Galan AI, Fernandez E, Moran D, Munoz ME, Jimenez R (1995) Cyclosporine A hepatotoxicity: effect of prolonged treatment with cyclosporine on biliary lipid secretion in the rat. Clin Exp Pharmacol Physiol 22: 260–265
10. Gurantz D, Laker MF, Hofmann AF (1981) Enzymatic measurement of choline-containing phospholipids in bile. J Lipid Res 22: 373–376
11. Kadman M, Klunemann C, Bohme M, Ishikawa T, Gorgas K, Otto G, Herfarth C, Keppler D (1993) Inhibition by cyclosporine A of adenosine triphosphate-dependent transport from the hepatocyte into bile. Gastroenterology 104: 1507–1514
12. Le Thai B, Dumont M, Michel A, Erlinger S, Houssin D (1988) Cholestatic effect of cyclosporine in the rat. An inhibition of bile acid secretion. Transplantation 46: 510–512
13. Lie RF, Schmitz JM, Pierre KJ, Gochman N (1976) Cholesterol oxidase-based determination, by continuous-flow analysis, of total and free cholesterol in serum. Clin Chem 22: 1627–1630
14. Maruyama K, Tanimura H, Hikasa Y (1980) Analysis of conjugated bile acids in bile by high-pressure liquid chromatography. Clin Chim Acta 100: 47–54
15. Mashige F, Imai K, Osuga T (1976) A simple and sensitive assay of total serum bile acids. Clin Chim Acta 70: 79–86
16. McCashland TM, Donovan JP, Amelsberg A, Rossi SS, Hofmann AF, Shaw BJ, Quigley EM (1994) Bile acid metabolism and biliary secretion in patients receiving orthotopic liver transplants: differing effects of cyclosporine and FK 506. Hepatology 19: 1381–1389
17. Mizuta K, Kobayashi E, Uchida H, Fujimura A, Kawarasaki H, Hashizume K (1998) Dose-dependent reduction of bile secretion in cyclosporine-treated rats. Transplantation 65: 758–759

18. Mizuta K, Hishikawa S, Yoshida T, Kobayashi E, Uchida H, Fujimura A, Kawarasaki H, Hashizume K (1999) Survival of rats undergoing continuous bile drainage depends on maintenance of circadian rhythm of bile secretion. Chronobiol Int 16 (in press)
19. Princen HM, Meijer P, Wolthers BG, Vonk RJ, Kuipers F (1991) Cyclosporin A blocks bile acid synthesis in cultured hepatocytes by specific inhibition of chenodeoxycholic acid synthesis. Biochem J 275: 501–505
20. Proia AD (1983) Du Pont aca and Dow AST/ALT-UV (PLP activated)-centrifugal analyzer serum aspartate aminotransferase assays compare well. Clin Chem 29: 1701–1702
21. Reese JC, Fairchild RB, Solomon H, Warty V, Kaminski DL (1993) The effect of FK506 on canine bile flow. Transplantation 56: 1162–1165
22. Richmond W (1976) Use of cholesterol oxidase for assay of total and free cholesterol in serum by continuous-flow analysis. Clin Chem 22: 1579–1588
23. Roman ID, Coleman R (1994) Disruption of canalicular function in isolated rat hepatocyte couplets caused by cyclosporin A. Biochem Pharmacol 48: 2181–2188
24. Sauer P, Stiehl A, Otto G, Theilmann L (1995) In patients with orthotopic liver transplantation, serum markers of cholestasis are unreliable indicators of biliary secretion. J Hepatol 22: 561–564
25. Smit JJ, Schinkel AH, Oude ER, Groen AK, Wagenaar E, van DL, Mol CA, Ottenhoff R, van dLN, van RM, et al (1993) Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 75: 451–462
26. Starzl TE, Porter KA, Mazzaferro V, Todo S, Fung J, Francavilla A (1991) Hepatotrophic effects of FK506 in dogs. Transplantation 51: 67–70
27. Sutherland FR, Preshaw RM, Shaffer EA (1993) The effect of cyclosporine and liver autotransplantation on bile flow and composition in dogs. Transplantation 55: 237–242
28. Takayama M, Itoh S, Nagasaki T, Tanimizu I (1977) A new enzymatic method for determination of serum choline-containing phospholipids. Clin Chim Acta 79: 93–98
29. Takeguchi N, Ichimura K, Koike M, Matsui W, Kashiwagura T, Kawahara K (1993) Inhibition of the multidrug efflux pump in isolated hepatocyte couplets by immunosuppressants FK506 and cyclosporine. Transplantation 55: 646–650
30. Tamura K, Kobayashi M, Hashimoto K, Kojima K, Nagase K, Iwasaki K, Kaizu T, Tanaka H, Niwa M (1987) A highly sensitive method to assay FK-506 levels in plasma. Transplant Proc 19: 23–29
31. Tocci MJ, Matkovich DA, Collier KA, Kwok P, Dumont F, Lin S, Degudicibus S, Siekierka JJ, Chin J, Hutchinson NI (1989) The immunosuppressant FK506 selectively inhibits expression of early T cell activation genes. J Immunol 143: 718–726
32. Vonk RJ, Van DA, Strubbe JH (1978) Bile secretion and bile composition in the freely moving, unanaesthetized rat with a permanent biliary drainage: influence of food intake on bile flow. Clin Sci Mol Med 55: 253–259
33. Wheeler HO (1972) Secretion of bile acids by the liver and their role in the formation of hepatic bile. Arch Intern Med 130: 533–541