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ORIGINAL ARTICLE

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Short-term treatment with mycophenolic acid increases bile flow in continuously perfused and cold-preserved rat livers and does not affect hepatic ischemia-reperfusion injury

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Abstract Mycophenolate mofetil (MMF) is a new immunosuppressive agent which has been used successfully after kidney and heart transplantation. Experience with MMF after liver transplantation is still limited. In particular, there is no information about influence on ischemia-reperfusion injury (IRI). Therefore, the aim of this investigation was to assess the effects of mycophenolic acid (MPA), the pharmacologically active metabolite of MMF, in the cold-preserved or normal rat liver. Livers of male Sprague-Dawley rats were subjected to cold ischemia in University of Wisconsin (UW) solution (24 h, 4°C) and reperfused for 2 h in the absence or presence of MPA (100 μg/ml, $n = 5$–6 each). Another group received MPA pretreatment for 20 min prior to ischemia ($n = 7$). In further experiments, livers were perfused with a bile salt-free Krebs-Henseleit buffer in a continuous fashion (controls, $n = 5$). MPA was infused from 20–40 min after starting perfusion in therapeutic concentrations (5 μg/ml, 10 μg/ml, 40 μg/ml, and 100 μg/ml; $n = 3$–6 each). There was no significant influence of MPA on portal pressure nor on postischemic efflux rates of LDH. MPA pretreatment resulted in a significant improvement of bile flow during reperfusion (0.32 ± 0.05 μl/min × g liver) compared with controls (0.17 ± 0.04 μl/min × g liver, mean ± SEM). In contrast, postischemic bile flow was not influenced by continuous administration of MPA during the reperfusion period only (0.18 ± 0.07 μl/min × g liver). In continuously perfused livers, MPA increased bile salt-independent bile flow (1.00 ± 0.06 μl/min × g liver) in a dose-dependent manner, reaching half-maximal effects around 5 μg/ml (1.66 ± 0.15 μl/min × g liver) and maximal effects at 40 μg/ml (2.61 ± 0.28 μl/min × g liver). In conclusion, neither preischemic nor postischemic administration of MPA influences IRI to hepatocytes significantly after hypothermic liver preservation in UW solution. In contrast to other immunosuppressive agents, MPA exhibits strong choleretic effects, which are related to a stimulation of bile salt-independent bile formation.

Keywords Mycophenolate mofetil · Mycophenolic acid · Hepatic ischemia-reperfusion injury · Cold ischemia

Abbreviations *CyA* Cyclosporine A · *IMPDH* Inosine monophosphate dehydrogenase · *IRI* Ischemia-reperfusion injury · *MPA* Mycophenolic acid · *KH* Krebs-Henseleit · *LDH* Lactate dehydrogenase · *MPAG* Mycophenolic acid-glucuronide · *MMF* Mycophenolate mofetil · *SEC* Sinusoidal endothelial cells · *UW* University of Wisconsin

Introduction

Ischemia-reperfusion injury (IRI) of the liver represents a serious clinical problem after liver transplantation. IRI contributes to problems such as primary nonfunction, dysfunction, and nonanastomotic biliary stenosis [17, 34], and these complications are major causes of retransplantation and mortality [29].

Preservation injury of the graft liver has been reduced by the introduction of the University of Wisconsin (UW) solution [2]. Recent reports have shown that the immunosuppressive agents azathioprine, cyclosporine (CyA), and FK 506 not only are powerful immunosuppressive substances, but also can protect against reperfusion injury following warm and cold ischemia, respectively [19, 21, 22, 23, 31, 33]. On the other hand, treatment with immunosuppressants can be associated with hepatotoxic effects. A recent report by Chan et al. [10] confirmed significant cholestatic effects of CyA which may contribute to early graft dysfunction. CyA-induced cholestasis is caused by inhibition of ATP-dependent bile acid carriers in the canalicular membrane, which leads to a reduction of bile salt-dependent bile flow [7]. Azathioprine, an inhibitor of purine synthesis, protects sinusoidal endothelial cells against IRI, but increases hepatocellular injury [25]. These findings suggest both toxic and protective effects of immunosuppressants on IRI.

Mycophenolate mofetil (MMF) is a new immunosuppressive agent which has been used successfully after kidney and heart transplantation [13, 24, 32, 37] and more recently after liver transplantation [15, 20]. However, there is no information about influence on IRI. MMF is rapidly and completely converted to the active metabolite mycophenolic acid (MPA) by plasma esterases [28]; the parent compound is not measurable in plasma. MPA is subsequently glucuronidated in the liver to MPA glucuronide (MPAG) [1]. MPAG is secreted into bile [16], suggesting a possible modulation of bile salt-independent bile flow by this substance. MPA is a noncompetitive and selective inhibitor of inosine monophosphate dehydrogenase (IMPDH) [28]. Inhibition of IMPDH blocks the de novo synthesis of guanosine nucleotides, which are necessary substrates for DNA and RNA synthesis. Recently, it has been shown that MPA can indirectly block nitric oxide synthase activity as a consequence of guanosine nucleotide depletion [35]. Because removal of the vasodilator NO aggravates reperfusion injury of the liver due to microcirculatory failure [38], early MPA administration could enhance reperfusion injury. On the other hand, removal of NO may lead to decreased formation of highly toxic peroxynitrite, thereby suggesting cytoprotective effects. Based on these studies, aim of this investigation was to assess the effects of MPA on cell damage and liver function in continuously perfused and cold-preserved rat livers. Experimental evidence suggests that sinusoidal endothelial cells (SEC) are more susceptible to cold ischemia than hepatocytes [9, 11, 30]. However, recent studies demonstrated considerable hepatocyte and SEC injury after cold ischemia [6, 18]. We therefore investigated the effect of MPA on hepatocyte injury and bile flow as an indicator of hepatocyte function.

Materials and methods

Perfusion of rat liver

Male Sprague-Dawley rats weighing 250–300 g were purchased from Savo (Kislegg, Germany) and housed in a climatized room with a 12-h light-dark cycle. The animals had free access to chow (Standard-Diet, Altromin 1314; Lage, Germany) and water up to the time of the experiments. The study was registered with the local animal welfare committee. Perfusion was performed as described previously [4, 36]. Rats were anesthetized with pentobarbital sodium (50 mg/kg body weight, intraperitoneally). After incision of the abdominal wall, the portal vein was cannulated with a 14-gauge Teflon intravenous catheter and the liver was perfused in situ with hemoglobin-free and albumin-free bicarbonate-buffered Krebs-Henseleit (KH) solution (pH 7.4, 37°C) gassed with 95% O_2 and 5% CO_2. The Krebs-Henseleit buffer contained 118 mmol NaCl/l, 4.8 mmol KCl/l, 1.2 mmol KH_2PO_4/l, 1.2 mmol $MgSO_4$/l × $7H_2O$, 1.5 mmol $CaCl_2$/l, and 25 mmol $NaHCO_3$/l. The perfusion medium was pumped through the livers with a membrane pump at a constant flow rate of 3.0–3.5 ml × min^{-1} × g liver in a nonrecirculating fashion. The inferior vena cava was then cannulated via the right atrium and ligated above the right renal vein. The bile duct was cannulated with polyethylene PE10 tubing, and bile was collected in preweighed tubes and measured gravimetrically. Portal pressure was monitored continuously during the total perfusion period.

Sinusoidal efflux of lactate dehydrogenase (LDH) was measured as index of hepatocyte damage [6, 36]. Activities of LDH in perfusate were analyzed according to standard photometric tests [3]. Sinusoidal LDH efflux rates were calculated from activities in perfusate multiplied by the rate of perfusate flow per minute and weight of liver in grams.

Experimental design

Three groups of rats were subjected to ischemia-reperfusion, and five groups of rats were studied with continuous liver perfusion without ischemia. In the experiments subjecting livers to cold ischemia, after 30 min of perfusion with KH buffer, livers were perfused with 30 ml of cold (4°C) UW solution for 1 min [18]. Then, the organs were kept in 150 ml of UW solution at 4°C for 24 h. Following this period of ischemia, the livers were reperfused with KH buffer (37°C) at the preischemic flow rate in a nonrecirculating fashion for 2 h.

Three groups of animals were subjected to ischemia-reperfusion as follows: (1) untreated ($n = 5$): 30 min of perfusion, 24 h of cold ischemia, and reperfusion for 120 min; (2) 100 μg MPA/ml during reperfusion ($n = 6$): 30 min of perfusion, 24 h of cold ischemia, and reperfusion for 120 min with 100 μg MPA/ml; μ(3) 100 μg MPA/ml before ischemia ($n = 7$): 100 μg of MPA/ml during 20 min until ischemia, 24 h of cold ischemia in UW solution containing 100 μg MPA/ml, and reperfusion for 120 min with KH buffer.

In further experiments, five groups of rats were studied with continuous liver perfusion without ischemia. Group 1 underwent

continuous perfusion for 100 min (controls, $n=7$). Groups 2–5 were subjected to different MPA concentrations (5 μg/ml, 10 μg/ml, 40 μg/ml, or 100 μg/ml, $n=3$–6 each) from 20 to 40 min after starting perfusion. MPA concentrations corresponded to the range of maximum plasma MPA concentrations following oral or intravenous administration of MMF in healthy individuals and patients with impaired liver function, respectively [8].

MPA was a gift from Roche Bioscience, Palo Alto, USA. MPA was dissolved in KH buffer. Stock solutions were infused into the portal inflow of the perfusion system by infusion pumps. UW solution was purchased from DuPont (Bad Homburg, Germany).

Statistics

All data are expressed as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) and the *t*-test of independent means were used to determine differences between multiple groups and two groups, respectively. When *F*-ratios were significant, means were compared using Bonferroni's test as the post hoc comparison. A *P*-value of 0.05 or below was considered statistically significant. The statistical analyses were performed with SPSS software, Release 6.1.3 (SPSS, Chicago, Ill., USA).

Results

Effects of preischemic or postischemic MPA administration on hepatic ischemia-reperfusion injury

During preischemic perfusion, sinusoidal efflux rates of LDH were low and at a constant level (3.1 ± 0.4 mU/min × g liver). Preischemic LDH efflux was not influenced by 100 μg MPA/ml (1.7 ± 0.8 mU/min × g liver). Following reperfusion, LDH release markedly increased up to about 100-fold (589 ± 133 mU/min × g liver) at the end of reperfusion, reflecting considerable cell damage (Fig. 1). This cell damage was neither influenced by preischemic (648 ± 206 mU/min × g liver) nor by postischemic administration of MPA (745 ± 193 mU/min × g liver).

Preischemic portal pressure was 5.7 ± 1.5 cm H_2O and similar in all three experimental groups (5.6 ± 1.0 cm H_2O in the group receiving MPA during reperfusion and 4.6 ± 0.5 cm H_2O in the group receiving MPA prior to ischemia). During reperfusion, portal pressure increased markedly (11.0 ± 1.8 cm H_2O), reflecting an increased hepatic vascular resistance (Fig. 2). The time course of portal pressure during the reperfusion period was not significantly affected by administration of MPA (Fig. 2).

Function of cold-preserved livers was assessed by recovery of bile flow. During reperfusion bile flow of the untreated livers remained markedly reduced and returned to a maximum of only 0.17 ± 0.04 μl/min × g liver after 30 min of reperfusion (Fig. 3). Postischemic bile flow was not influenced by continuous administration of 100 μg MPA/ml during the reperfusion period (0.18 ± 0.07 μl/min × g liver after 30 min of reperfusion).

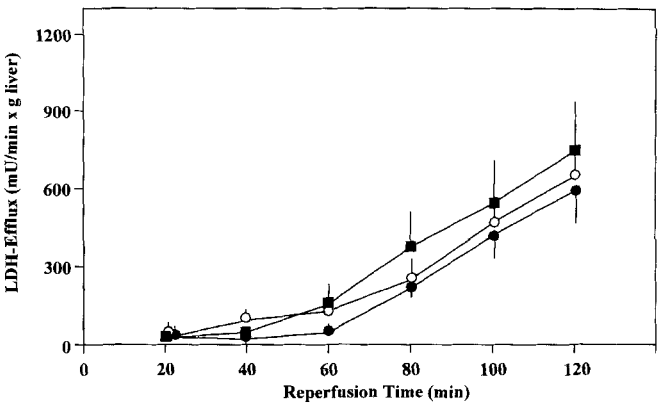

Fig. 1. Release of LDH into the perfusate during reperfusion of livers after 24 h of cold ischemia in the presence or absence of MPA. MPA (100 μg/ml) was infused for 20 min until ischemia or was infused only during the reperfusion period of 120 min. Administration of MPA during the preischemic or postischemic perfusion period did not influence the time course of postischemic LDH release (mean ± SEM). *Filled circles* controls ($n=5$); *filled squares* 100 μg MPA/ml during reperfusion ($n=6$); *empty circles* 100 μg MPA/ml prior to ischemia ($n=7$)

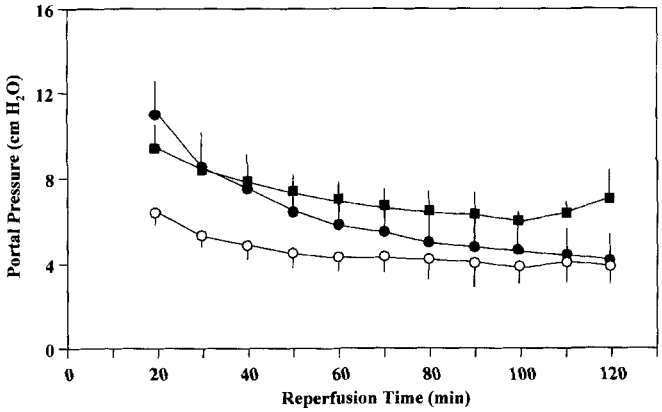

Fig. 2. Time course of portal pressure after cold ischemia in the presence or absence of MPA. MPA was infused 20 min during the preischemic perfusion period or given only during the reperfusion period. Portal pressure (mean ± SEM) was not significantly influenced by MPA during reperfusion or by MPA prior to ischemia compared with untreated livers. *Filled circles* controls ($n=5$); *filled squares* 100 μg MPA/ml during reperfusion ($n=6$); *empty circles* 100 μg MPA/ml prior to ischemia ($n=7$)

In contrast, MPA pretreatment induced a significant increase in bile flow during the preischemic period (2.72 ± 0.26 μl/min × g liver) compared with controls (1.11 ± 0.06, $P<0.05$). In addition, MPA pretreatment of cold-preserved livers resulted in a significant ($P<0.05$) increase in postischemic bile flow to a maximum of 0.32 ± 0.05 μl/min × g liver (Fig. 3), suggesting improvement of postischemic liver function. Because MPA pretreatment did not prevent cell damage, the increase in bile flow could be the consequence of

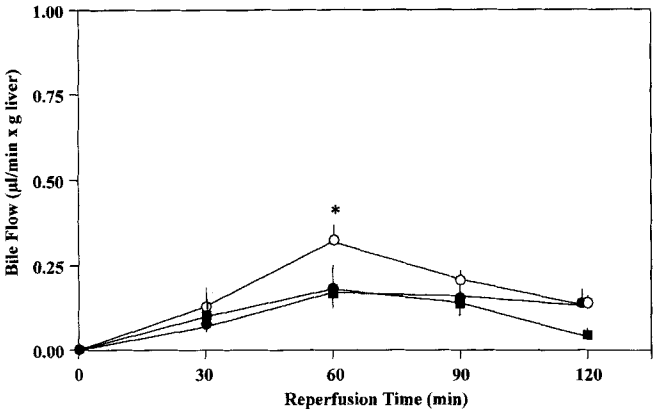

Fig. 3. The effect of MPA on bile flow after 24 h of cold ischemia. MPA (100 µg/ml) was infused before or after ischemia as described in Fig. 1. MPA administered prior to ischemia significantly augmented bile flow (mean ± SEM) during reperfusion. *Filled circles* controls ($n=5$); *filled squares* 100 µg/ml MPA during reperfusion ($n=6$); *empty circles* 100 µg/ml MPA prior to ischemia ($n=7$). *$P<0.05$ compared with untreated livers

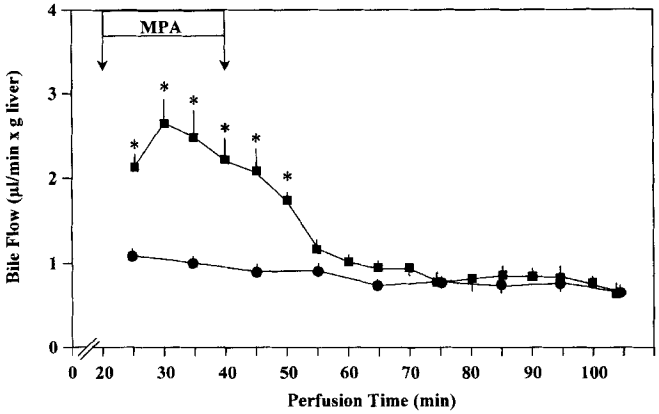

Fig. 4. The effect of MPA on bile flow in continuously perfused rat livers. Infusion of MPA (100 µg/ml) resulted in a 2.5-fold increase in bile flow. Upon termination of MPA infusion bile flow declined to baseline within 15–20 min. Results are expressed as mean ± SEM. *Filled circles* controls ($n=7$); *filled squares* 100 µg/ml MPA ($n=5$). *$P<0.05$ compared with untreated livers

choleretic effects, thereby mimicking improved liver function. This hypothesis was tested by experiments in continuously perfused rat livers.

MPA administration and continuous nonischemic liver perfusion

During the perfusion period of 100 min, LDH release of untreated livers increased very slightly (6.2 ± 0.8 mU/min × g liver), indicating negligible cell damage until the end of perfusion. Portal pressure remained nearly constant during the perfusion period (4.4 ± 0.4 cm H_2O after 100 min of perfusion). Bile flow declined slowly during the perfusion period (Fig. 4), which is comparable with previous results [27, 28, 29]. When MPA was infused over 20 min, neither portal pressure (3.5 ± 0.9 cm H_2O, 3.7 ± 0.4 cm H_2O, 3.5 ± 0.2 cm H_2O, and 2.8 ± 0.2 cm H_2O after 100 min of perfusion) nor sinusoidal efflux of LDH were significantly affected by any tested concentration of MPA (6.2 ± 0.7 mU/min × g liver, 11.2 ± 0.7 mU/min × g liver, 27.9 ± 8.3 mU/min × g liver, and 16.2 ± 7.7 mU/min × g liver with 5 µg MPA/ml, 10 µg MPA/ml, 40 µg MPA/ml, and 100 µg MPA/ml, respectively).

Interestingly, infusion of MPA (100 µg/ml) significantly ($P<0.05$) increased bile flow from 1.00 ± 0.06 µl/min × g liver to 2.48 ± 0.32 µl/min × g liver (Fig. 4). Upon termination of MPA administration, bile flow declined to baseline values within 15–20 min. MPA at concentrations observed in human plasma increased bile flow in a dose-dependent fashion, reaching half-maximal effects around 5 µg/ml and maximal effects at 40 µg/ml (Fig. 5). These findings indicate strong choleretic properties of MPA at therapeutic concentrations.

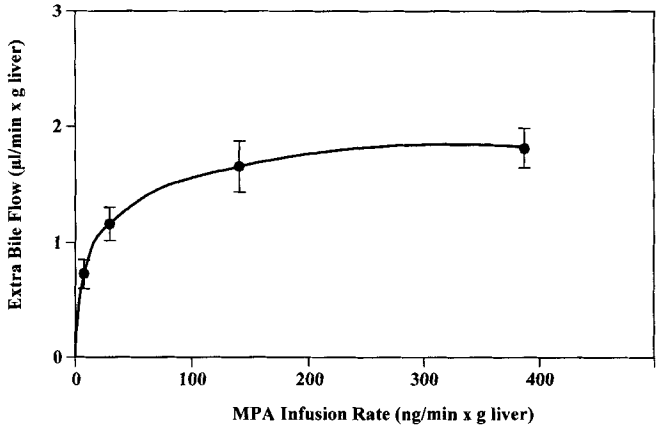

Fig. 5. Relation between extra bile flow and MPA infusion rates in continuously perfused rat livers. Twenty minutes after starting perfusion, MPA was infused for 20 min. Final concentrations of MPA in the influent perfusate were 5 µg/ml, 10 µg/ml, 40 µg/ml, and 100 µg/ml, which corresponded to infusion rates of 16.9 ng/min × g liver, 31.9 ng/min × g liver, 139.3 ng/min × g liver, and 396.2 ng/min × g liver, respectively. Extra bile flow was calculated from the difference between maximal bile flow and baseline values (mean ± SEM). MPA 5 µg/ml, $n=3$; 10 µg MPA/ml, $n=5$; 40 µg MPA/ml, $n=6$; 100 µg MPA/ml, $n=5$

Discussion

Experience with MMF after liver transplantation is still limited, but MMF appears to be a safe and useful adjuvant immunosuppressive agent for rescue and maintenance therapy. However, no information is available about influence on ischemia-reperfusion damage, which can be affected by the established

immunosuppressive calcineurin inhibitors CyA and FK 506, and the purine antimetabolite azathioprine [19, 21, 22, 23, 31, 33].

To investigate a possible influence of MPA on ischemia-reperfusion damage of the liver, we chose the model of the isolated perfused rat liver with a 24-h period of cold ischemia (4°C) which has been extensively characterized by recent studies [6, 18]. Upon reperfusion, portal pressure increased markedly, reflecting a disturbed hepatic microcirculation. Furthermore, reperfusion resulted in a sustained increase in LDH efflux up to about 100-fold, indicating considerable cell damage. As shown recently, postischemic LDH efflux following hypothermic preservation is strongly associated with hepatocyte injury rather than SEC damage [6]. Accordingly, bile flow remained markedly reduced, indicating an impaired hepatocyte function. Thus, a model of considerable ischemia-reperfusion injury to hepatocytes was applied. Using this approach, the results of our investigation show that the administration of 100 μg MPA/ml during the preischemic or postischemic perfusion period did not significantly influence the time course of portal pressure or of postischemic LDH release. In addition, postischemic bile flow was not influenced by administration of MPA during the reperfusion period. Therefore, MPA given during the reperfusion period has no significant effect on hepatocyte injury and dysfunction caused by hypothermic preservation in UW solution. However, these findings do not rule out possible influences on SEC injury, which has not been investigated in the present study.

CyA and FK 506 showed protective effects against the hepatic injury associated with warm ischemia and reperfusion when given prior to ischemia [12, 13, 15]. In most studies, the immunosuppressive compound was given 24 h or more before the operation and induction of ischemia. In contrast, postischemic treatment (immediately upon reperfusion) of the organ seems unsuccessful [12]. Based on these studies, it seems likely that there is an association of the interval between application and induction of the experimental hepatic ischemia and the salutary effects on ischemia-reperfusion injury of the liver. Therefore, we may have missed these effects by applying MPA 20 min prior to ischemia only. However, long-term pretreatment of donors with immunosuppressants seems unfeasible in the clinical setting, due to potential side effects, and practical and ethical concerns.

Nevertheless, MPA pretreatment resulted in a significant improvement of bile flow during reperfusion. Since there were no significant influences on cell damage, we assume that this reflects to some extent a MPA-induced stimulation of bile flow, which could mimic improved liver function. This view is supported by the strong choleretic effect of MPA in continuously perfused rat livers. When MPA was administered at concentrations detected in human plasma, we observed a dose-dependent increase in bile flow. Maximal stimulation of bile flow was reached at a concentration of 40 μg/ml. Because we used a bile acid-free perfusion buffer, the bile flow determined mainly represents the bile acid-independent bile formation, which accounts for about half of the total canalicular bile flow in the rat [26]. MPA is glucuronidated by UDP glucuronyltransferases to a stable phenolic glucuronide (MPAG), which has been detected in bile [16]. Therefore, it is possible that MPAG release into bile and subsequent increase in water flow by osmotic effects contribute to MPA-induced choleresis. Thus, improvement of postischemic bile flow by MPA pretreatment could be caused by the washout of accumulated MPAG into bile during reperfusion. In contrast, MPA infusion upon starting reperfusion did not increase postischemic bile flow. This could be due to a deterioration of sinusoidal uptake or glucuronidation of MPA in cold-preserved livers during the early reperfusion period. On the other hand, ischemia-reperfusion injury of the liver impairs preferentially bile acid-independent bile flow, which is determined by the biliary transport of reduced glutathione [4]. Therefore, it might be possible that MPA pretreatment preserves biliary glutathione transport, which requires further investigation. In contrast to MPA, recent animal studies confirmed significant cholestatic effects by CyA [10]. In a clinical study tacrolimus-based immunosuppression resulted in significantly higher bile flow rates than therapy with CyA [14]. These findings suggest possible choleretic effects or the lack of cholestatic effects in tacrolimus-treated patients. However, infusion of tacrolimus to isolated perfused rat livers at concentrations detected in human plasma did not reveal any influences on bile flow, which clearly argues against relevant choleretic properties of this compound [5]. Thus, in contrast to CyA and tacrolimus, MPA should be considered a choleretic immunosuppressant which may potentially influence the clearance of endogenous compounds and drugs eliminated in the bile.

In conclusion, neither preischemic nor postischemic administration of MPA influences ischemia reperfusion injury to hepatocytes after hypothermic liver preservation in UW solution. In contrast to other immunosuppressive agents, MPA exhibits strong choleretic effects which are related to a stimulation of bile salt-independent bile formation.

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References

1. Allison AC, Eugui EM (1993) Mycophenolate mofetil, a rationally designed immunosuppressive drug. Clin Transplant 7:96–112
2. Belzer FO, Southard JH (1988) Principles of solid-organ preservation by cold storage. Transplantation 45:673–676
3. Bergmeyer HU (1974) Methods of enzymatic analysis. Academic Press, New York
4. Bilzer M, Gerbes AL (1998) Prevention of oxidative liver injury by the immunosuppressant tacrolimus (FK 506). Hepatology 28:660A
5. Bilzer M, Witthaut R, Paumgartner G, Gerbes AL (1994) Prevention of ischemia/reperfusion injury in the rat liver by atrial natriuretic peptide. Gastroenterology 106:143–151
6. Bilzer M, Paumgartner G, Gerbes AL (1999) Glutathione protects the rat liver against reperfusion injury after hypothermic preservation. Gastroenterology 117:200–210
7. Bohme M, Jedlitschky G, Leier I (1994) ATP-dependent export pumps and their inhibition by cyclosporins. Adv Enzyme Regul 34:371–380
8. Bullingham RES, Nicholls AJ, Kamm BR (1998) Clinical pharmacokinetics of mycophenolate mofetil. Clin Pharmacokinet 34:429–455
9. Caldwell-Kenkel JC, Currin RT, Tanaka Y, Thurmann RG, Lemasters JJ (1989) Reperfusion injury to endothelial cells following cold ischemic storage of rat livers. Hepatology 10:292–299
10. Chan FKL, Zhong Y, Lee SS, Shaffer EA (1998) The effects of liver transplantation and cyclosporine on bile formation and lipid composition: an experimental study in the rat. J Hepatol 28:329–336
11. Clavien PA (1998) Sinusoidal endothelial cell injury during hepatic preservation and reperfusion. Hepatology 28:281–285
12. Dhar DK, Nagasue N, Kimoto T, Uchida M, Takemoto Y, Nakamura T (1992) The salutary effect of FK 506 in ischemia-reperfusion injury of the canine liver. Tranplantation 54:583–586
13. Ensley RD, Bristow MR, Olsen SL, Taylor DO, Hammond EH, O'Connell JB, Dunn D, Osburn L, Jones KW, Kauffman RS (1993) The use of mycophenolate mofetil (RS-61 443) in human heart transplant recipients. Transplantation 56:75–82
14. Ericzon BG, Esufzai S, Soederdahl G, Duraj F, Einarsson K, Angelin B (1997) Secretion and composition of bile after human liver transplantation. Transplantation 63:74–80
15. Fisher RA, Ham JM, Marcos A, Shiffman ML, Luketic VA, Kimball PM, Sanyal AJ, Wolfe L, Chodorov A, Posner MP (1998) A prospective randomized trial of mycophenolate mofetil with neoral or tacrolimus after orthotopic liver transplantation. Transplantation 66:1616–1621
16. Fulton B, Markham A (1996) Mycophenolate mofetil. A review of its pharmacodynamic and pharmacokinetic properties and clinical efficacy in renal transplantation. Drugs 51:278–298
17. Furukawa H, Todo S, Imventarza O, Casavilla A, Wu YM, Scotti-Foglieni C, Broznick B, Bryant J, Day R, Starzl TE (1991) Effect of cold ischemia time on the early outcome of human hepatic allografts preserved with UW solution. Transplantation 51:1000–1004
18. Gerbes AL, Vollmar AM, Kiemer AK, Bilzer M (1998) The guanylate cyclase-coupled natriuretic peptide receptor: a new target for prevention of cold ischemia-reperfusion damage of the rat liver. Hepatology 28:1309–1317
19. Goto S, Kim YI, Kamada N, Kawano K, Kobayashi M (1990) The beneficial effect of pretransplant cyclosporine therapy on recipient rats grafted with a 12-h cold-stored liver. Transplantation 49:1003–1005
20. Hebert MF, Ascher NL, Lake JR, Emond J, Nikolai B, Linna TJ, Roberts JP (1999) Four-year follow-up of mycophenolate mofetil for graft rescue in liver allograft recipients. Transplantation 67:707–712
21. Kawano K, Kim YI, Kaketani K, Kobayashi M (1989) The beneficial effect of cyclosporine on liver ischemia in rats. Transplantation 48:759–764
22. Kawano K, Kim YI, Ono M, Goto S, Kai T, Kobayashi M (1993) Evidence that both cyclosporin and azathioprine prevent warm ischemia reperfusion injury to the rat liver. Transplant Int 6:330–336
23. Kawano K, Bowers JL, Clouse ME (1995) Protective effect of FK 506 on hepatic injury following cold ischemic preservation and transplantation: influence on hepatic microcirculation. Transplant Proc 27:362–363
24. Keown P (for the Tricontinental Mycophenolate Mofetil Renal Transplantation Study Group) (1996) A blinded, randomized clinical trial of mycophenolate mofetil for the prevention of acute rejection in cadaveric renal transplantation. Transplantation 61:1029–1037
25. Kim YI, Kawano K, Goto S, Ono M, Iwao Y, Kobayashi M (1991) Amelioration of 3.5-h warm ischemia time for pig livers by cyclosporine pretherapy, compared with azathioprine. Transplant Proc 23:655–656
26. Klaassen CD, Watkins III JB (1984) Mechanisms of bile formation, hepatic uptake, and biliary excretion. Pharmacol Rev 36:1–67
27. Konno H, Hardison WG, Miyai K (1991) Reoxygenation injury following anoxic perfusion preferentially impairs bile acid-independent bile flow. Eur Surg Res 23:151–157
28. Lee WA, Gu L, Miksztal AR, Chu N, Leung K, Nelson PH (1999) Bioavailability improvement of mycophenolic acid through amino ester derivatization. Pharm Res 7:161–166
29. Lemasters JJ, Bunzendahl H, Thurman RG (1995) Reperfusion injury to donor livers stored for transplantation. Liver Transplant Surg 1:124–138
30. McKeown CMB, Edwards V, Phillips MJ, Harvey PRC, Petrunka CN, Strasberg SM (1988) Sinusoidal lining cell damage: the critical injury in cold preservation of liver allografts in the rat. Transplantation 46:178–191
31. Okano K, Hamamoto I, Izuishi K, Wakabayashi H, Akram HM, Maeba T, Tanaka S (1994) Ameliorative effect of FK 506 on cold ischemia reperfusion injury of the rat liver. Transplant Proc 26:2367–2369
32. Pichlmayr R (for the European Mycophenolate Mofetil Cooperative Study Group) (1995) Placebo-controlled study of mycophenolate mofetil combined with cyclosporin and corticosteroids for prevention of acute rejection. Lancet 345:1321–1325
33. Sakr MF, Zetti GM, Hassanein TI, Farghali H, Nalesnik MA, Gavaler JS, Starzl TE, Van Thiel DH (1991) FK 506 ameliorates the hepatic injury associated with ischemia and reperfusion in rats. Hepatology 13:947–951
34. Sanchez-Urdazpal L, Gores GJ, Ward EM, Maus TP, Wahlstrom HE, Moore SB, Wiesner RH, Krom RA (1992) Ischemic-type biliary complications after orthotopic liver transplantation. Hepatology 16:49–53
35. Senda M, DeLustro B, Eugui E, Natsumeda Y (1995) Mycophenolic acid, an inhibitor of IMP dehydrogenase that is also an immunosuppressive agent, suppresses the cytokine-induced nitric oxide production in mouse and rat vascular endothelial cells. Transplantation 60:1143–1148

36. Sies H (1978) The use of perfusion of liver and other organs for the study of microsomal electron-transport and cytochrome P-450 systems. Methods Enzymol 52:48–59

37. Sollinger HW (for the US Renal Transplant Mycophenolate Mofetil Study Group) (1995) Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. Transplantation 60:225–232

38. Wang CY, Mathews WR, Guido DM, Farhood A, Jaeschke H (1995) Inhibition of nitric oxide synthesis aggravates reperfusion injury after hepatic ischemia and endotoxemia. Shock 4:282–288