Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical Pharmacology 69 (2005) 561–568
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Transient up-regulation of P-glycoprotein reduces tacrolimus absorption after ischemia–reperfusion injury in rat ileum

Takanori Omae, Maki Goto, Masahiro Shimomura, Satohiro Masuda, Kimitaka Ito, Masahiro Okuda, Ken-ichi Inui*

Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

Received 10 September 2004; accepted 27 October 2004

Abstract

Ischemia–reperfusion injury is an unavoidable problem for organ transplantation including small bowel transplantation, and causes a large intra-individual variation of tacrolimus (FK506) pharmacokinetics. Little information is available about the regulation of the intestinal P-glycoprotein expression during tissue regeneration. In the present study, we have examined the molecular and functional variations of ileum P-glycoprotein using rats after ischemia–reperfusion treatment. Morphological study revealed a rapid regeneration of the intestinal wall during 24 h after reperfusion. A reverse transcription-coupled competitive PCR and Western blot analysis revealed that the intestinal expression of P-glycoprotein recovered with time after reperfusion. At 24 h after reperfusion, the ileum P-glycoprotein level was transiently increased to two-fold, and the absorption rate of *dihydro*-[^{3}H]FK506 from in situ ileum loop into portal vein was markedly low in comparison with the control. P-glycoprotein was detected in the crypt area as well as in villous cells at 6 h after reperfusion, and then localized to the apical surface at 24 h consistent with the cell proliferation and differentiation. However, the P-glycoprotein level returned to normal at 48 h. The intra-individual variation in the absorptive rate of tacrolimus was suggested to be regulated by the morphological status of the intestinal epithelium and enterocyte expression level of P-glycoprotein. Therefore, the monitoring of the enterocyte P-glycoprotein level would provide useful information for determining the dosage of tacrolimus immediately after small bowl transplantation.

Keywords: MDR1; Small bowel transplantation; Proliferation; C219 monoclonal antibody; FK506; Transporter

1. Introduction

The transplantation of a solid organ requires discontinuation of the blood supply to that organ, and graft organs are exposed to ischemia–reperfusion [1]. Therefore, the graft intestine is damaged by ischemia–reperfusion injury in the case of small bowel transplantation, immediately before the immunological actions.

Although the intestinal epithelium is sensitive to ischemia–reperfusion injury, it is one of the most rapidly regenerating tissues; epithelial cells completely regenerate within 3–4 days in mice [2]. Reperfusion after temporal ischemia induces rapid morphological damage accompanied by alterations of the intestinal barrier and digestive functions [3,4]. Thereafter, a rapid regeneration occurs: a transient increase of cell production in the crypt, the migration of new cells from the crypt to villous, and the subsequent differentiation of villous epithelial cells [5]. The intestine also plays an important role in the active absorption of drugs as well as nutrients. A complete restoration of villi was observed 12 h after reperfusion in the rat small intestine [6]. However, information about the functional recovery of drug absorption should be clarified, considering the absorptive barrier function of the small intestine.

The immunosuppressant tacrolimus, used to prevent the acute rejection of grafted small intestine, shows wide inter-individual variation in bioavailability ranging 4–89% (with an average of about 25%) in the kidney and liver transplant

Abbreviations: AUC, area under the concentration-time curve; BBM, brush-border membrane; *C/D* ratio, concentration/oral dose ratio; CYP3A, cytochrome P450 IIIA; DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, glyceraldehydes 3-phosphate dehydrogenase; MDR1, multidrug resistance; Pgp, P-glycoprotein

* Corresponding author. Tel.: +81 75 751 3577; fax: +81 75 751 4207.
E-mail address: inui@kuhp.kyoto-u.ac.jp (K.-i. Inui).

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.10.016

patients [7,8]. This variable bioavailability is considered to be caused by enterocyte absorptive barriers, namely, an efflux pump P-glycoprotein (Pgp, a product of multidrug resistance (*MDR1*) gene) and a metabolic enzyme cytochrome P450 IIIA subfamilies (CYP3A) [9]. Brady et al. [10] reported the segmental distribution of rat Pgp along the small intestine. Moreover, we reported that the segmental barrier functions in the absorption of tacrolimus were regulated by both Pgp and CYP3A [11]. In that study, the bioavailability of tacrolimus was mainly decreased by CYP3A activity in the upper region of the intestine (duodenum), the Pgp expression level in the lower segment (ileum), and a concerted contribution of both molecules in the jejunum [11]. In living-donor partial small bowel transplantation, the length of the graft intestine is a serious problem for the donor. Since the distal ileum segment shows better fat absorption, this region has been selected in segmental small bowel transplantation [12,13]. Therefore, the functional alteration of ileum Pgp may explain the intra-individual variation of tacrolimus pharmacokinetics immediately after the ileum transplantation.

Recently, we have reported two small bowel transplant recipients who showed a transient up-regulation in the expression of the MDR1 mRNA coding Pgp 2–3 weeks after surgery [14]. In these cases, the blood concentration of tacrolimus was mainly controlled by intravenous administration during increase in the MDR1 mRNA in the graft intestine to avoid a markedly lowered bioavailability of tacrolimus. The expression level of MDR1 mRNA is inversely correlated to the concentration/dose (*C*/*D*) ratio of tacrolimus in the recipient of a living-donor liver transplantation as well as small bowel transplantation [12,14,15]. Therefore, we hypothesized that the transient up-regulation of MDR1 expression and lowered absorptive rate of tacrolimus were due to the recovery of the grafted intestinal epithelium from ischemia–reperfusion injury.

In the present study, considering clinical phenomena such as the transient enhancement of MDR1 mRNA expression and large intra-individual variation of tacrolimus observed in two recipients of small bowel transplantation, we used rats after ischemia–reperfusion injury of the small intestine as a model of the conditions within the graft intestine immediately after the transplantation. Using this animal model, we examined the expression and localization of Pgp, and the alteration of the absorptive property of tacrolimus in accordance with the recovery from the injury.

2. Materials and methods

2.1. Materials

Dihydro-[^{3}H]FK506 (3219 GBq/mmol) was obtained from PerkinElmer Life Science Products and inulin carboxyl, [carboxyl-^{14}C] (70.3 MBq/g) was obtained from Moravek Biochemicals. All other chemicals were of the highest purity available.

2.2. Animals and surgical procedure

Seven-week-old male Wistar rats were used for this study. Prior to the experiments, the rats were housed in a temperature- and humidity-controlled room with free access to water and standard rat chow. The animal experiments were performed in accordance with *The Guidelines for Animal Experiments of Kyoto University*. The intestinal ischemia–reperfusion injury was induced by the method of Udassin et al. [6]. Briefly, rats were anesthetized with diethylether, the superior mesenteric artery was occluded with a micro vascular clamp for 30 min, and then the clamp was removed. At the specified periods after the reperfusion, the rats were killed by bleeding from aorta. Immediately after the killing of rats, the 15 cm ileal specimens were obtained. After removing 1 cm of samples for histology, the mucosa was collected using the cover glasses on ice-cold plate, mixed well and divided into two as follows: about 20 mg for mRNA preparation and the other for crude plasma membrane preparation.

2.3. Histological examination

A bowel specimen was harvested from the ileum (beginning at 3 cm from the ileocecal valve) for histological evaluation. Each specimen was embedded in paraffin, sectioned 5 μm thick, and stained with hematoxylin–eosin.

2.4. Evaluation of mRNA expression

The mRNA expression was quantified using the competitive PCR method as described previously [12]. The total RNA fraction of the ileum was extracted using an RNeasy spin column (QIAGEN GmbH). Aliquots of 1 μg of total RNA were reverse-transcribed using Superscript™ II reverse transcriptase (Invitrogen). The single-stranded DNA was used for subsequent competitive PCR. The primer sets and PCR conditions specific for mdr1a, mdr1b and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were as reported previously [16,17]. Because the PCR-fragment for mdr1b could not be detected in the single-stranded DNA derived from the ileum mRNA, quantification of the mRNA expression level for mdr1a and GAPDH, but not mdr1b, was performed. The value for mdr1a and GAPDH was taken as the ratio to the fixed amount of the competitor c DNA specific for mdr1a (50 zmol/reaction, 1 zmol $= 10^{-21}$ mol) and GAPDH (100 zmol/reaction), respectively. Following competitive PCR for GAPDH with the same batch of single-stranded DNA used to detect the cellular mRNA of mdr1a, the densitometry data were normalized for each batch of RNA by correcting for the amount of GAPDH as an internal control.

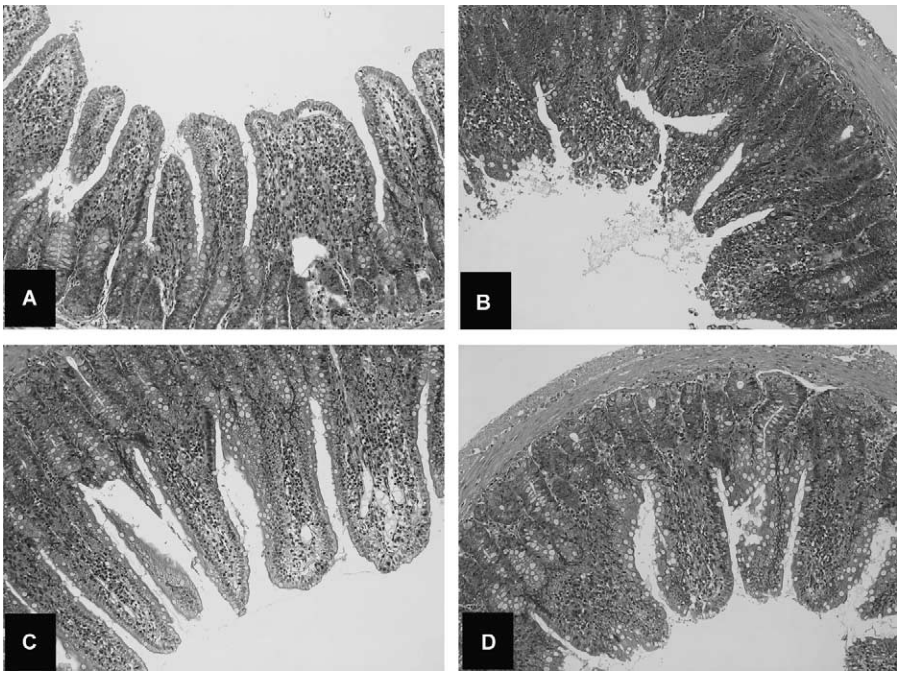

Fig. 1. Histological evaluation of mucosal injury after ischemia–reperfusion. The alteration of the villous structure after ischemia–reperfusion was evaluated by hematoxylin–eosin staining using 5 μm thick ileum sections. Normal intestine (A) and the ileum sections at 1 h (B), 6 h (C) and 24 h (D) after reperfusion were stained. Each image was taken at ×400 magnification.

2.5. Western blotting

The crude plasma membrane fractions from the ileum were isolated as described previously [11]. The protein expression levels of Pgp and villin in each crude membrane fraction were evaluated by Western blotting. Western blotting using C219 monoclonal antibody (CIS bio international) for Pgp was performed as described [11], and a polyclonal antibody for villin (Santa Cruz Biotechnology, Inc.) was used according to the manufacturer's instructions.

2.6. Immunohistochemistory

For indirect immunofluorescent staining, 1 cm bowel specimens were taken in each rat from the ileum. These specimens were fixed in 4% paraformaldehyde, and the frozen specimens were sectioned 5 μm thick. The immunofluorescent staining was performed by using C219 monoclonal antibody for Pgp and anti-villin polyclonal antibody for villin as primary antibody. The fluorescent signals for Pgp and villin were detected using Alexa488 anti-mouse IgG

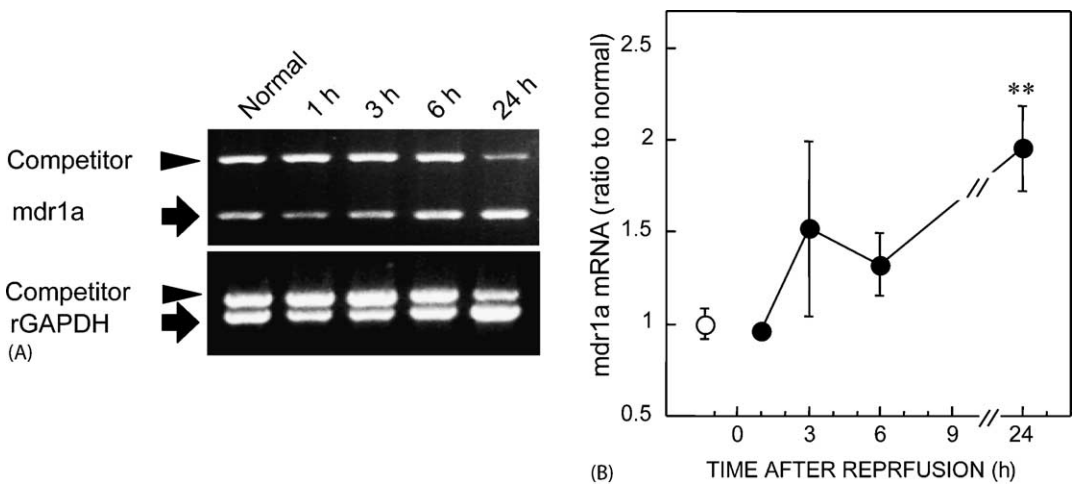

Fig. 2. Ileum mdr1a mRNA expression after ischemia–reperfusion. (A) Representative results of competitive PCR for mdr1a and GAPDH are shown. The arrow and arrowhead indicate the bands derived from single-stranded DNA from intestinal mucosa and competitor DNA, respectively. (B) mRNA expression level was evaluated by competitive PCR as a ratio to a fixed amount of competitor DNA (50 zmol/assay for mdr1a and 100 zmol/assay for GAPDH). Each point shows mdr1a mRNA expression as a ratio to that in normal (open circle) intestine after a correction based on GAPDH expression. Each point represents means ± S.E. of five rats. $^{**}P < 0.01$, significantly different from normal.

conjugated (Invitrogen) and Alexa546 anti-goat IgG conjugated (Invitrogen), respectively. The staining for nuclei was performed with 4′,6-diamidino-2-phenylindole (DAPI) (DOJINDO Lab.). Images were captured with a DP-50 CCD camera (Olympus) using Studio Lite Software (Olympus).

2.7. Drug transport experiment

The rats were fasted 18 h before the experiment. Water was available ad libitum. They were anesthetized with diethylether, subjected to intestinal ischemia for 30 min, and then divided into four experimental groups: normal rats, 1 h after reperfusion, 6 h after reperfusion, and 24 h after reperfusion. A catheter was inserted into the portal vein with polyethylene tubing (Intramedic PE-10, Becton Dickinson and Co.) for blood sampling. An in situ ileum loop (15 cm length) was made in each rat, and phosphate-buffered saline (pH 7.4) containing *dihydro*-[^{3}H]FK506 (100 μg/mL, 370 kBq/mL) and [^{14}C]inulin (1 mg/mL, 74 kBq/mL) was administered into the loop (0.5 mL) at the desired time after the reperfusion. Blood samples (200 μL) were drawn before and 1, 3, 5, 10 and 15 min after the drug administration. Whole blood samples (100 μL) were mixed with a precipitation reagent (100 μL) consisting of methanol (50%, v/v), ethylene-glycol (30%, v/v), water (20%, v/v) and zinc sulfate (100 mM), and centrifuged for 10 min at 10,000 rpm. The radioactivity of the supernatants was measured in ACSII (Amersham Biotech) by liquid scintillation counting. The radioactivity of [^{14}C]Inulin in the portal vein was used as paracellular/non-specific diffusion, and that of *dihydro*-[^{3}H]FK506 was evaluated as total absorption including transcellular and paracellular route.

2.8. Statistics

The statistical significance of differences between mean values was calculated using the non-paired *t*-test. A *p*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Histological examination

The histological features of the ileum at different post-reperfusion times are shown in Fig. 1. Upper villous cells were sloughed by ischemia–reperfusion injury, and the most severe damage to the intestinal mucosa was observed

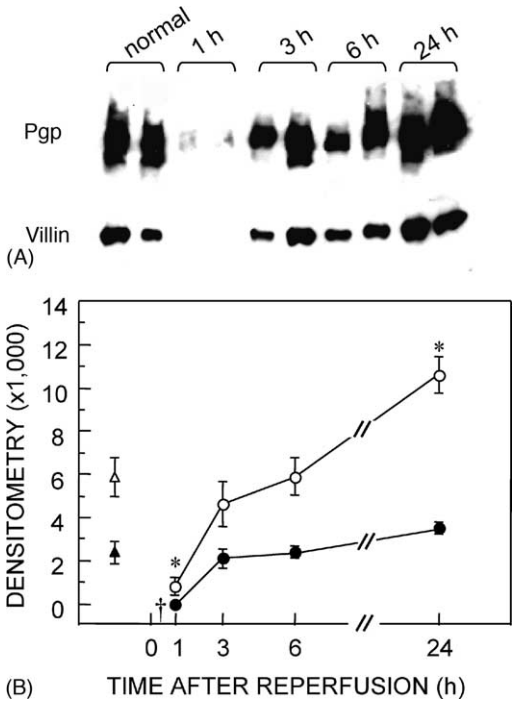

Fig. 3. Ileum Pgp expression immediately after ischemia–reperfusion. Protein expression after ischemia–reperfusion was evaluated by Western blotting using crude plasma membrane. (A) Representative results of Western blotting for Pgp and villin. (B) The expression of Pgp (open circle) and villin (closed circle) after reperfusion was evaluated. The expression levels of Pgp and villin in normal intestine were showed as open triangle and closed triangle, respectively. Each point represents means ± S.E. of five rats. $^{*}P < 0.05$ and $^{\dagger}P < 0.05$, significantly different from normal at the expression levels of Pgp and villin, respectively.

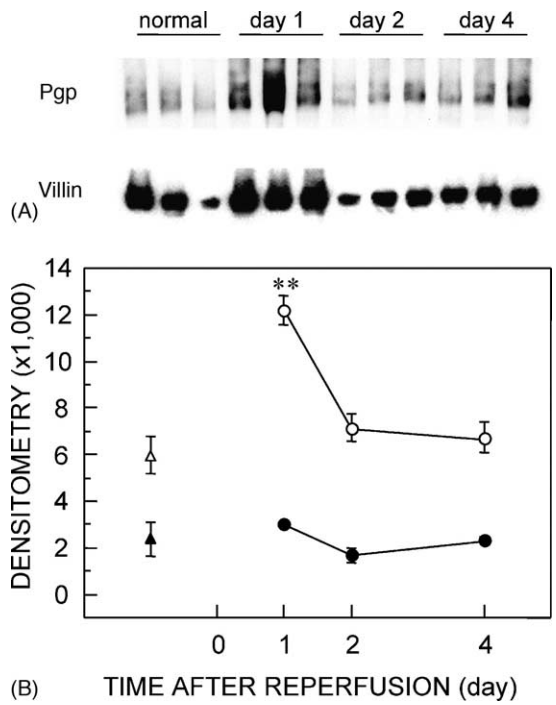

Fig. 4. Ileum Pgp expression after ischemia–reperfusion. (A) Protein expression of Pgp and villin after ischemia–reperfusion was evaluated by Western blotting using crude plasma membrane at 1, 2 and 4 days after reperfusion. (B) The expression of Pgp (open circle) and villin (closed circle) after reperfusion was evaluated. The expression levels of Pgp and villin in normal intestine were showed as open triangle and closed triangle, respectively. Each point represents means ± S.E. of three rats. $^{**}P < 0.01$, significantly different from normal at the expression levels of Pgp.

at 1 h after reperfusion (Fig. 1B). Almost all of the epithelia were improved 6 h after the ischemia–reperfusion treatment, and the mucosal histological features had normalized by 24 h after the treatment.

3.2. Expression of mdr1a mRNA and Pgp

Fig. 2 shows the expression profile of mdr1a mRNA in the ileum after reperfusion. The expression was unchanged even at 1 h after reperfusion, and increased until 24 h after reperfusion in comparison with the normal rats.

Figs. 3 and 4 show the protein expression of Pgp and villin in the ileum sections for 4 days. The expression of Pgp and villin was depressed at 1 h after reperfusion. After that, the expression of villin increased with time, and had recovered to normal levels at 24 h after reperfusion. In contrast, the expression of Pgp increased with time, and was approximately two-fold higher than normal at 24 h after reperfusion (Fig. 3). The up-regulation of Pgp expression at 24 h after reperfusion had nearly recovered to normal levels at 48 h (Fig. 4).

3.3. Immunohistochemistry

To examine the localization of Pgp after ischemia–reperfusion injury, we performed indirect immunofluorescent staining with the ileal specimens. In normal ileal specimens, the signal for Pgp was predominantly found at the brush-border membrane (BBM) of the villous epithelial cells along the crypt–villous axis (Fig. 5A). At 1 h after reperfusion, immunoreactive Pgp was faintly detected in the remaining villous epithelial cells (Fig. 5D). At 6 h after reperfusion, the signal for Pgp was weakly observed in the regenerated villous cell, on the BBM as well as in the intracellular region (Fig. 5E). At 24 h after reperfusion, the signal for Pgp was localized to the BBM (Fig. 5F). The expression of villin was detected at the villous tip similar to Pgp in the normal intestine (Fig. 5B). After ischemia–reperfusion, the signal for villin was almost completely diminished, and not until 24 h was a clear signal at the BBM, although a weak signal was detected at 6 h after reperfusion (Fig. 5H–J). The expressions of Pgp and villin were also detected at the BBM similar to both the proteins in the normal ileum at time 0, immediately before the reperfusion (Fig. 5C and G).

3.4. Transport experiment

Fig. 6 shows the portal blood concentrations of *dihydro*-[^{3}H]FK506 as a substrate for Pgp and inulin as a marker compound for the permeability of the paracellular route after the intraintestinal administration. The profile of the portal vein concentration and area under the concentration-time curve (AUC) of *dihydro*-[^{3}H]FK506 was increased at 1 and 6 h after reperfusion, but markedly decreased at 24 h (Fig. 6A). The value of inulin at 1 h after reperfusion was

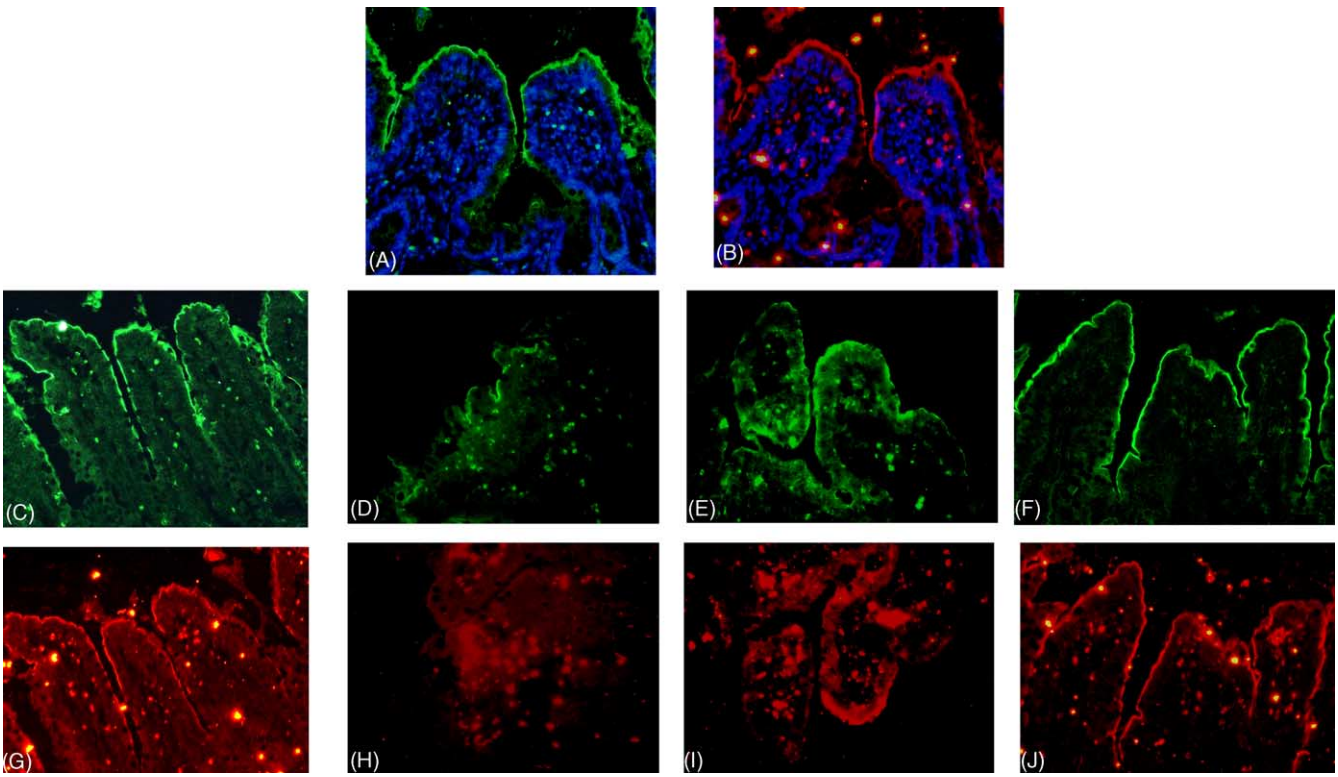

Fig. 5. Immunohistochemical evaluation of protein expression. Localization of Pgp and villin in normal intestine was represented in (A) and (B), respectively, with double staining with DAPI. The Pgp expression was determined at zero time (C), and 1 h (D), 6 h (E) and 24 h (F) after reperfusion. The expression of villin was determined at zero time (G), and 1 h (H), 6 h (I) and 24 h (J), respectively.

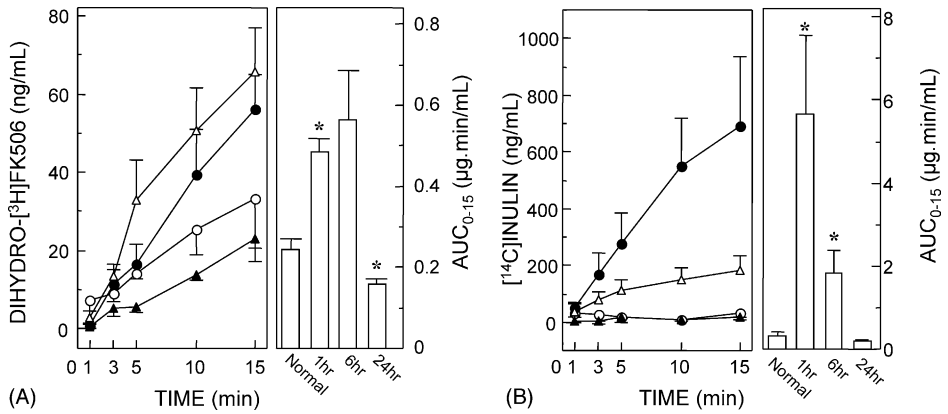

Fig. 6. The absorption rate of *dihydro*-[^{3}H]FK506 after ischemia–reperfusion. (A) The *dihydro*-[^{3}H]FK506 blood concentration in the portal vein was measured in normal ileum (open circle) and at 1 h (closed circle), 6 h (open triangle) and 24 h (closed triangle) after reperfusion. The AUC_{0-15} value was estimated from the cumulative amount of *dihydro*-[^{3}H]FK506 during 15 min. (B) The inulin blood concentration in the portal vein was measured in normal intestine (open circle) and at 1 h (closed circle), 6 h (open triangle) and 24 h (closed triangle) after reperfusion. The AUC_{0-15} value was estimated from the cumulative amount of inulin during 15 min. Each point represents means ± S.E. of five rats. $^*P < 0.05$, significantly different from normal rats.

significantly high, and then decreased toward the control level with time (Fig. 6B).

4. Discussion

In living-donor partial small bowel transplantation, the ileum is often selected as a graft to ensure the safety of the donor [12,13]. In addition, the ileum was suggested to be a more suitable graft than the jejunum for post-transplant immunosuppressive therapy [7]. In a recipient of living-donor ileum transplantation, the variation in the mucosal MDR1 mRNA level was suggested to be a key pharmacokinetic factor explaining the intra-individual variation of tacrolimus trough concentrations [12]. Recently, we have reported that the monitoring of mucosal MDR1 mRNA in the graft intestine provided useful information for modifying the dosage of tacrolimus [14]. In those three cases, the expression level of MDR1 was depressed immediately after the transplantation, and then gradually increased to be higher than that at surgery [14]. However, the relationship between the variation in the tacrolimus blood level and the enterocyte expression level of MDR1 immediately after the small bowel transplantation was not clear. In the present study, the expressional change of mucosal Pgp and the absorption of intra-intestinally administered tacrolimus was performed immediately after ischemia–reperfusion injury in rats as an animal model to examine the relationship between morphological variation of the ileum.

The enterocyte expression of villin as a marker protein of intestinal epithelial cells was depressed and gradually increased at the apical surface, which was comparable with the morphological features (Figs. 1 and 5). In addition, the expression level and membrane localization were also recovered at 24 h after the reperfusion (Figs. 3 and 5). Although the mucosal Pgp was diminished at 1 h after the reperfusion, the expression level of the transporter was two-fold higher than at surgery (Figs. 3 and 4). The expression of Pgp was limited to the apical surface at time 0, but some intracellular Pgp was found at 6 and 24 h after the reperfusion (Fig. 4). These results suggested that the expression level of Pgp after reperfusion was the sum of the apical- and intracellular-Pgp levels.

The heightened absorption of inulin at 1 and 6 h after the reperfusion indicated a loss of barrier function of the small intestine caused by the sloughed epithelial cells. Despite the recovered expression of Pgp at 6 h after reperfusion, the portal vein concentration of *dihydro*-[^{3}H]FK506 was higher than the control value. However, the AUC of *dihydro*-[^{3}H]FK506 was significantly lower than the control level at 24 h after reperfusion, suggesting enhanced back-flux activity with the increased expression of Pgp (Figs. 2, 3 and 6). At 48 h after reperfusion, the expression level of Pgp had decreased to normal (Fig. 4). These results suggest that the enhanced permeability of *dihydro*-[^{3}H]FK506 via the intestinal barrier is due to the transient depression of enterocytes after ischemia–reperfusion rather than the decreased expression of Pgp. However, a transient enhancement of Pgp according to the regeneration of intestine was suggested to act as an absorptive barrier and decrease the absorption of *dihydro*-[^{3}H]FK506 at 24 h after reperfusion.

Both the *mdr1a* and *mdr1b* genes provide Pgp in rats and mice [18]. In the present study, mdr1b mRNA could not be detected by competitive PCR. Therefore, the expressional change of Pgp after ischemia–reperfusion treatment was indicated to be derived from mdr1a mRNA. The mdr1b mRNA and Pgp expression were transiently up-regulated after partial hepatectomy in the rats. However, the expression level of mdr1a mRNA did not change [16]. In addition, mdr1b mRNA and Pgp, but not mdr1a mRNA, were also up-regulated after intra-peritoneal administration of lipopolysaccalide [19]. These reports suggest that the mdr1a mRNA is a constitutive type, but mdr1b is an inducible type

activated by a states of stress such as inflammation. Interestingly, the basal expression level of mdr1b was markedly low in the rat ileum, and the Pgp was indicated to be a product of mdr1a. In addition, the mdr1b mRNA was not detected after reperfusion (data not shown). Therefore, the expressional change of Pgp after ischemia–reperfusion injury would reflect the variation in the mdr1a mRNA level, and the mdr1a could be induced during the tissue regeneration. The expression of the *mdr* gene is reported to be up-regulated by a growth factor, EGF [20]. In experiments in vitro using human intestinal Caco-2 cells, the up-regulation of Pgp expression corresponding to the proliferation was due to stabilization of the mRNA [21]. Therefore, the transient up-regulation of Pgp after reperfusion may be explained by the tissue regeneration or cell growth and differentiation rather than the inflammatory response.

Pgp is localized at the BBM along the crypt–villous axis in the normal intestine. The villous structure of the ileum was rapidly sloughed, and the expression of Pgp and villin at the BBM of enterocytes was diminished at 1 h after reperfusion, but a slight C219 positive signal was also detected around the crypt area. Signal for C219 corresponding to Pgp was detected in the intracellular region of enterocytes at 6 h after reperfusion, and strongly localized to the BBM at 24 h. From the result of in situ hybridization using mouse intestine, the signal for mdr mRNA was detected in the villous differentiated epithelium, but not proliferating crypt cells [22]. However, the expression of Pgp in the Caco-2 monolayers corresponded to the proliferating activity [21]. We assumed that Pgp appeared in the villous epithelial cells and crypt undifferentiated cells following ischemia–reperfusion injury. Therefore, the enterocytes migrate from the proliferating crypt area to the villous tip with differentiation after ischemia–reperfusion injury. According to the migration and/or proliferation–differentiation of enterocytes, the intracellularly diffused Pgp may be localized to the BBM.

The present results suggested that the bioavailability of tacrolimus was influenced by intestinal barrier functions including the expression and localization of Pgp after ischemia–reperfusion injury. In a recipient of living-donor partial small bowel transplantation, the mRNA expression of MDR1 was significantly decreased on postoperative day 3, at which time the oral bioavailability of tacrolimus was estimated to be more than 80%, and the expression of MDR1 mRNA was increased and the *C/D* ratio of tacrolimus was decreased early in the postoperative period [14]. Therefore, the present examination using an animal model demonstrated the pathological and molecular mechanisms behind the intra-individual variation in the absorption of orally administered tacrolimus. In addition, the enterocyte mRNA level of MDR1 would be an important marker of the morphological status of graft intestine and absorptive rate of tacrolimus, because the variation of Pgp in the intestine was comparable with the morphological status of the recovering intestine. The present findings also provide useful information for further studies of postoperative control including immunosuppressive therapy with tacrolimus.

Acknowledgments

This work was supported by a Grant-in-Aid from the Japan Health Sciences Foundation, by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan, by a Grant-in-Aid from the Japan Research Foundation for Clinical Pharmacology, and by the 21st Century COE program ''Knowledge Information Infrastructure for Genome Science''. M.S. is supported as a Research Fellow by the 21st Century COE program ''Knowledge Information Infrastructure for Genome Science''.

References

[1] Massberg S, Messmer K. The nature of ischemia–reperfusion injury. Transplant Proc 1998;30:4217–23.
[2] Gordon JI. Intestinal epithelial differentiation: new insights from chimeric and transgenic mice. J Cell Biol 1989;108:1187–94.
[3] Zhi-Yong S, Dong YL, Wang XH. Bacterial translocation and multiple system organ failure in bowel ischemia and reperfusion. J Trauma 1992;32:148–53.
[4] Yeh KY, Yeh M, Glass J. Expression of intestinal brush-border membrane hydrolases and ferritin after segmental ischemia–reperfusion in rats. Am J Physiol 1998;275:G572–83.
[5] Rijke RP, Hanson WR, Plaisier HM, Osborne JW. The effect of ischemic villous cell damage on crypt cell proliferation in the small intestine: evidence for a feedback control mechanism. Gastroenterology 1976;71:786–92.
[6] Udassin R, Vromen A, Haskel Y. The time sequence of injury and recovery following transient reversible intestinal ischemia. J Surg Res 1994;56:221–5.
[7] Farmer DG, McDiarmid SV, Yersiz H, Cortina G, Amersi F, Vargas J, et al. Outcome after intestinal transplantation: results from one center's 9-year experience. Arch Surg 2001;136:1027–31 [Discussion 1031–2].
[8] Venkataramanan R, Swaminathan A, Prasad T, Jain A, Zuckerman S, Warty V, et al. Clinical pharmacokinetics of tacrolimus. Clin Pharmacokinet 1995;29:404–30.
[9] Hebert MF. Contributions of hepatic and intestinal metabolism and P-glycoprotein to cyclosporine and tacrolimus oral drug delivery. Adv Drug Deliv Rev 1997;27:201–14.
[10] Brady JM, Cherrington NJ, Hartley DP, Buist SC, Li N, Klaassen CD. Tissue distribution and chemical induction of multiple drug resistance genes in rats. Drug Metab Dispos 2002;30:838–44.
[11] Shimomura M, Masuda S, Saito H, Sakamoto S, Uemoto S, Tanaka K, et al. Roles of the jejunum and ileum in the first-pass effect as absorptive barriers for orally administered tacrolimus. J Surg Res 2002;103:215–22.
[12] Masuda S, Uemoto S, Hashida T, Inomata Y, Tanaka K, Inui K. Effect of intestinal P-glycoprotein on daily tacrolimus trough level in a living-donor small bowel recipient. Clin Pharmacol Ther 2000;68:98–103.
[13] Kiyozaki H, Kobayashi E, Toyama N, Miyata M. Segmental small bowel transplantation in the rat: comparison of lipid absorption between jejunal and ileum grafs. J Parenter Enteral Nutr 1996;20:67.
[14] Masuda S, Uemoto S, Goto M, Fujimoto Y, Tanaka K, Inui K. Tacrolimus therapy according to mucosal MDR1 levels in small-bowel transplant recipients. Clin Pharmacol Ther 2004;75:352–61.

[15] Hashida T, Masuda S, Uemoto S, Saito H, Tanaka K, Inui K. Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. Clin Pharmacol Ther 2001;69:308–16.
[16] Vos TA, Ros JE, Havinga R, Moshage H, Kuipers F, Jansen PL, et al. Regulation of hepatic transport systems involved in bile secretion during liver regeneration in rats. Hepatology 1999;29:1833–9.
[17] Beiche F, Scheuerer S, Brune K, Geisslinger G, Goppelt-Struebe M. Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. FEBS Lett 1996;390: 165–9.
[18] Zhang F, Riley J, Gant TW. Intrinsic multidrug class 1 and 2 gene expression and localization in rat and human mammary tumors. Lab Invest 1996;75:413–26.
[19] Cherrington NJ, Slitt AL, Li N, Klaassen CD. Lipopolysaccharide-mediated regulation of hepatic transporter mRNA levels in rats. Drug Metab Dispos 2004;32:734–41.
[20] Hirsch-Ernst KI, Ziemann C, Schmitz-Salue C, Foth H, Kahl GF. Modulation of P-glycoprotein and mdr1b mRNA expression by growth factors in primary rat hepatocyte culture. Biochem Biophys Res Commun 1995;215:179–85.
[21] Goto M, Masuda S, Saito H, Inui K. Decreased expression of P-glycoprotein during differentiation in the human intestinal cell line Caco-2. Biochem Pharmacol 2003;66:163–70.
[22] Trezise AE, Romano PR, Gill DR, Hyde SC, Sepulveda FV, Buchwald M, et al. The multidrug resistance and cystic fibrosis genes have complementary patterns of epithelial expression. EMBO J 1992;11: 4291–303.