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Comparison of Cyclosporin A and Tacrolimus Concentrations in Whole Blood between Jejunal and Ileal Transplanted Rats

YOSHINORI OGINO, EIJI KOBAYASHI AND AKIO FUJIMURA

Department of Clinical Pharmacology and Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan

Abstract

Most immunosuppresive drugs are absorbed from the intestine after oral administration, although there is some difference of bioavailability between ileum and jejunum. Using an orthotopic segmental small bowel transplantation (SBT) model in rats, we studied the pharmacokinetic profiles of cyclosporin A and tacrolimus concentrations after oral intake, comparing jejunal and ileal transplanted rats.

Two types of segmental SBT (jejunal and ileal SBT) in a syngeneic combination were performed. After oral administration of cyclosporin A ($10\,mg\,kg^{-1}$) or tacrolimus ($5\,mg\,kg^{-1}$), pharmacokinetic data were obtained from the long-surviving rats transplanted with segmental SBT. To determine the effect of additional bile on cyclosporin absorption, an emulsion of cyclosporin A with fresh bile juice was re-challenged on segmental SBT rats before killing. A histological study was also performed by use of the intestinal grafts from the killed SBT rats.

A higher concentration of cyclosporin A was observed in the ileum-grafted rats than in the rats which received the jejunal grafts. Oral bioavailability of cyclosporin A in ileal SBT rats tended to be increased by addition of fresh bile juice, but that in jejunal SBT rats did not change. On the other hand, there was no significant difference of tacrolimus concentration between jejunum- and ileum-transplanted rats. Histological studies showed that the superficial mucosal layer of both grafts, but especially the ileal graft, was markedly elongated compared with that of normal intestine.

The present study showed that cyclosporin A was more actively absorbed from ileum than from jejunum in SBT, but tacrolimus was absorbed equally from both sites. These data suggest that cyclosporin A concentration is satisfactorily controlled in the segmental ileal graft, while there is no difference of tacrolimus absorption between ileal and jejunal graft.

Small bowel transplantation (SBT) is a radical therapy for short bowel syndrome, although total parenteral nutrition has improved patients' prognoses. As donor shortage is a serious problem, especially in paediatric transplantation, segmental SBT is a feasible clinical option; use of living related donors overcomes the shortage of donor intestine for clinical SBT (Pollard et al 1996; Gruessner & Sharp 1997; Jaffe et al 1997; Tesi et al 1997).

To study immunological and physiological difference between ileum and jejunum, we developed a simple rat model of orthotopic segmental SBT (Kobayashi et al 1994), demonstrating that lipid absorption was predominantly better in the ileum than in the jejunum (Kiyozaki et al 1996). When cyclosporin A is used for SBT patients, it is important to compare its relative absorption for jejunal and ileal grafts. Experimental and clinical evidence has shown that tacrolimus is better than cyclosporin A for immunosuppression in SBT (Gruessner et al 1995; Grant 1996; Pirenne et al 1996). Tacrolimus has been reported to be absorbed mainly from the upper part of the small intestine using an in-situ rat model (Kagayama et al 1993). Thus, it would be beneficial to confirm this phenomenon in more detail using the model of segmental SBT.

Correspondence: E. Kobayashi, Division of Organ Replacement Research, Center for Molecular Medicine, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan. E-Mail: eijikoba@jichi.ac.jp

In this study, we investigate the pharmacokinetics of cyclosporin A and tacrolimus in rats with segmental SBT.

Materials and Methods

Rats

Male rats of the inbred LEW ($RT1^1$) strain (Japan CLEA, Tokyo, Japan), weighing 230–270 g, were used for orthotopic segmental SBT models in a syngeneic combination. Donor rats were fasted one day before the operation. All recipient rats were allowed free access to water and food (CE-2 food, Nippon Clea Co. Ltd, Tokyo Japan) after the postoperative period. All operations were carried out under ether anaesthesia. This work was performed according to the guidelines for animal research at Jichi Medical School.

Operation

Syngeneic orthotopic SBT was carried out using the two-step method as described previously (Kobayashi et al 1993). The jejunum from the ligament of Treitz to the middle of intestine (approximately 35 cm length measured by a string scale) was prepared from the donor with a vascular pedicle consisting of aorta, superior mesenteric artery and portal vein. The ileum from the middle of the small intestine to the ileocaecal valve (the same length as jejunum) was prepared from the donor in the same way. The aorta and portal vein of the graft were anastomosed to the left renal artery and vein, respectively, of the recipient using a cuff technique. For obtaining a high success rate of orthotopic transplantation, the second-step method was performed as described previously (Kobayashi et al 1995); in brief, both ends of the grafted small bowel were exteriorized as stomata and then, one week after transplantation, the intestinal tract was re-constructed. After the small intestine of the recipient was entirely removed from the ligament of Treitz to the ileocaecal valve, the jejunal or ileal grafts exteriorized as stomata were anastomosed orthotopically with 6-0 silk using continuous sutures.

Drugs

Cyclosporin A solution (Sandimmune oral solution, 100 mg mL^{-1}; Sandoz Ltd, Basel, Switzerland) (0·5 mL) was diluted with 20 mL of olive oil (Kanto Chemical Co., Tokyo, Japan) to produce a 2·5 mg mL^{-1} solution, which was orally administered at a dose of 10 mg kg^{-1}. Tacrolimus powder was kindly provided by Fujisawa Ltd (Tokyo, Japan), suspended at 5 mg mL^{-1} in distilled water and given orally to recipient rats.

Experimental protocol

The rats transplanted with jejunal or ileal graft were divided into two groups 4 months after grafting ($n = 5$ in each group). Cyclosporin A or tacrolimus was administered at 0100 h and blood samples were collected from the tail vein at 1, 2, 4, 7, 12, 14, 17 and 24 h or 1, 4, 8 and 12 h after administration. Untreated animals were tested according to the same protocol, as controls ($n = 6$).

At the end of observation period (6 months after reconstruction), cyclosporin A in combination with 4 mL kg^{-1} fresh bile juice collected from normal rats was orally administered to the recipient rats and blood samples were collected at the same times as mentioned above. All of the SBT rats were finally killed for histological evaluation (non-operative rats at the same weeks were also killed as a control group). The blood samples were fixed in 10% formalin and stained with haematoxylin & eosin, and histologically evaluated for villus height and crypt depth of the intestine at three randomly selected fields by an expert (F. Uchiyama, Mizayaki Medical College) in a blind fashion. The ratio of villus vs crypt was calculated as previously reported (Rahman et al 1996).

Drug concentration

The concentration of cyclosporin A in whole blood was measured by fluorescence polarization immunoassay (Jolley et al 1981; Pescovitz et al 1992) using a TDX analyser (Dinabot Co. Ltd, Tokyo, Japan), which has an assay limit of 25–1500 ng mL^{-1}. The concentration of tacrolimus in whole blood was measured by immunoabsorption assay (Japanese FK 506 Study Group 1991), with a limit of detection of 0·5 ng mL^{-1}.

Pharmacokinetic analysis

The maximum concentration in whole blood (C_{max}) and time to maximum concentration (t_{max}) were determined directly from the concentration–time curve. The area under the whole blood concentration–time curve from 0 to 24 h (AUC_{0-24}) and that from 0 to 12 h (AUC_{0-12}) was determined using the trapezoidal rule. The results are expressed as the mean ± s.d.

Statistical analysis

Data obtained were analysed by two-way analysis of variance. Histological data were analysed by an

unpaired Student's *t*-test. The difference between groups was considered to be significant when the *P* value was $< 0{\cdot}05$.

Results

The rats receiving ileal or jejunal grafts increased in weight, and there was no statistical difference between the two groups during the time of drug challenge. Whole blood concentration of cyclosporin A or tacrolimus after oral administration in segmental SBT rats are shown in Figures 1 and 2, respectively. The C_{max} of cyclosporin A in the ileal SBT group significantly decreased, compared with that in the control. The blood level in the ileal group tended to be higher than that in the jejunal group, although the difference was not significant ($547{\cdot}5 \pm 118{\cdot}2$ vs $169{\cdot}7 \pm 183{\cdot}4$ ng mL^{-1}, respectively). The t_{max} in jejunal SBT tended to be delayed compared with that in ileal SBT ($12{\cdot}0 \pm 3{\cdot}5$ vs $10{\cdot}3 \pm 2{\cdot}6$ h, respectively). The AUC_{0-24} of cyclosporin A was markedly lower in the jejunal SBT group than that for the control group ($P = 0{\cdot}0009$), and there was also a significant difference between values for ileal and jejunal SBT rats ($P = 0{\cdot}028$).

As segmental SBT rats were reported to have altered bile acid compositions (Kiyozaki et al 1996; Rahman et al 1996), fresh bile was added to study its effect on cyclosporin A blood concentration in the long-surviving SBT rats. The concentration in ileal SBT rats was slightly increased by the addition of fresh bile juice (C_{max}: $933{\cdot}2 \pm 669{\cdot}4$ ng mL^{-1} and AUC_{0-24}: $9506{\cdot}5 \pm 6837{\cdot}2$ ng h mL^{-1}), however, no statistically significant difference was seen between the rats treated with cyclosporin A alone and those with cyclosporin A plus bile juice. In addition, no influence on the pharmacokinetics of cyclosporin A at a low level was observed in jejunal SBT rats even by the addition of bile juice.

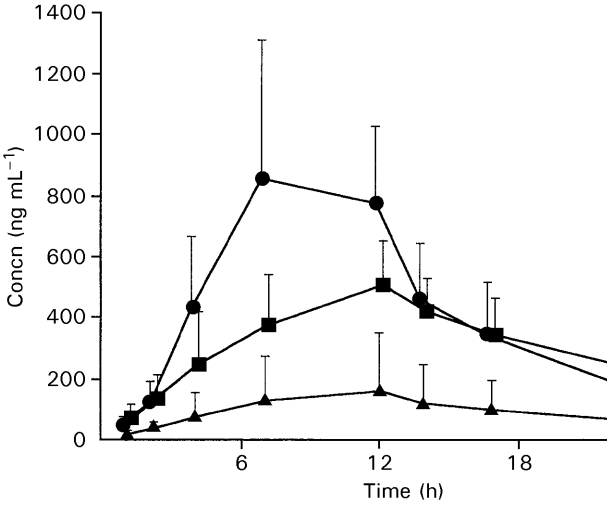

Figure 1. Cyclosporin A concentration in whole blood obtained from non-operative control (●), jejunal (▲) and ileal (■) SBT rats.

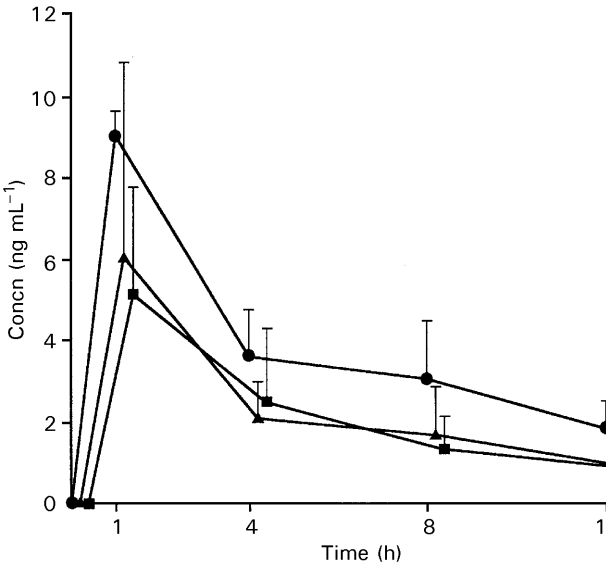

Figure 2. Tacrolimus concentration in whole blood obtained from non-operative control (●), jejunal (▲) and ileal (■) SBT rats.

The C_{max} in the tacrolimus-treated group was reduced to approximately 40% of that in the control rats, without any statistical difference (Figure 2). The C_{max} of ileum and jejunal groups was the same. There was also no significant difference of AUC_{0-12} among these three groups.

Histological findings of intestinal grafts in the long-surviving SBT rats showed significant elongation of superficial mucosa of the two experimental groups ($P < 0{\cdot}05$, compared with control group; Table 1). Better adaptation was observed in the ileal grafts, resulting in an increase of villus height/crypt depth ratio.

Discussion

Whole intestinal transplantation is not required for the treatment of the patients with short gut. In

Table 1. Histological evaluation of the long-surviving segmental SBT rats.

	Jejunum		Ileum	
	Control	Graft	Control	Graft
Villus height (μm)	448 ± 24	579 ± 25*	286 ± 23	593 ± 45*
Crypt depth (μm)	199 ± 7	279 ± 33*	176 ± 16	301 ± 30*
Villus/crypt ratio	2·25	2·07	1·63	1·97

*$P < 0{\cdot}05$ compared with controls.

addition, shortage of cadaver donor remains a major problem in Japan. Thus, living related segmental SBT was recently tried in two paediatric patients (Prof. Tanaka, Kyoto University Faculty of Medicine, Japan, personal communication).

Jejunum and ileum have been evaluated separately by other investigators (LaRosa et al 1989; Rahman et al 1996). We have developed a model of segmental SBT in rats, showing that ileal grafts are better than jejunal grafts from the aspect of lipid absorption (Kiyozaki et al 1996). Using this model, in the present study we evaluated the difference in pharmacokinetic profile of cyclosporin A or tacrolimus in recipients with jejunal and ileal grafts. In the cyclosporin A-treated groups, the C_{max} and AUC_{0-24} in the recipients after ileal grafting were approximately three times higher than those after jejunal grafting. In contrast, there was no difference in tacrolimus absorption between ileal- and jejunal-grafted rats.

It has been reported that the bioavailability of cyclosporin A is approximately 20–25% of the oral dose (Gupta & Benet 1989) and is dependent on several factors, including length of small intestine, bowel transit time, lymphatic disruption/reconstruction, and autonomic nervous system (Mackenzie et al 1982; Lear et al 1988; Roberts et al 1988; LaRosa et al 1989; Whitington et al 1990). The absorption site in the intestine has been studied using a tube insertion method in humans (Drewe et al 1992) and animals (Sawchuk & Awni 1986; Tarr & Yalkowsky 1989; Cakaloglu et al 1993), with results indicating that a high blood concentration of cyclosporin A was obtained when the drug was administered into the upper intestinal tract. However, the most preferable part of the intestine for cyclosporin A absorption has not yet been determined in segmental SBT, except for one report by LaRosa et al (1989), which demonstrated there to be no difference in serum levels of cyclosporin A between the recipients with heterotopic jejunal and ileal grafts. However, they measured cyclosporin A concentrations not when the drug was given orally but when it was administered directly into the heterotopically transplanted grafts. Our present study using the long-term surviving orthotopic SBT rats, showed that ileal gut absorbed cyclosporin A better than jejunal gut. This phenomenon was also confirmed using an intestinal resection model in our preliminary study (Ogino et al 1999).

The higher cyclosporin A absorption from the ileal segment than from the jejunal segment might be due to effects of bile salts. It is well-known that cyclosporin A levels markedly increased in liver transplant patients when the T-tube was clamped (Mehta et al 1988). Cakaloglu et al (1993) also experimentally showed that cyclosporin A absorption significantly increased by 23% in duodenum and proximal jejunum, and by 50% in distal ileum when bile juice was administered into the intestinal lumen. In our study, the addition of fresh bile resulted in a tendency to improve cyclosporin A absorption in ileal SBT rats, but not in jejunal SBT rats. Pathophysiological adaptation of the grafted ileum also supported this phenomenon. Recently, a new formulation of cyclosporin A (Neoral; Sandoz Pharma, Basel, Switzerland) has been introduced, which is a microemulsion composed of lipophilic and hydrophilic components together with a surfactant. The bioavailability of this drug is markedly enhanced and its pharmacokinetic profile is more consistent and predictable than that of the original agent (Kovarik et al 1994). The beneficial effect of better absorption might be observed on ileal SBT with the use of Neoral.

Recent clinical and experimental studies for SBT showed that tacrolimus is more effective than cyclosporin A for graft survival (Gruessner et al 1995; Grant 1996; Pirenne et al 1996). Use of an in-situ loop absorption test showed that tacrolimus was absorbed predominantly from the duodenum and proximal jejunum (Kagayama et al 1993). In the present study, however, unlike cyclosporin A absorption, there was no difference in tacrolimus absorption between jejunal and ileal segments under physiological conditions. Although the mechanism by which there is a significant difference of bioavailability between jejunal and ileal grafts with cyclosporin A, but not tacrolimus, remains unclear, it might be that cyclosporin A inhibits the activity of the multidrug efflux pump (P-glycoprotein), but tacrolimus is less effective in the isolated hepatocyte couplets (Takeguchi et al 1993). The liver first-pass effect of these drugs also requires further study. While the vein of the graft intestine in our model was drained to the inferior vena cava of the recipient, study of the pharmacokinetic profiles is needed in the portally-drained rats (manuscript in preparation).

In conclusion, our data indicate that cyclosporin A absorption is predominantly higher in the ileum than the jejunum after orthotopic SBT. Ileal segmental transplantation may be more appropriate in patients treated with cyclosporin A. However, tacrolimus is absorbed equally from jejunal and ileal segments.

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