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The Effect of P-Glycoprotein Inhibition and Activation on the Absorption and Serum Levels of Cyclosporine and Tacrolimus in Rats*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Permeability glycoprotein (P-glycoprotein or P-gp) plays an important role in the intestinal absorption of the immunosuppressive agents: cyclosporine and tacrolimus.

Objectives. The aim of this study was to determine how the intestinal absorption of cyclosporine and tacrolimus is affected when they are used with P-gp activating or inhibiting agents.

Material and Methods. In *in vitro* experiments, everted parts of rat small intestines were used to evaluate the effects of verapamil (a P-gp inhibitor) and rifampicin (a P-gp inducer) on the intestinal absorption of cyclosporine and tacrolimus. In *in vivo* experiments, the effects of verapamil and rifampicin on the plasma concentrations of cyclosporine and tacrolimus were evaluated.

Results. In *in vitro* experiments, the absorption of cyclosporine and tacrolimus from the small intestine increased in a time-dependent manner when the drugs were administered with or without verapamil or rifampicin. There was no difference in the absorption of cyclosporine ± verapamil/rifampicin between the jejunum and ileum; however, ileal absorption of tacrolimus + rifampicin was significantly higher than jejunal absorption ($p < 0.05$). Plasma concentrations of cyclosporine and tacrolimus were significantly increased when they were co-administered with verapamil ($p < 0.001$) and significantly decreased when co-administered with rifampicin ($p < 0.05$).

Conclusions. P-gp may play an important role in the absorption of immunosuppressive drugs, and it may contribute to drug-drug interactions that may lead to inadequate drug response or toxicity (Adv Clin Exp Med 2016, 25, 2, 237–242).

Key words: P-glycoprotein, cyclosporine, tacrolimus, everted sac.

Permeability glycoprotein (P-glycoprotein or P-gp) is an efflux transporter that is present in the apical side of the intestinal epithelium and prevents intracellular accumulation of its substrates by reducing their influx and increasing their efflux [1]. The main function of intestinal P-gp is to alter the pharmacokinetics of substrates by reducing their intestinal absorption.

Although P-gp expression and activity has been reported to gradually increase in the small intestine from the proximal to the distal regions [2, 3], there are also some other reports demonstrating just the opposite [4, 5].

P-glycoprotein exports structurally and pharmacologically diverse hydrophobic compounds from cells. The most important substrates of P-gp include anti-cancer agents, immunosuppressive agents, steroid hormones, calcium channel blockers, β -blockers and cardiac glycosides [6–9].

The pump function of P-gp may be affected by some modulating or inhibiting agents [10]. The modulating agents usually have similar features to the P-gp substrates or may themselves be substrates of P-gp. For example, verapamil, a calcium-channel blocking agent, is a P-gp substrate when used in low doses, while high doses of verapamil

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strongly inhibit P-gp [8]. On the other hand, P-gp can easily be activated by some agents, such as rifampicin [11, 12].

P-glycoprotein plays an important role in the intestinal absorption of immunosuppressive agents, including cyclosporine and tacrolimus [13–15]. These two immunosuppressive agents are substrates of P-gp and their absorption increases or decreases when they are used concomitantly with P-gp inhibiting or activating agents. Because many organ transplant patients using immunosuppressive agents are elderly and use multiple drugs for comorbidities, and because many of these drugs are P-gp substrates, these patients are at great risk for drug-drug interactions.

The aim of this study was to determine how the intestinal absorption of two commonly used immunosuppressive agents (cyclosporine and tacrolimus) is affected when they are used with P-gp activating or inhibiting agents. To be effective, these agents have to be at a certain concentration in the body. However, because of their narrow therapeutic index, these agents may easily cause toxic effects. Predicting how and to what degree the bioavailability of immunosuppressive drugs will be affected when used concomitantly with many other commonly used drugs can help to reduce the risk of inadequate treatment or overdose toxicity in this group of patients.

Material and Methods

The Animals

Male Sprague-Dawley rats weighing 200–250 g were used in this study. The animals were fasted for 16 h with free access to tap water *ad libitum*. All the animal studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals at Eskisehir Osmangazi University. The study was financially supported by Scientific Research Projects Unit of Eskisehir Osmangazi University (project no. 2010/11045).

In Vitro Experiments

Following administration of overdose of ether, the animals were killed by cervical dislocation. The abdominal wall was incised and 15–20 cm of proximal jejunum and distal ileum were removed. The intestinal parts were turned inside out by the method described by Wilson and Wiseman [16]. The everted parts of the small intestine were cut into 5–6 cm long segments and put into oxygenated Tyrode's solution (composition: NaCl 136.9 mM, KCl 2.68 mM, CaCl₂ 1.8 mM, MgCl₂ 1.05 mM,

NaHCO₃ 1.19 mM, NaH₂PO₄ 0.42 mM, glucose 0.55 mM; pH 7.3). One end of the intestinal segment was ligated and the other end was tied around a plastic cannula. The resulting intestinal sac was mounted in 20 mL isolated organ baths in Tyrode's solution oxygenated with 95% O₂ + 5% CO₂ at 37°C. Then 0.4 mL of Tyrode's solution was injected through the cannula into the intestinal sac.

Cyclosporine or tacrolimus at a concentration of 1 µM was added to the Tyrode's solution on the outer side of the sacs with and without rifampicin (100 µg/mL) as an activator of P-gp or verapamil (200 µM) as an inhibitor of P-gp. The fluid samples were taken from the internal side of the sacs at 30, 60 and 120 min after the administration of the test drugs. The samples were stored at –20°C until the analysis.

In Vivo Experiments

After a fasting period of 16 h, eight animals from each group were treated with a single dose of cyclosporine (1 mg/kg) or tacrolimus (1 mg/kg) with and without rifampicin (250 mg/kg) or verapamil (25 mg/kg) by gavage. Under light ether anesthesia, a 1–2 mL blood sample was withdrawn from the heart of the animals 120 min after the administration of the test drugs. The blood samples were put into tubes containing EDTA and stored at –20°C until the analysis.

Drug Concentrations

The concentrations of cyclosporine and tacrolimus in the fluid and blood samples were determined on a Hitachi 912 analyzer (Roche Diagnostics, Mannheim, Germany) by using commercially available radioimmunoassay kits (CEDIA® Cyclosporine PLUS Assay and CEDIA® Tacrolimus Assay, Microgenics Co, CA, USA).

Statistical Analysis

The data were analyzed using SPSS 15.0 statistical software (SPSS Inc, Chicago, IL, USA) and were expressed as mean ± SEM. Student's *t*-test and a one-way ANOVA were used for the statistical analyses. A *p*-value of < 0.05 was considered significant.

Results

Cyclosporine

In the *in vitro* experiments, the absorption of cyclosporine from the ileum (Fig. 1) and jejunum (Fig. 2) increased in a time-dependent man-

Fig. 1. Time-dependent changes in the ileal absorption of cyclosporine (CSA) when administered alone, with verapamil (VER) or with rifampicin (RIF)

* different from CSA ($p < 0.05$).

Fig. 2. Time-dependent changes in the jejunal absorption of cyclosporine (CSA) when administered alone, with verapamil (VER) or with rifampicin (RIF)

ner when it was administered alone or with verapamil or rifampicin. It is noteworthy that the mean drug concentration in the fluid samples taken at 30 min was significantly higher in the cyclosporine + verapamil group compared to the cyclosporine only group ($p < 0.05$).

The difference in the absorption of cyclosporine $\pm$ verapamil or rifampicin was not significantly different between the ileum and jejunum ($p > 0.05$).

In the *in vivo* experiments, the plasma concentration of cyclosporine was found to be significantly increased in the cyclosporine + verapamil group ($p < 0.05$) and significantly decreased in the cyclosporine + rifampicin group ($p < 0.001$) compared to the cyclosporine only group (Fig. 3).

Tacrolimus

In the *in vitro* experiments, the absorption of tacrolimus from the ileum (Fig. 4) and jejunum

Fig. 3. Plasma cyclosporine level in blood samples obtained 120 min after the administration of cyclosporine with/without verapamil (VER) or rifampicin (RIF)

* different from CSA ($p < 0.05$); *** different from CSA ($p < 0.001$).

Fig. 4. Time-dependent changes in the ileal absorption of tacrolimus (TACRO) when administered alone, with verapamil (VER) or with rifampicin (RIF)

Fig. 5. Time-dependent changes in the jejunal absorption of tacrolimus (TACRO) when administered alone, with verapamil (VER) or with rifampicin (RIF)

(Fig. 5) increased in a time-dependent manner when it was administered alone or with verapamil or rifampicin.

In the fluid samples obtained at 60 min, ileal absorption of tacrolimus was significantly higher

Fig. 6. Ileal and jejunal absorption of tacrolimus (TACRO) when co-administered with rifampicin (RIF)

* different from jejunal absorption ($p < 0.05$)

Fig. 7. Plasma tacrolimus (TACRO) level in blood samples obtained 120 min after the administration of tacrolimus with/without verapamil (VER) or rifampicin (RIF)

* different from TACRO ($p < 0.05$)

than jejunal absorption when it was administered with rifampicin ($p < 0.05$) (Fig. 6).

In the *in vivo* experiments, the plasma concentration of tacrolimus was found to be significantly increased in the tacrolimus + verapamil group ($p < 0.05$) and significantly decreased in the tacrolimus + rifampicin group ($p < 0.05$) compared to the tacrolimus only group (Fig. 7).

Discussion

This study showed that the absorption of the commonly used immunosuppressive agents cyclosporine and tacrolimus increased when they were co-administered with the P-gp inhibitor verapamil, and decreased when co-administered with the P-gp activator rifampicin.

P-glycoprotein is known to play an important role in the intestinal absorption of cyclosporine and tacrolimus [14, 15]. Efflux transporter molecules, particularly P-gp, alter the plasma concentration of the drugs by pumping them back into

the intestinal lumen [1]. However, the function of P-gp may be affected by some modulating or inhibiting agents [10]. Verapamil, which increased the intestinal absorption of cyclosporine and tacrolimus in this study, has previously been reported to be a substrate of P-gp when used in low doses and to strongly inhibit P-gp when used high doses [8]. Furthermore, it has been suggested that rifampicin activates P-gp *in vivo* and *in vitro* [11, 12]. Although there are some other studies about the effects of P-glycoprotein modulation on the bioavailability of several drugs [16–18], to best of the authors' knowledge, this is the first study to investigate the effects of P-gp-inhibiting and -activating drugs on the intestinal absorption of immunosuppressive drugs.

The “inverted sac” method used for the evaluation of intestinal drug absorption is a simple, inexpensive and well-established *in vitro* experimental method. The method provides valuable information about the drug absorption mechanisms as well as the effects of substances increasing or decreasing intestinal absorption [19, 20]. It has been suggested that drug transporter molecules are distributed unevenly throughout the small intestine, and the absorption of some drugs has been reported to be higher in the distal parts of the intestine (the ileum and jejunum) compared to the proximal intestinal segments [5, 6, 21]. However, in the present study, the absorption of drugs from the ileum did not differ from absorption from the jejunum, except for the significantly higher absorption of tacrolimus from the jejunum than from the ileum at 60 min.

In the *in vitro* experiments, the drug concentration in the fluid samples increased in a time-dependent manner. Although the cyclosporine and tacrolimus concentrations tended to increase with the co-administration of the P-gp inhibitor verapamil and to decrease with the co-administration of the P-gp activator rifampicin, the difference was not statistically significant. Because P-gp is not evenly distributed throughout the intestine [5, 21], evaluation of absorption from 4–5 cm intestinal segments prepared from different parts of the small intestine might give inconsistent results. In addition, because there are multiple factors affecting drug absorption, the effect of P-gp on drug bioavailability may not be adequately apparent *in vitro*. Although some authors have reported that P-gp plays an important role in intestinal absorption of tacrolimus [22, 23], there are also some studies reporting that P-gp has only limited effect [24] or no effect [25] effect on intestinal absorption of tacrolimus. With respect to cyclosporine, it has been suggested that P-gp may be responsible for changes in the clearance of cyclosporine after oral administration [15, 26].

In addition to the *in vitro* experiments, the present study also evaluated the absorption of cyclosporine and tacrolimus by *in vivo* experiments in order to take into account the additional effects of the cytochrome P450 enzyme system on the bioavailability of the drugs. In the *in vivo* experiments, the plasma cyclosporine and tacrolimus levels were found to be significantly increased when they were co-administered with verapamil, and significantly decreased when they were co-administered with rifampicin.

The cytochrome P450 enzyme system is the major site for drug metabolism and catalyzes the oxidative biotransformation of lipophilic substrates to hydrophilic metabolites [27]. There are numerous drugs, including immunosuppressive drugs, that are metabolized by the CYP450 system. Drug therapies can interact with the CYP450 system by inhibiting, activating or acting as a substrate for CYP enzymes. Drugs that inhibit or activate the CYP450 enzymes cause a decrease or increase in the metabolism of other drugs metabolized by the same enzyme, resulting in increased or decreased plasma levels of that drug [28]. Because verapamil and rifampin are also known to inhibit and activate the p450 enzymes [29], and the influence of rifampicin or verapamil was more pronounced on the serum level than on the intestinal

absorption of cyclosporine and tacrolimus, it can be suggested that these drugs may have a stronger effect on the P450 enzyme system than on P-gp.

In conclusion, P-gp may play an important role in the absorption of immunosuppressive drugs and may contribute to drug-drug interactions that can lead to inadequate drug response or toxicity. Because many organ transplant patients using immunosuppressive agents are elderly and use multiple drugs for co-morbidities, they are at great risk for drug-drug interactions. Because of the long-term use of immunosuppressive drugs, it is preferable for them to be taken orally. However, the oral bioavailability and pharmacokinetics of these drug are quite variable. Moreover, because their therapeutic ranges are narrow, the absorption and thus the oral bioavailability of the drugs will increase or decrease with the co-administration of agents inhibiting or activating the P-gp function, resulting in transplanted organ toxicity, damage or rejection. In addition to P-gp, there are many other factors and mechanisms affecting the pharmacokinetics of these drugs. Therefore, it should be kept in mind that patients on immunosuppressive drug treatment may have individual differences in drug response and the plasma drug concentrations should be closely monitored during the treatment.

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