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Regular Article

Long-term Levothyroxine Treatment Decreases the Oral Bioavailability of Cyclosporin A by Inducing P-glycoprotein in Small Intestine

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Summary: We have noticed that the trough level of blood concentration of cyclosporin A (CyA) tends to be lower in patients receiving long-term oral levothyroxine (LTX) than in patients not receiving LTX. We confirmed this clinical observation in experiments using Wistar rats orally given LTX (8 μg/kg) or saline (control) for 3 weeks, followed by CyA (10 mg/kg). The LTX treatment had little effect on the blood concentrations of CyA after i.v. administration, whereas they were decreased significantly after p.o. administration. After p.o. administration, the value of the area under the blood concentration-time curve from 0 to 24 hr and the bioavailability of CyA in the LTX group were decreased to only about one-fifth and a quarter of those in the control group, respectively. After treatment with LTX, the expression levels of mdr1a, mdr1b and CYP3A2 mRNAs in the duodenum were markedly increased to about twice the control, but in jejunum, ileum and liver the expression levels were little changed. These findings suggest that the absorption of CyA, which occurs mainly from the upper intestine, is reduced as a result of efflux transport *via* P-glycoprotein induced by LTX. In conclusion, careful monitoring of CyA levels is required in the event of LTX administration to patients receiving immunotherapy with CyA.

Key words: levothyroxine; cyclosporin A; drug interaction; oral bioavailability; P-glycoprotein; CYP3A2

Introduction

It is well established that the induction of gene expression by dexamethasone (DEX), rifampicin, phenobarbital, and various other drugs is mediated by nuclear receptors such as pregnane X receptor (PXR) and constitutive androstane receptor (CAR).[1–4] We previously reported that the decrease in the blood concentrations of cyclosporin A (CyA) and FK506 caused by high-dose steroid therapy is a consequence of the induction of P-glycoprotein (P-gp) and CYP3A in the liver and intestine, and these changes were reversed within 2 weeks after the cessation of steroid therapy.[5,6] Hormones also regulate transcription factors by binding to nuclear receptors, so it is possible that drug disposition kinetics can be altered by the induction of transporter and CYP genes in patients with hormone-related disease or patients receiving long-term hormone therapy.

There are some reports indicating that patients with hyperthyroidism or heart failure might require larger-than-normal doses of digitalis to regulate their heart rate.[7,8] Siegmund *et al*.[9] found that the expression of P-glycoprotein (P-gp) was increased in duodenum after oral administration of levothyroxine (LTX, 200 μg/day) for 17 days in healthy volunteers, and the half life of talinolol, a substrate of P-glycoprotein, was slightly decreased. Recently, we have noted that the blood CyA concentration tends to be decreased in patients receiving oral immunotherapy with CyA in combination with LTX, in comparison with similar patients not receiving LTX (**Fig. 1**). In this study, we attempted to clarify the mechanism of the drug interaction between CyA and LTX, by means of animal experiments.

Materials and Methods

Chemicals: Thyradin S® tablets (levothyroxine sodium, LTX) and Sandimmun® injection (cyclosporin

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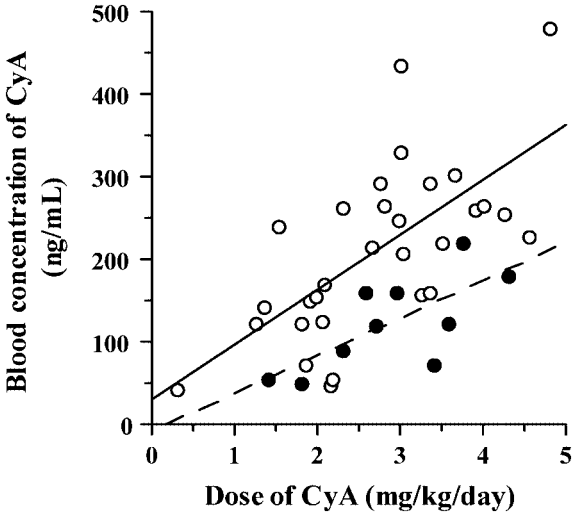

Fig. 1. Relationship between the blood concentration and the dose of CyA in patients with (closed symbol) and without (open symbol) LTX treatment. There is a significant difference between the two groups ($p < 0.05$).

A, CyA) were purchased from Teikoku Hormone Co. Ltd. (Tokyo, Japan) and Novartis Pharma Co. Ltd. (Tokyo, Japan), respectively. Oligonucleotide primers were custom-synthesized by Amersham Pharmacia Biotech (UK). Primary antibody (mouse anti-P-glycoprotein C219) and secondary antibody (anti-mouse IgG HRP-linked antibody) for P-gp were purchased from DAKO Co., Ltd. (CA, USA) and Cell Signaling (MA, USA), respectively. Primary antibody (goat anti-rat CYP3A2 antibody) and secondary antibody (peroxidase-labeled anti-goat IgG) for CYP3A2 were purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan) and Vector Laboratories, Inc. (CA, USA), respectively. Other reagents were purchased from Sigma Co. (MO, USA).

Animal Experiments: All animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Kanazawa.

Male 7-week-old Wistar rats (Japan SLC Co., Hamamatsu, Japan) were orally administered a suspension of LTX (8 μg/kg/day), prepared by grinding Thyradin S® tablets in distilled water, daily for 3 weeks. The control rats received distilled water alone. A 100 μL aliquot of CyA (10 mg/kg) was injected *via* the femoral vein at 24 h after the last treatment with LTX. Blood samples (200 μL each) were collected at designated time intervals from the jugular vein of untreated rats and LTX-treated rats under light ether anaesthesia.

Measurement of blood concentration of CyA: Blood concentration of CyA was measured with a TDx analyzer using a commercial kit according to the manufacturer's instructions (Dainabot Co. Ltd., Tokyo, Japan). The TDx assay is a fluorescence polarization immunoassay (FPIA) reagent system for the measurement of CyA.[10] The measurement range of blood concentration was 25–1500 ng/mL. The cross-reactivities with the metabolites of CyA were 19.4% for M1 and less than 5% for other metabolites.

Measurement of plasma concentrations of T_3, T_4 and TSH: Blood samples were collected under light ether anesthesia from the jugular vein of rats before the LTX treatment and at 24 h after the 3-week LTX treatment. Serum was separated by centrifugation and stored at −30°C. Measurements of triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) were conducted by SRL Co. Ltd. (Tokyo, Japan).

Reverse Transcriptase-polymerase Chain Reaction (RT-PCR)

Assay: Total RNA was isolated from the liver and intestine by using an Isogen Kit (Wako, Osaka). Synthesis of cDNA from the isolated total RNA was carried out using RNase H-reverse transcriptase (Invitrogen Co., Tokyo, Japan). Reverse transcription (RT) reactions were carried out in 40 mM KCl, 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM each of dATP, dCTP, dGTP, and dTTP, 10 units of RNase inhibitor (Promega, Madison, WI), 100 pmol of random hexamer, total RNA and 200 units of the Moloney murine leukemia virus reverse transcriptase (Invitrogen Co.) in a final volume of 22 μL (1 μg/μL) at 37°C for 60 min. Polymerase chain reaction (PCR) was carried out in a final volume of 20 μL, containing 1 μL of RT reaction mixture, 50 mM KCl, 20 mM Tris-HCl (pH 8.3), 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 10 μM each of the mixed oligonucleotide primers, and 1 unit of Taq DNA polymerase (Invitrogen Co.). Primers were used for rat mdr1a (511 bp),[11] rat mdr1b (451 bp),[11] rat CYP3A2 (252 bp),[12] and rat β-actin (456 bp),[13] respectively. Each cycle consisted of 30 sec at 94°C, 60 sec at 60°C, and 75 sec at 72°C for mdr1a and mdr1b, 30 sec at 94°C, 60 sec at 55°C, and 75 sec at 72°C for CYP3A2, and 30 sec at 94°C, 60 sec at 58°C, and 75 sec at 72°C for β-actin. The PCR reaction was run for 26 cycles for mdr1a, mdr1b and CYP3A2, and for 22 cycles for β-actin.

Preparation of plasma membrane fraction and microsomes: For the preparation of plasma membrane fraction, the liver was homogenized in 10 mM Tris-HCl buffer (pH 7.5) containing 2 mM $CaCl_2$ at 4°C. The homogenate was centrifuged at 3,500 × g for 10 min, and the supernatant was then centrifuged at 15,000 × g for 30 min. The pellet was washed, resuspended in 50 mM Tris-HCl buffer (pH 7.2), and was twice centrifuged at 10,000 × g for 5 min, and stored at −80°C until use.

The intestine was quickly removed and washed with

ice-cold isotonic saline containing 1 mM phenylmethylsulfonyl fluoride (PMSF). Mucosa was scraped on ice with a slide glass and homogenized in a buffer containing 250 mM sucrose, 50 mM Tris-HCl (pH 7.4), 1 mM PMSF. The homogenate was centrifuged at 3,000×g for 10 min, and the supernatant was again centrifuged at 15,000×g for 30 min. The pellet was resuspended in 0.5 mL of a buffer containing 50 mM mannitol, 50 mM Tris-HCl (pH 7.4), 1 mM PMSF, and stored at −80°C until use.

For the preparation of microsomes fraction, the intestine was quickly removed and washed with buffer containing 2 mM HEPES, 0.9% NaCl and 0.5 mM phenylmethylsulfonyl fluoride (PMSF). Mucosa was scraped on ice with a slide glass and homogenized in a buffer containing 300 mM mannitol, 5 mM EDTA, 5 mM HEPES and 1 mM PMSF (pH 7.1). The homogenate was centrifuged at 10,000×g for 20 min, and the supernatant was centrifuged at 105,000×g for 60 min. The pellet was added same buffer and the homogenate was again centrifuged at 105,000×g for 60 min, and stored at −80°C until use.

Rat liver microsomes were prepared as described previously[14] and stored at −80°C until use. Protein concentrations were measured according to the method of Lowry *et al.*[15]

SDS-PAGE and immunoblotting: SDS-PAGE and immunoblotting with peroxidase/antiperoxidase staining of the plasma membrane for P-gp and of the microsomes for CYP3A2 were carried out essentially as described by Laemmli[16] and Guengerich *et al.*[17] The sample protein of liver and intestine was used 4 or 200 μg for CYP3A2, and 30 or 300 μg for P-gp, respectively. The sample protein was electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel and transferred onto nitrocellulose membrane filters (Schleicher & Schuell, Dassel, Germany). After having been blocked with 5% skim milk, the filters were incubated overnight with primary antibody, C219 and goat anti-rat CYP3A2 antibody, and for 1 h with secondary antibody, anti-mouse IgG HRP-linked antibody and peroxidase-labeled anti-goat IgG. Thereafter, the sample was extensively washed with phosphate-buffered saline. The immunopositive band was detected by means of a light-emitting nonradioactive detection system (Amersham International plc, Little Chalfont, Buckinghamshire, UK) with Kodak X-Omat R film (Eastman Kodak Co., Rochester, NY).

Data Analysis: The pharmacokinetic parameters were estimated according to model-independent moment analysis as described by Yamaoka *et al.*[18] Where appropriate, data were analyzed using the Mann-Whitney U test to compare the unpaired mean values of two sets of data (**Fig. 1**). Other data were analyzed using Student's *t* test to compare the unpaired mean values of two sets of data (**Fig. 4**, **Tables 1, 2**). The number of determinations is noted in each table and figure. A value of $P<0.05$ or 0.01 was taken to indicate a significant difference. The electrophoresis results were analyzed by using NIH Image software.

Table 1. Plasma concentration of triiodthyronine (T_3), levothyroxine (T_4) and thyroid stimulating hormone (TSH) before and after the treatment with LTX (8 μg/kg/day for 3 weeks)

	before treatment	after treatment
T_3 (ng/dL)	73±6	83±12
T_4 (μg/dL)	4.5±1.1	8.1±1.0**
TSH (μU/mL)	5.3±0.3	5.3±0.7

Each value represents the mean±SD (n=4).
**Significantly different from before treatment at $p<0.01$.

Table 2. Pharmacokinetic parameters of CyA after an i.v. or p.o. administration of CyA (10 mg/kg) in untreated and LTX-treated rats (8 μg/kg/day, p.o. for 3 weeks)

Parameter	no treatment	LTX-treatment
i.v. administration		
AUC (μg h/mL)[a]	48.3±4.4	39.7±7.7
MRT (h)[b]	5.74±1.22	5.54±1.92
Vd_{ss} (L/kg)[c]	1.19±0.32	1.39±0.68
CL_{tot} (mL/h/kg)[d]	207±56	252±52
p.o. administration		
AUC (μg h/mL)	13.8±4.8	2.71±0.78**
Bioavalability (%)	28.6	6.8

Each value represents the mean±SD (n=4).
Pharmacokinetic parameters were estimated according to model-independent moment analysis.
**Significantly different from no treatment at $p<0.01$.
a) area under the blood concentration-time curve from zero to 24 h for CyA.
b) mean residence time from 0 to 24 h.
c) distribution volume at the steady-state.
d) blood total clearance.

Case Report

In 40 patients who had received immunotherapy with oral administration of Neoral® capsule (CyA, Novartis Pharma Co. Ltd., Tokyo, Japan), (twice a day, daily, for over one year), the blood concentrations of CyA were measured at the trough level. The blood samples were collected from 30 patients without LTX treatment (16 men and 14 women) aged 4 to 61 (32.6±16.9, mean ±SD) years and weighing 17 to 75 (51.4±13.3) kg, and from 10 patients with LTX treatment (100 μg/day, p.o., once a day, daily, over 3 months), (6 men and 4 women) aged 6 to 58 (25.2±16.5) years and weighing 12 to 70 (44.6±16.0) kg. The patients were selected for study because their hepatic and renal functions were at normal condition and they were not administered a drug which

may influence on the disposition kinetics of CyA.

Figure 1 shows the relationship between the dose and blood concentration of CyA in 40 patients with or without LTX treatment. The blood concentrations of CyA represented the mean value ($n=2$–6) in every patients collected for 6 months. The correlation coefficients were $r=0.669$ in the non-LTX group and $r=0.719$ in the LTX group, but the blood concentrations of CyA in the LTX group were significantly lower than those in the non-LTX group ($p<0.05$).

Experimental Results

Plasma concentrations of T_3, T_4 and TSH: **Table 1** shows the plasma concentrations of T_3, T_4 and TSH before and at 24 h after administration of LTX (8 μg/kg/day, p.o.) for 3 weeks. The plasma concentrations of T_4 were significantly increased, but the values of T_3 and TSH were little changed.

Pharmacokinetics of CyA after LTX treatment in rats: The blood concentration-time courses of CyA after i.v. or p.o. administration of CyA (10 mg/kg) in control rats and in rats treated with LTX (8 μg/kg/day, p.o.) for 3 weeks are shown in **Fig. 2**. The blood concentrations of CyA after i.v. administration in the LTX group tended to be decreased compared with those of the control group, but the difference was not significant. After p.o. administration, the blood concentrations of CyA in the LTX group were significantly lower than those of the control group.

The pharmacokinetic parameters of CyA in the two groups are listed in **Table 2**. There were no significant differences between the two groups after i.v. administration of CyA. But, after p.o. administration the value of the area under the blood concentration-time curve from 0 to 24 hr (AUC) of the LTX group was only about one-fifth of that of the control group ($p<0.01$). The oral bioavailability of CyA of the LTX group was decreased to about a quarter of that in the control group.

RT-PCR Analysis of mdr1a, mdr1b and CYP3A2 mRNAs in the tissues: **Figures 3 and 4** show the effect of LTX at 24 h after the last treatment (8 μg/kg/day, p.o., for 3 weeks) on the expression of mdr1a, mdr1b and CYP3A2 mRNAs in the duodenum, jejunum, ileum and liver. The expression levels of mdr1a and mdr1b mRNAs in small intestine of the control rats were greatest in the ileum, followed by jejunum and duodenum, but that of CYP3A2 mRNA was not different among the tissues. On the other hand, following treatment with LTX, the expression of mdr1a and mdr1b mRNAs was increased more in tissues initially showing lower expression, and consequently, expression in the duodenum was increased to about 2-fold of the untreated control.

Western blot analysis of P-gp and CYP3A2 in the

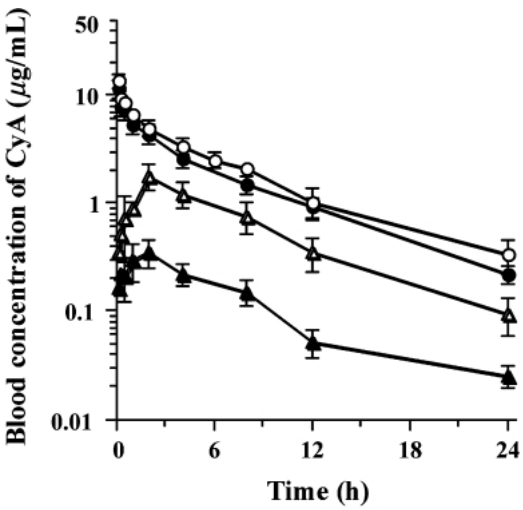

Fig. 2. Blood concentration-time courses of CyA after i.v. or p.o. administration of CyA (10 mg/kg) at 24 h after the last treatment in the control rats and in rats orally treated daily for 3 weeks with LTX (8 μg/kg/day). Each point and bar represents the mean ± SE of four rats.

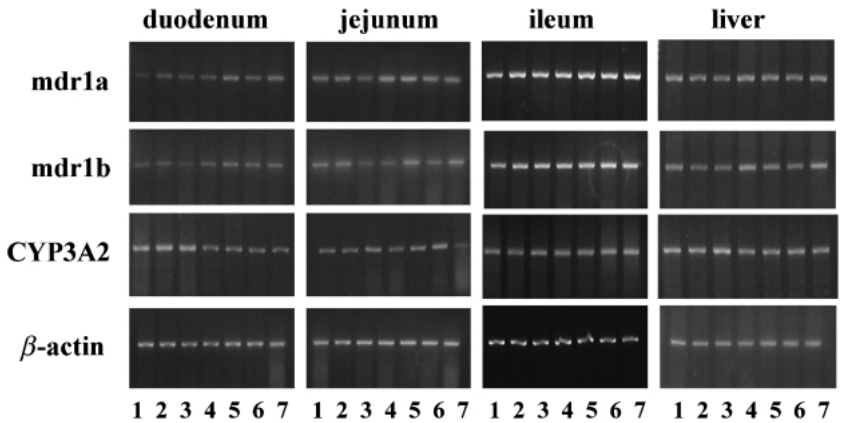

Fig. 3. The expression of mdr1a, mdr1b and CYP3A2 mRNAs in duodenum, jejunum, ileum and liver of control and LTX-treated rats. Rats were orally given LTX (8 μg/kg/day) for 3 weeks. Lanes 1–3, control; Lanes 4–7; at 24 h after the last administration of LTX.

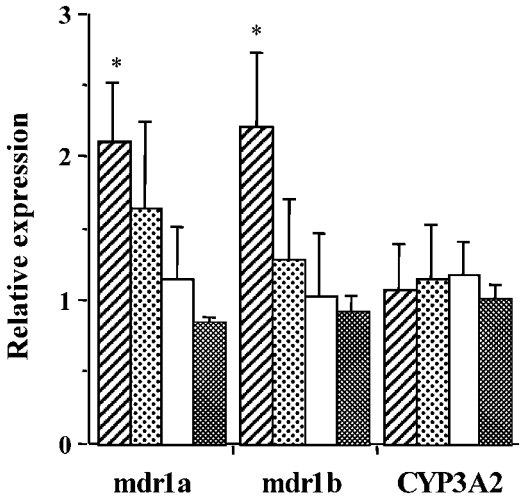

Fig. 4. Effect of LTX on the expression of mdr1a, mdr1b and CYP3A2 mRNAs. The data represent the relative expression of the mRNAs given by dividing mRNA/β-actin ratios in the LTX-treated group by that in the control group. Each column and bar represents the mean ± SD of four rats. *Significantly different from the control group at $p < 0.05$.
Duodenum, ▨; jejunum, ⁙; ileum, □; liver, ▩

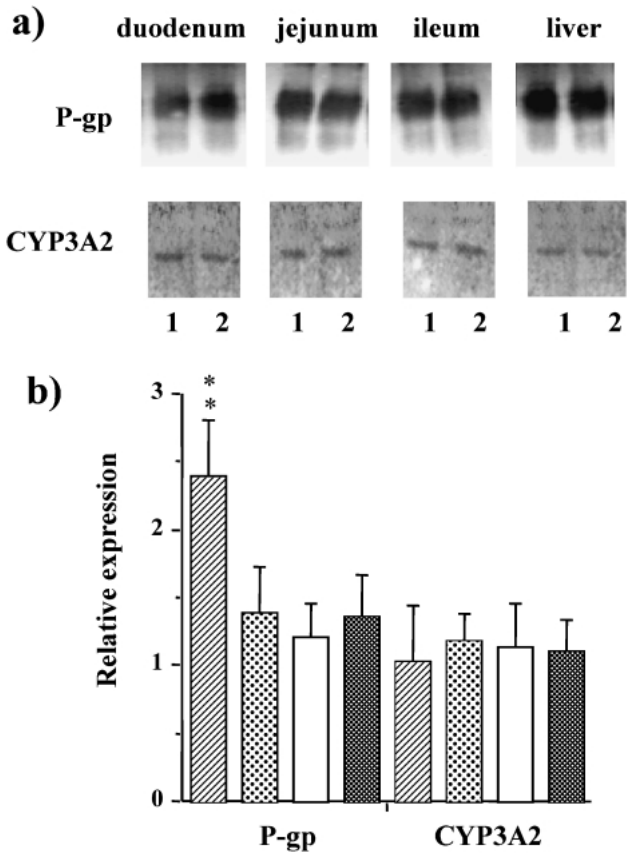

Fig. 5. Western blot analysis of P-gp and CYP3A2 in the duodenum, jejunum, ileum and liver of control and LTX-treated rats. a) Rats were orally given LTX (8 μg/kg/day) for 3 weeks. Lane 1, control; Lane 2, at 24 h after the last administration of LTX. b) Relative expression of P-gp and CYP3A2 proteins. Each column and bar represents the mean ± SD of four rats. *Significantly different from the control group at $p < 0.05$.
Duodenum, ▨; jejunum, ⁙; ileum, □; liver, ▩

tissues: **Figure 5** shows the protein levels of P-gp and CYP3A2 in duodenum, jejunum, ileum and liver at 24 h after the last LTX treatment (8 μg/kg/day, p.o., for 3 weeks). The protein level of P-gp in duodenum was elevated to about 2-fold of the control by LTX treatment, but the levels in other tissues showed no marked change. The protein level of CYA3A2 showed little change in all of the tissues.

Discussion

We had noticed that the trough blood concentrations of CyA tended to be lower in patients receiving long-term oral LTX treatment than in those not receiving LTX. Therefore, in this study, we attempted to clarify the mechanism of the decrease of blood CyA concentration in patients receiving CyA immunotherapy in combination with LTX, by means of animal experiments.

First, we examined the influence of LTX on the expression of mdr1a, mdr1b and CYP3A2 mRNAs in small intestine and liver of rats. The expression of mdr1a and mdr1b mRNAs in the control group was in the order of ileum > jejunum > duodenum. The 3-week administration of LTX increased the expression of these mRNAs by about 2-fold specifically in the duodenum, where the expression was initially lowest (**Fig. 4**). It has also been reported that the expression of P-gp is induced in duodenum of LTX-treated volunteers.[9] These results may reflect the fact that the duodenum is the initial site of exposure after the p.o. administration of LTX, although CYP3A2 mRNA expression was not different among the tissues. We confirmed that the changes of protein expression of P-gp and CYP3A2 corresponded to the extents of induction of the mRNAs (**Fig. 5**). Mitin *et al.*[19] reported that P-gp, but not CYP3A2, is induced by LTX (100 mM) in human colon carcinoma cells (LS180 and Caco-2) *in vitro*, and the induction is mediated by thyroid hormone receptor (TR) rather than PXR. Our results are consistent with this. Thus, it seems that LTX more strongly affects tissues that exhibit a lower basal gene expression by acting *via* TR, although this requires confirmation.

Siegmund *et al.*[9] reported that, although the expression of P-gp in duodenum was increased 3.8-fold by LTX treatment, the change in P-gp expression was associated with only minor alterations in the half-life of talinolol after both oral and intravenous administration. However, in our case, the increase of about 2-fold in the duodenal expression of P-gp induced by LTX greatly decreased the bioavailability of CyA to about a

quarter of that in the control rats (**Table 2**). It has been reported that CyA is absorbed predominantly from the upper small intestine.[20] Because CyA is a good substrate of P-gp, its absorption is likely to be highly vulnerable to modulation of P-gp expression on the brush border membranes of the upper small intestine. Previously, we showed that the bioavailability of CyA greatly decreased from 22.2% to 6.8% by the DEX treatment (1 mg/kg/day) for 7 days, probably due to the markedly enhanced level of P-gp expression in the intestine[5]. But, in this study, even though the induction of P-gp expression in the duodenum by the LTX treatment was smaller compared with that in the case of the previous DEX treatment, the extent of decrease in CyA bioavailability was roughly the same. These findings may indicate that CyA bioavailability is greatly influenced independent of the extent of induction of P-gp, because the expression of P-gp in duodenum is initially very low and CyA is mainly absorbed from the upper small intestine.

On the other hand, in the case of i.v. administration of CyA, the disposition kinetics of CyA was little changed by LTX treatment (LTX 8 μg/kg/day, p.o., for 3 weeks), presumably because the expression of mdr1 and CYP3A mRNAs in the liver did not change almost. Moreover, Matsubara *et al.*[21] reported that the mRNAs and proteins of CYP3A1 and CYP3A2 were induced in the rat liver after the DEX treatment (100 mg/kg/day) for 3 days, while those of CYP3A62 and CYP3A9 were induced in the intestine. In this study, although we did not evaluate the effect of LTX treatment on the expression of CYP3A62 and CYP3A9, we believe that the influence of LTX treatment on these proteins was slight, if any, because the CL_{tot} value of CyA after the i.v. administration of CyA was little changed by LTX treatment.

Taken together, we think that the disposition of CyA is influenced by the induction of P-gp only, but not of CYP. However, interaction between CyA and LTX might well occur when these proteins are induced under certain conditions, depending on dosage, physiology, and other factors. We previously reported that the low-dose DEX treatment (1 mg/kg) induced only the expression of P-gp in the liver and intestine, while the high-dose DEX-treatment (75 mg/kg) induced expressions of both P-gp and CYP3A.[5,6] Therefore, the effect of LTX treatment may also be dose-dependent.

In conclusion, our results indicate that the decrease of blood CyA concentration in patients receiving CyA together with LTX for hypothyroidism is due to the induction of P-gp expression in the small intestine by LTX. Therefore, blood concentration of drugs that are substrates of P-gp, such as CyA, should be carefully monitored in patients when these drugs are administered in combination with LTX.

References

1) Pascussi, J. M., Drocourt, L., Gerbal-Chaloin, S., Fabre, J. M., Maurel, P. and Vilarem, M. J.: Dual effect of dexamethasone on CYP3A4 gene expression in human hepatocytes. Sequential role of glucocorticoid receptor and pregnane X receptor. *Eur. J. Biochem.*, **268**: 6346–6358 (2001).
2) Quattrochi, L. C. and Guzelian, P. S.: Cyp3A regulation: from pharmacology to nuclear receptors. *Drug Metab. Dispos.*, **29**: 615–622 (2001).
3) Geick, A., Eichelbaum, M. and Burk, O.: Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J. Biol. Chem.*, **276**: 14581–14587 (2001).
4) Chen, Y., Ferguson, S. S., Negishi, M. and Goldstein, J. A.: Induction of human CYP2C9 by rifampicin, hyperforin, and phenobarbital is mediated by the pregnane X receptor. *J. Pharmacol. Exp. Ther.*, **308**: 495–501 (2004).
5) Yokogawa, K., Shimada, T., Higashi, Y., Itoh, Y., Masue, T., Ishizaki, J., Asahi, M. and Miyamoto, K.: Modulation of mdr1a and CYP3A gene expression in the intestine and liver as possible cause of changes in the cyclosporin A disposition kinetics by dexamethasone. *Biochem. Pharmacol.*, **63**: 777–783 (2002).
6) Shimada, T., Terada, A., Yokogawa, K., Kaneko, H., Nomura, M., Kaji, K., Kaneko, S., Kobayashi, K. and Miyamoto, K.: Lowered blood concentration of tacrolimus and its recovery with changes in expression. *Transplantation*, **74**: 1419–1424 (2002).
7) Frye, R. L. and Braunwald, E.: Studies on digitalis. III. The influence of triiodothyronine on digitalis requirements. *Circulation*, **23**: 376–382 (1961).
8) O'Connor, P. and Feely, J.: Clinical pharmacokinetics and endocrine disorders. Therapeutic implications. *Clin. Pharmacokinet.*, **13**: 345–364 (1987).
9) Siegmund, W., Altmannsberger, S., Paneitz, A., Hecker, U., Zschiesche, M., Franke, G., Meng, W., Warzok, R., Schroeder, E., Sperker, B., Terhaag, B., Cascorbi, I. and Kroemer, H. K.: Effect of levothyroxine administration on intestinal P-glycoprotein expression: consequences for drug disposition. *Clin. Pharmacol. Ther.*, **72**: 256–264 (2002).
10) David-Neto, E., Ballarati, C. A., Freitas, O. J., Lemos, F. C., Nahas, W. C., Arap, S. and Kalil, J.: Comparison of the fluorescent polarization (TDx) and the enzymatic competitive (EMT 2000) immune assays for the measurement of cyclosporin A blood concentration. *Rev. Hosp. Clin. Fac. Med. Sao. Paulo.*, **55**: 207–212 (2000).
11) Chin, J. E., Soffir, R., Noonan, K. E., Choi, K. and Roninson, I. B.: Structure and expression of the human MDR (P-glycoprotein) gene family. *Mol. Cell Biol.*, **9**: 3808–3820 (1989).
12) Oinonen, T. and Lindros, K. O.: Hormonal regulation of the zonated expression of cytochrome P-450 3A in rat liver. *Biochem. J.*, **309**: 55–61 (1995).
13) Waki, Y., Miyamoto, K., Kasugai, S. and Ohya, K.: Osteoporosis-like changes in Walker carcinoma 256-bearing rats, not accompanied with hypercalcemia or

parathyroid hormone-related protein production. *Jpn. J. Cancer Res.*, **86**: 470–476 (1995).
14) Kamataki, T. and Kitagawa, H.: Effects of lyophilization and storage of rat liver microsomes on activity of aniline hydroxylase, contents of cytochrome b5 and cytochrome P-450 and aniline-induced P-450 difference spectrum. *Jpn. J. Pharmacol.*, **24**: 195–203 (1974).
15) Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193**: 265–275 (1951).
16) Laemmli, U. K.: Cleavage of structure proteins during the assembly of the head of bacteriophage T4. *Nature (London)*, **227**: 680–685 (1970).
17) Guengerich, F. P., Wang P. and Davidson, N. K.: Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits and humans using immunochemical staining coupled with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Biochemistry*, **21**: 1698–1706 (1982).
18) Yamaoka, K., Nakagawa, T. and Uno, T.: Statistical moments in pharmacokinetics. *J. Pharmacokinet. Biopharm.*, **6**: 547–558 (1978).
19) Mitin, T., Von Moltke, L. L., Court, M. H. and Greenblatt, D. J.: Levothyroxine up-regulates P-glycoprotein independent of the pregnane X receptor. *Drug Metab. Dispos.*, **32**: 779–782 (2004).
20) Cakaloglu, Y., Marinos, G., Marsden, J., Peters, T. J., Williams, R. and Tredger, J. M.: Localization of cyclosporin A absorption in rat small bowel and the effect of bile. *Clin. Sci. (London)*, **84**: 675–679 (1993).
21) Matsubara, T., Kim, H. J., Miyata, M., Shimada, M., Nagata, K. and Yamazoe, Y.: Isolation and characterization of a new major intestinal CYP3A form, CYP3A62, in the rat. *J. Pharmacol. Exp. Ther.*, **309**: 1282–1290 (2004).