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Effect of imatinib mesilate on the disposition kinetics of ciclosporin in rats

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Abstract

The purpose of this study was to investigate the effect of imatinib mesilate on the disposition kinetics of ciclosporin in rats. The blood concentration–time course and pharmacokinetic parameters of ciclosporin did not significantly change after intravenous injection of ciclosporin (10 mg kg^{-1}) in rats treated with imatinib mesilate (50 mg kg^{-1}) as compared with a control. When ciclosporin (10 mg kg^{-1}) was orally administered, the time course, area under the curve, bioavailability and peak blood concentration of ciclosporin were significantly increased in rats that had been treated with imatinib mesilate 2 h before ciclosporin administration as compared with the control. Because both drugs are transported via P-glycoprotein and breast cancer resistance protein and metabolized by cytochrome P450 3A2, the interaction of imatinib mesilate with these proteins may be responsible for the increased intestinal absorption of ciclosporin in rats. These results indicate that imatinib mesilate enhanced the intestinal absorption of ciclosporin in rats with only the oral administration of ciclosporin, suggesting that our results support clinical data. In addition, imatinib mesilate may increase the pharmacological effects and possibly toxicity of ciclosporin.

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Introduction

Ciclosporin is a powerful immunosuppressive agent, widely used in organ transplantation therapy and the treatment of autoimmune diseases. It has a narrow therapeutic window and, in excess, can cause severe nephritis and neurotoxic disease. The bioavailability of ciclosporin is extremely poor (Yokogawa et al 2002; Lee et al 2005). A possible factor in the poor absorption is the intestinal first-pass metabolism by cytochrome P450 (CYP) 3A4 systems and P-glycoprotein (P-gp), which is a member of the ATP-binding-cassette (ABC) superfamily of membrane transporters. It has been reported that CYP3A4 is expressed in the intestine as well as the liver (de Waziers et al 1990), while CYP3A4 mRNA in man corresponds to CYP3A2 mRNA in rats (Tomlinson et al 1997). P-gp is expressed not only in multidrug-resistant cancer cells but also in various normal tissues, such as the adrenals, kidney, liver, small intestine, colon and brain capillary endothelium (Thiebaut et al 1987; Tsuji et al 1992; Miyamoto et al 1996; Terao et al 1996). Additionally, it was reported that ciclosporin inhibited breast cancer resistance protein (BCRP), an ABC transporter and abundantly expressed in the placenta, liver, intestine and so on (Maliepaard et al 2001; Özvegy-Laczka et al 2001).

Imatinib mesilate, a tyrosine kinase inhibitor, is predominantly metabolized by CYP3A4 (O'Brien et al 2003). Illmer et al (2004) recently showed that P-gp mediated drug efflux was a resistance mechanism of chronic myelogenous leukemia cells to treatment with imatinib mesilate. Also, imatinib mesilate interacted with BCRP (Özvegy-Laczka et al 2004).

Ciclosporin and imatinib mesilate are clinically co-administered to patients after bone-marrow transplantation. Our clinical data showed that the ciclosporin concentration in blood increased in combination with imatinib only when ciclosporin was orally administered, but not when intravenously administered. The interaction mechanism might be ascribed mainly to the inhibition of these proteins by imatinib

on the absorption process of ciclosporin. In this study, we examined the effects of imatinib mesilate on the disposition kinetics of ciclosporin in rats with the aim of relating to the results as presented in the clinical data.

Materials and Methods

Materials

Ciclosporin (Sandimune injection, 50 mg mL^{-1}) and imatinib mesilate (Glivec capsules, 100 mg) were kindly provided by Novartis Pharma Co. Ltd (Tokyo, Japan). Ciclosporin was diluted with saline and water for intravenous injection and oral administration, respectively. Imatinib mesilate suspension (25 mg mL^{-1}) was prepared with water after the contents were taken out of the capsule.

Animals

Male Wistar rats were obtained from Sankyo Laboratory Animal (Toyama, Japan) and treated in accordance with the guidelines of the Institutional Animal Care and Use Committee of Hokuriku University. A 0.4 and 2 mL kg^{-1} dose of ciclosporin (10 mg kg^{-1}) was intravenously injected and administered orally, respectively, to untreated rats or rats at 0.5 or 2 h after an oral treatment with imatinib mesilate suspension (50 mg kg^{-1}, 2 mL kg^{-1}). Rats were fasted for 12 h before ciclosporin administration, while water was given freely. Under light anaesthetization by diethyl ether, each rat was serially sampled from the jugular vein at designated time intervals. Each blood sample (each 150 μL) was stored at 4°C until assaying. The numbers of rats used for intravenous injection and oral administration of ciclosporin were 3 and 6, respectively.

Measurement of ciclosporin

The blood concentration of ciclosporin was measured with a TDx analyser using a commercial kit according to the manufacturer's instructions (Abbott Japan Co. Ltd, Tokyo, Japan). The TDx assay is a fluorescence polarization immunoassay for whole blood (David-Neto et al 2000). The measurement range of ciclosporin in blood was 65–2000 ng mL^{-1}. The coefficient of variation and the recovery were 2.8–5.5% and 88.8–103.5%, respectively. Samples expressing the over-concentration ($>$ 2000 ng mL^{-1}) were diluted with drug-free blood obtained from normal rats. The cross-reactivity with the metabolites of ciclosporin was 19.4% for M1 and $<$ 5% for other metabolites.

Data analysis

The area under the blood concentration–time curve from zero to 24 h (AUC_{0-24}) and the mean residence time from zero to 24 h (MRT) for ciclosporin were calculated by the trapezoidal rule. The absolute bioavailability of ciclosporin was estimated by dividing AUC_{0-24} after oral administration by AUC_{0-24} after intravenous injection. The peak blood concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined from the actual data obtained after oral administration. Data are expressed as the mean $\pm$ s.d. The data were analysed using Student's *t*-test to compare the unpaired mean values of the two sets of data. $P < 0.05$ was taken to indicate a significant difference between the sets of data.

Results

Blood concentration–time course of ciclosporin after intravenous injection or oral administration of ciclosporin alone and after co-administration with imatinib mesilate in rats

Figure 1A shows the blood concentration–time course of ciclosporin after the intravenous injection of ciclosporin alone and after co-administration with imatinib mesilate. The time courses of ciclosporin in rats treated with imatinib mesilate 0.5 and 2 h before ciclosporin administration were consistent with that of the control. As shown in Figure 1B, the blood concentration–time courses of ciclosporin after oral administration of ciclosporin alone and after co-administration of ciclosporin and imatinib mesilate were investigated. The time course of ciclosporin significantly increased in rats treated with imatinib 2 h before ciclosporin administration compared with the control, although an obvious change did not appear in rats treated 0.5 h earlier with imatinib.

Effect of imatinib mesilate on the pharmacokinetic parameters of ciclosporin in rats

The pharmacokinetic parameters of ciclosporin estimated by the time courses expressed in Figures 1A and 1B are listed in Table 1. With intravenous injection of ciclosporin, the difference between the parameters of the control and rats treated with imatinib mesilate was not significant. With oral administration of ciclosporin, the values of AUC_{0-24} and C_{max} of ciclosporin in rats treated with imatinib mesilate 2 h previously significantly increased as compared with those in the control. The bioavailability value of ciclosporin increased to 58.1% from 38.6%. No changes in T_{max} and MRT values were observed. On the other hand, in rats treated with imatinib mesilate 0.5 h previously, the parameters of ciclosporin did not significantly change as compared with the control.

Discussion

Ciclosporin and imatinib mesilate are co-administered to patients after bone-marrow transplantation. Our clinical data showed that only oral administration of imatinib

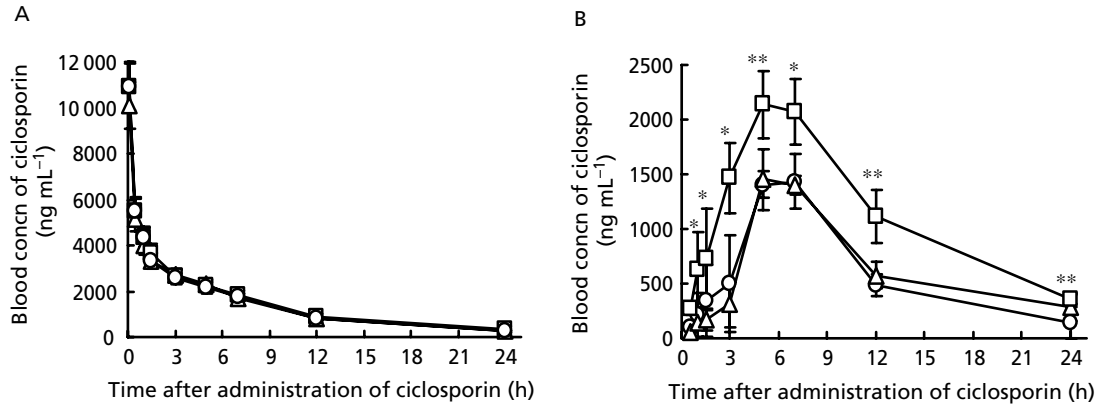

Figure 1 Blood concentration–time courses of ciclosporin after intravenous injection (A) or oral administration (B) of ciclosporin alone and after co-administration with imatinib mesilate in rats. Ciclosporin (10 mg kg^{-1}) was intravenously injected or orally administered into the control and rats treated with imatinib mesilate (50 mg kg^{-1}) 0.5 and 2 h before the administration. ○, control; △, rats treated with imatinib mesilate 0.5 h before ciclosporin administration; □, rats treated with imatinib mesilate 2 h before ciclosporin administration. Each point represents the mean ± s.d. of three (A) to six (B) rats. *$P < 0.05$, **$P < 0.01$ compared with control.

Table 1 Pharmacokinetic parameters of ciclosporin after intravenous injection or oral administration of ciclosporin alone (10 mg kg^{-1}) and after co-administration with imatinib mesilate (50 mg kg^{-1}) in rats

Parameters	Control	Rats treated with imatinib mesilate	
		0.5 h prior	2 h prior
Intravenous injection			
AUC_{0-24} (μg h mL^{-1})	36.8 ± 4.0	37.4 ± 4.5	43.9 ± 6.1
MRT (h)	5.76 ± 0.63	5.99 ± 0.83	7.12 ± 1.12
Per oral administration			
AUC_{0-24} (μg h mL^{-1})	14.2 ± 1.9	16.1 ± 4.0	25.5 ± 4.9**
Bioavailability (%)	38.6	43.0	58.1
C_{max} (μg mL^{-1})	1.56 ± 0.17	1.52 ± 0.28	2.16 ± 0.30**
T_{max} (h)	5.67 ± 1.63	5.67 ± 1.63	4.75 ± 1.78
MRT (h)	8.65 ± 1.35	10.9 ± 2.7	9.34 ± 2.06

Each value represents the mean ± s.d. of three (intravenous injection) to six (oral administration) rats. **$P < 0.01$ compared with control.

mesilate increased ciclosporin blood concentration, indicating that the intestinal first-pass metabolism of ciclosporin might be mainly responsible for the mechanism. This paper describes the drug–drug interaction between ciclosporin and imatinib mesilate in rats in a dose route-dependent manner.

The blood concentration–time course of ciclosporin was examined after intravenous injection of ciclosporin in rats and the pharmacokinetic parameters were estimated. In rats treated with imatinib mesilate 0.5 and 2 h before ciclosporin administration, the kinetic behaviour of ciclosporin did not change. However, with the oral administration of ciclosporin, we found that the ciclosporin blood concentration was significantly increased, and the values of AUC_{0-24}, bioavailability and C_{max} of ciclosporin increased by 80, 51 and 38%, respectively, in rats treated with imatinib mesilate 2 h before the ciclosporin administration. Thus, a dose route-dependent drug–drug interaction was observed between ciclosporin and imatinib mesilate in rats. Yokogawa et al (2002) showed a drug–drug interaction between orally administered ciclosporin and an intraperitoneal injection of dexamethasone as observed in rats. The increased P-gp level in the rat intestine was considered as the possible cause. However, the ciclosporin concentration in blood did not change with the intravenous injection of ciclosporin and intraperitoneal injection of dexamethasone. These data imply that orally administered ciclosporin more easily influences an interaction with a co-administered drug and that side effects should be more strictly monitored than with intravenously injected ciclosporin.

It is well known that both ciclosporin and imatinib mesilate are metabolized by CYP3A2 and transported via P-gp in rats (Terao et al 1996; Yokogawa et al 2002; O'Brien et al 2003; Illmer et al 2004). Moreover, BCRP was inhibited by ciclosporin as well as imatinib mesilate (Özvegy-Laczka et al 2001, 2004). Although our results could not demonstrate which system was mainly responsible for the effects of imatinib mesilate on the

intestinal first-pass metabolism of ciclosporin, Özvegy-Laczka et al (2004) showed that in several in-vitro assay systems imatinib mesilate interacted with BCRP at a submicromolar concentration, whereas P-gp was much less sensitive to imatinib. Further in-vivo and in-vitro studies are needed for the contribution ratio of these proteins on the interaction of ciclosporin and imatinib mesilate.

In rats treated with imatinib mesilate 0.5 h previously, no change in ciclosporin blood concentration was observed. Factors in the intestinal first-pass mechanism of ciclosporin might be dysfunctional or down regulated 2 h after imatinib pretreatment. To determine the responsibility for the pharmacokinetic change of ciclosporin at 2 h after imatinib mesilate administration, these protein levels and the imatinib concentration in plasma, liver and intestine should be determined.

On the other hand, since imatinib mesilate capsules contain such inert ingredients as stearic acid magnesium salt and cellulose, we cannot ignore the possibility of these other ingredients being responsible for pharmacokinetic changes seen for ciclosporin. Further study is needed on this point.

In summary, imatinib mesilate enhanced the intestinal absorption of ciclosporin in rats only with orally administered ciclosporin. In addition, imatinib mesilate may increase the pharmacological effects and, possibly, the toxicity of ciclosporin. Our results are greatly consistent with the clinical data.

References

David-Neto, E., Ballarati C. A., Freitas, O. J., Lemos, F. C., Nahas, W. C., Arap, S., Kalil, J. (2000) Comparison of the fluorescent polarization (TDx) and the enzymatic competitive (EMIT 2000) immune assays for the measurement of cyclosporin A blood concentration. *Rev. Hosp. Clin. Fac. Med. Sao. Paulo*. **55**: 207–212

de Waziers, I., Cugnenc, P. H., Yang, C. S., Leroux, J. P., Beaune, P. H. (1990) Cytochrome P 450 isoenzymes, epoxide hydrolase and glutathione transferases in rat and human hepatic and extrahepatic tissues. *J. Pharmacol. Exp. Ther*. **253**: 387–394

Illmer, T., Schaich, M., Platzbecker, U., Freiberg-Richter, J., Oelschlagel, U., von Bonin, M., Pursche, S., Bergemann, T., Ehninger, G., Schleyer, E. (2004) P-glycoprotein-mediated drug efflux is a resistance mechanism of chronic myelogenous leukemia cells to treatment with imatinib mesylate. *Leukemia* **18**: 401–408

Lee, Y. J., Chung, S. J., Shim, C. K. (2005) Limited role of P-glycoprotein in the intestinal absorption of cyclosporin A. *Biol. Pharm. Bull*. **28**: 760–763

Maliepaard, M., Scheffer, G. L., Faneyte, I. F., van Gastelen, M. A., Pijnenborg, A. C., Schinkel, A. H., van De Vijver, M. J., Scheper, R. J., Schellens, J. H. (2001) Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. *Cancer Res*. **61**: 3458–3464

Miyamoto, K., Koga-Takeda, K., Koga, K., Ohshima, T., Nomura, M. (1996) Saturable function of P-glycoprotein as a drug-efflux pump in multidrug-resistant tumour cells. *J. Pharm. Pharmacol*. **48**: 522–525

O'Brien, S. G., Meinhardt, P., Bond, E., Beck, J., Peng, B., Dutreix, C., Mehring, G., Milosavljev, S., Huber, C., Capdeville, R., Fischer, T. (2003) Effects of imatinib mesylate (STI571, Glivec) on the pharmacokinetics of simvastatin, a cytochrome p450 3A4 substrate, in patients with chronic myeloid leukaemia. *Br. J. Cancer* **89**: 1855–1859

Özvegy-Laczka, C., Litman, T., Szakacs, G., Nagy, Z., Bates, S., Varadi, A., Sarkadi, B. (2001) Functional characterization of the human multidrug transporter, ABCG2, expressed in insect cells. *Biochem. Biophys. Res. Commun*. **285**: 111–117

Özvegy-Laczka, C., Hegedüs, T., Várady, G., Ujhelly, O., Schuetz, J. D., Váradi, A., Kéri, G., Orfi, L., Német, K., Sarkadi, B. (2004) High-affinity interaction of tyrosine kinase inhibitors with the ABCG2 multidrug transporter. *Mol. Pharmacol*. **65**: 1485–1495

Terao, T., Hisanaga, E., Sai, Y., Tamai, I., Tsuji, A. (1996) Active secretion of drugs from the small intestinal epithelium in rats by P-glycoprotein functioning as an absorption barrier. *J. Pharm. Pharmacol*. **48**: 1083–1089

Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M. M., Pastan, I., Willingham, M. C. (1987) Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl Acad. Sci. USA* **84**: 7735–7738

Tomlinson, E. S., Maggs, J. L., Park, B. K., Back, D. J. (1997) Dexamethasone metabolism in vitro: species differences. *J. Steroid Biochem. Mol. Biol*. **62**: 345–352

Tsuji, A., Terasaki, T., Takabatake, Y., Tenda, Y., Tamai, I., Yamashima, T., Moritani, S., Tsuruo, T., Yamashita, J. (1992) P-glycoprotein as the drug efflux pump in primary cultured bovine brain capillary endothelial cells. *Life Sci*. **51**: 1427–1437

Yokogawa, K., Shimada, T., Higashi, Y., Itoh, Y., Masue, T., Ishizaki, J., Asahi, M., Miyamoto, K. (2002) 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