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Effect of Oral Adsorbent AST-120 on Cyclosporin Absorption in Rats

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Abstract

The oral adsorbent AST-120 is used to inhibit the progression of renal failure by adsorbing uraemic toxins in the gastrointestinal tract. When AST-120 is administered to patients receiving immunosuppressive medicines, it is important to study the effect of AST-120 on the amount of these and other drugs absorbed. We have, therefore, studied the in-vitro adsorption of cyclosporin by AST-120 and investigated the effect of oral administration of AST-120 on the absorption of cyclosporin in rats.

The in-vitro adsorption ratios of AST-120 for cyclosporin were more than 80%. When pure cyclosporin powder was administered with AST-120, blood cyclosporin concentrations were significantly higher than when cyclosporin was administered alone. When cyclosporin dissolved in medium-chain triglyceride was administered to rats by intramuscular injection there was no significant difference in the blood cyclosporin concentration of rats given combined AST-120 and cyclosporin and those given cyclosporin alone. There was no significant difference between the serum concentration of total bile acids, in rats receiving combined oral AST-120 and cyclosporin dissolved in olive oil, and those receiving orally solely a solution of cyclosporin dissolved in olive oil.

These results suggest that oral administration of AST-120 accelerates the absorption of orally administered cyclosporin from the gastrointestinal tract and does not affect the metabolism of cyclosporin. When a solution of cyclosporin in olive oil is administered orally, however, oral administration of AST-120 has no influence on cyclosporin absorption and does not affect the enterohepatic circulation of bile acids.

The oral adsorbent AST-120 (kremezin) is a medicine used to inhibit the progression of renal failure by adsorbing the uraemic toxins secreted or produced in the gastrointestinal tract (Yamazaki et al 1980). In clinical practice AST-120 is quite efficacious against chronic renal failure in patients at the predialytic stage; it results in improvement of uraemic manifestations and delays the need to introduce dialysis (Takara et al 1985; Sanaka et al 1988). There is, however, concern about the effect of AST-120 on the quantity of other medicines absorbed when they are administered orally in combination with AST-120. When AST-120 is administered to patients receiving immunosuppressive medicines for organ transplantation or autoimmune diseases, it is important to study the effect of AST-120 on the quantity of these medicines absorbed.

Cyclosporin is a potent immunosuppressant widely used in organ transplantation and various autoimmune diseases; it has significantly improved graft survival rates in organ transplantation. The therapeutic range of blood cyclosporin concentration is narrow, and cyclosporin has various toxic effects which are mostly dependent on blood cyclosporin concentration. It is necessary to keep the blood cyclosporin concentration within the therapeutic range to provide adequate immunosuppressive effects. Therefore, monitoring of blood cyclosporin concentrations is important.

In this study we have examined the effect of AST-120 on the in-vitro absorption of cyclosporin, and investigated the effect of oral administration of AST-120 on cyclosporin absorption in rats.

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Materials and Methods

Drug preparation

The adsorbent AST-120 was kindly provided by the Kureha Chemical Industry, Tokyo, Japan. It consists of fine spherical particles, approximately 0·2–0·4 mm in diameter, of porous microcrystalline carbon with an oxygen complex including a surface oxide. AST-120 was desiccated at 105°C for 4 h, and then used in the in-vitro adsorption test and in the animal study described below.

Pure cyclosporin powder and cyclosporin dissolved in olive oil (100 mg mL^{-1}) were kindly provided by Japan Sandoz (Tokyo, Japan). The solution in olive oil was diluted with olive oil for oral administration at a dose of 5 mg cyclosporin mL^{-1}; a solution (25 mg mL^{-1}) of the pure cyclosporin powder in medium-chain triglyceride was used for intramuscular injection.

In-vitro AST-120 adsorption of cyclosporin

Pure cyclosporin powder (5 mg) was dissolved in phosphate buffer solution (pH 7·4; 0·05 M; 50 mL) and the solution obtained after removing undissolved cyclosporin by membrane filtration (pore size 0·45 μm; Japan Millipore, Tokyo, Japan) was designated the pre-adsorption solution. AST-120 (100 mg) was added to the pre-adsorption solution in a stoppered 200-mL conical flask and the mixture was shaken at 37°C for 3 h. The solution obtained after removing AST-120 and adsorbed cyclosporin (0·45-μm pore size membrane filter) was designated the post-adsorption solution. Samples (0·5 mL) were taken from these solutions for cyclosporin assay. These samples were immediately frozen and then stored at −20°C until analysis.

The cyclosporin concentrations in the pre- and post-adsorption solutions were measured by the method described below and the adsorption ratio was calculated according to the formula:

$$\text{Adsorption ratio} = (\text{Pre-concn} - \text{Post-concn})/\text{Pre-concn} \times 100(\%)$$

where Pre-concn is the concentration of cyclosporin in the pre-adsorption solution and Post-concn is the concentration of cyclosporin in the post-adsorption solution. The above procedures were performed on three specimens.

Animal study

Eleven-week-old female Sprague–Dawley rats, 220–280 g, were used in the study. The rats were bred at a constant temperature of 20°C and humidity of 60%. Food was withdrawn completely for 6 h before cyclosporin administration but the animals had free access to water. Cyclosporin (10 mg kg^{-1}) and AST-120 (120 mg kg^{-1}) were administered to the rats once a day for five consecutive days. AST-120 and the powdered and olive oil-dissolved forms of cyclosporin were forcibly administered via the mouth into the stomach of the rats by use of a 4 Fr steel tube. Intramuscular administration of cyclosporin was achieved by injecting a solution of pure cyclosporin powder in medium-chain triglyceride into the muscle of the rat's thigh. The animals were divided into eight groups according to the methods of drug administration. Group 1: cyclosporin powder and AST-120 were orally administered at the same time (n = 10). Group 2: AST-120 was administered orally 2 h after oral administration of the cyclosporin powder (n = 10). Group 3: cyclosporin powder was administered orally (n = 10). Group 4: cyclosporin was injected intramuscularly and AST-120 was administered orally at the same time (n = 8). Group 5: cyclosporin was injected intramuscularly (n = 8). Group 6: the solution of cyclosporin in olive oil and AST-120 were administered orally at the same time (n = 7). Group 7: AST-120 was administered orally 2 h after oral administration of the solution of cyclosporin in olive oil (n = 7). Group 8: the solution of cyclosporin in olive oil was administered orally (n = 7).

On the fifth day of the experiment, blood samples were obtained from the jugular vein, under ether anaesthesia, immediately before cyclosporin administration and 2, 6 and 12 h thereafter. A sample of blood (0·3 mL) was obtained from groups 1 to 5 for cyclosporin assay. For groups 6 to 8 a sample of blood (0·3 mL) was obtained for cyclosporin assay immediately before cyclosporin administration and 2 and 6 h thereafter. Another sample of blood (1 mL) was taken 12 h after cyclosporin administration; this sample was divided into two portions, 0·3 mL for cyclosporin assay and 0·7 mL for serum total bile acids assay. Each blood sample for cyclosporin assay was collected into a glass tube containing ethylenediaminetetraacetic acid as anticoagulant. Each blood sample for serum total-bile-acids assay was kept at room temperature (20–25°C) for 2 h and centrifuged at 3000 rev min^{-1} for 15 min to furnish supernatant serum (0·3 mL). These blood and serum samples were immediately frozen and then stored at −20°C until use. The whole-blood cyclosporin concentrations were measured for all groups. The serum concentrations of total bile acids were measured for groups 6 to 8.

Chemical assay

The cyclosporin concentrations in in-vitro samples and in whole blood were measured by means of a Cyclo-Trac SP radioimmunoassay kit (Baxter, Tokyo, Japan), using a specific anti-cyclosporin monoclonal antibody labelled with ^{125}I (Donatsch et al 1981).

The serum concentration of total bile acids was measured by an enzymic spectrophotometric assay using 3α-hydroxysteroid dehydrogenase and 3-oxo-5β-steroid Δ^4-dehydrogenase (Mashige et al 1981).

Statistical analysis

Data were expressed as mean ± s.d. The data obtained were analysed for statistical differences by one- and two-way factorial analysis of variance. If significant, multiple comparison was performed by Scheffé's F method. A P-value of less than 0·05 was regarded as indicative of statistical significance.

Results

The in-vitro adsorption test demonstrated that the AST-120 adsorption ratios for cyclosporin were more than 80% (average 86·4%) in all the three specimens (Table 1).

The blood cyclosporin concentrations in group 1 (simultaneous oral administration of AST-120 and cyclosporin) were significantly higher than those in group 3 (oral administration of cyclosporin alone) until 12 h after cyclosporin administration (Fig. 1; $P < 0·01$). The blood cyclosporin concentrations in group 2 (oral administration of AST-120 2 h after oral administration of cyclosporin) showed a significantly higher value after 2 h in comparison with those in group 3 only (Fig. 1, $P < 0·01$). In addition, compared with blood cyclosporin concentrations in group 2, those in group 1 were significantly higher 0, 6, and 12 h after cyclosporin administration (Fig. 1, $P < 0·01$). Although the in-vitro adsorption test revealed high adsorption ratios of AST-120 for cyclosporin, oral administration of both drugs resulted in higher blood cyclosporin concentrations. The blood cyclosporin concentrations in group 4 (intramuscular injection of cyclosporin and simultaneous oral administration of AST-120) and group 5 (intramuscular injection of cyclosporin alone) showed no significant difference until 12 h after cyclosporin administration (Fig. 2).

There was no significant difference among the blood cyclosporin concentrations in groups 6, 7 and 8 (Fig. 3). The blood cyclosporin concentrations in groups 6 to 8 (oral administration of the solution of cyclosporin in olive oil) were significantly higher than those in groups 1 to 3 (oral administration of cyclosporin) 6 h after cyclosporin administration (Table 2, $P < 0·01$).

Table 1. In-vitro AST-120 adsorption ratio for cyclosporin.

No.	Cyclosporin concn of pre-adsorption solution (ng mL^{-1})	Adsorption ratio (%)
1	6000	81·7
2	4900	82·5
3	3600	95·0
Mean		86·4

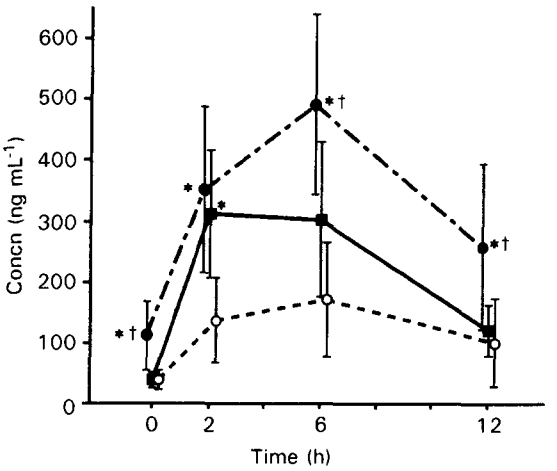

FIG. 1. Changes in whole-blood cyclosporin concentrations (mean ± s.d.) after oral administration of cyclosporin. ●, Simultaneous oral administration of cyclosporin and AST-120 (group 1, n = 10); ■, AST-120 was orally administered 2 h after oral cyclosporin administration (group 2, n = 10); ○, oral administration of cyclosporin alone (group 3, n = 10). *$P < 0.01$ compared with group 3. † $P < 0.01$ compared with group 2.

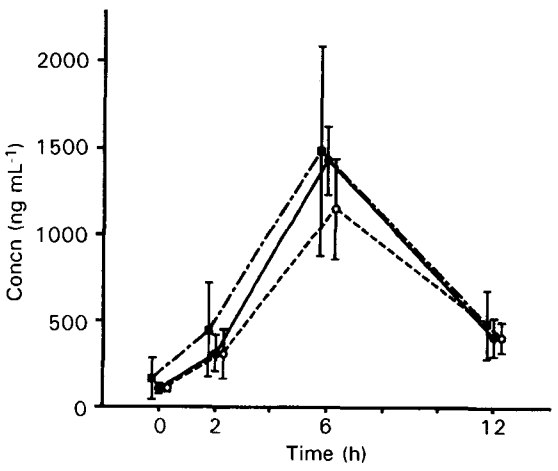

FIG. 3. Changes in whole-blood cyclosporin concentrations (mean ± s.d.) after oral administration of the solution of cyclosporin in olive oil. ●, Simultaneous oral administration of cyclosporin and AST-120 (group 6, n = 7); ■, AST-120 was administered orally 2 h after oral cyclosporin administration (group 7, n = 7); ○, oral administration of cyclosporin alone (group 8, n = 7). There was no significant difference among results from groups 6, 7 and 8.

The serum concentrations of total bile acids 12 h after oral administration of the solution of cyclosporin in olive oil were 87·9 ± 44·9 mg dL^{-1} (mean ± s.d.) for group 6, 90·9 ± 47·4 mg dL^{-1} for group 7 and 61·4 ± 37·6 mg dL^{-1} for group 8. There was no significant difference among these concentrations in groups 6, 7 and 8.

Discussion

The adsorptive capacity of AST-120 depends on the molecular weight of the adsorbates (Honda & Nakano 1994). This capacity rapidly increases when the molecular weight of the adsorbates increases from 100 to 1000 and then gradually decreases. On oral administration of AST-120, this characteristic adsorptive capacity has the advantage of leading to preferential adsorption and elimination of the low-to-moderate molecular-weight uraemic toxins that accumulate in the digestive tract of renal failure patients without the simultaneous adsorption of digestive enzymes and polysaccharides of large molecular weight. Because the molecular weights of many clinically used medicines range from several hundreds to approximately 1000, their combined administration with AST-120 might result in reduced absorption of these medicines because of their adsorption by AST-120.

Cyclosporin is widely used in clinical practice for organ transplantation and treating autoimmune diseases; its use has strikingly improved graft survival rates.

Because of its molecular weight of 1203 (Wenger et al 1986), cyclosporin is easily adsorbed by AST-120. The in-vitro AST-120 adsorption ratios for cyclosporin were more than 80% in our study. In our animal study, when cyclosporin and AST-120 were simultaneously administered orally blood cyclosporin concentrations were significantly higher than those after oral administration of cyclosporin alone. Moreover, in combined oral administration of AST-120 and a solution of

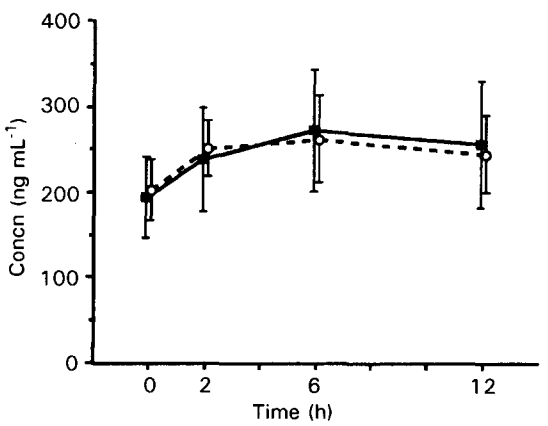

FIG. 2. Changes in whole-blood cyclosporin concentrations (mean ± s.d.) after intramuscular injection of cyclosporin dissolved in medium-chain triglyceride. ■, Intramuscular injection of cyclosporin and simultaneous oral administration of AST-120 (group 4, n = 8); ○, intramuscular injection of cyclosporin alone (group 5, n = 8). There was no significant difference between results from groups 4 and 5.

Table 2. Whole-blood cyclosporin concentrations (mean ± s.d.) in rats 6 h after oral administration of cyclosporin (10 mg kg^{-1}).

	Cyclosporin concn (ng mL^{-1})
Group 1	483 ± 148
Group 2	294 ± 127
Group 3	163 ± 100
Group 6	1429 ± 198*
Group 7	1486 ± 612†
Group 8	1143 ± 293‡

Groups 1–3 were administered cyclosporin orally (n = 10). Groups 6–8 were administered the solution of cyclosporin in olive oil orally (n = 7). Groups 1 and 6 were administered cyclosporin and AST-120 orally simultaneously. Groups 2 and 7 were administered AST-120 orally 2 h after cyclosporin administration. Groups 3 and 8 were administered cyclosporin alone orally. *$P < 0.01$ compared with group 1. †$P < 0.01$ compared with group 2. ‡$P < 0.01$ compared with group 3.

cyclosporin in olive oil in conventional clinical practice, blood cyclosporin concentrations were rather higher than those when cyclosporin was administered orally alone.

Cyclosporin is metabolized mainly by microsomal cytochrome P450 enzymes in the liver (Maurer 1985). It has been reported that when cyclosporin is administered simultaneously with drugs affecting the activity of microsomal cytochrome P450 enzymes in the liver, the blood cyclosporin concentration fluctuates (Freeman et al 1984). Our study revealed that there was no difference between the blood cyclosporin concentration in the group receiving intramuscular injection of cyclosporin combined with oral administration of AST-120 and that of the group receiving intramuscular injection of cyclosporin alone. This finding suggests that oral administration of AST-120 has no effect on cyclosporin metabolism in the liver. Our results, therefore, indicate that combined oral administration of AST-120 and cyclosporin might lead to augmented absorption of cyclosporin from the gastrointestinal tract.

Bile plays an important role in cyclosporin absorption in the digestive tract (Venkataramanan et al 1985). In the current experiment the serum concentration of total bile acids did not significantly change after oral administration of AST-120. The effect of oral administration of AST-120 on the enterohepatic circulation of bile can be ignored.

The in-vitro AST-120 adsorption ratio for nicaldipine, a calcium-antagonist used in the treatment of hypertension, was shown by Honda & Nakano (1994) to be more than 80%. In their experiment with dogs, AST-120 administered orally in combination with oral nicaldipine, however, did not inhibit absorption of nicaldipine from the digestive tract. Conversely, its blood concentration tended to increase. They supposed that these findings resulted from physical stimulation of gastrointestinal function by oral administration of AST-120, and consequent augmentation of nicaldipine absorption. In our experiments on oral administration of AST-120 2 h after oral administration of cyclosporin (group 2), the blood cyclosporin concentration immediately before administration of AST-120 was already significantly high compared with that after administration of cyclosporin alone (group 3). Administration of AST-120 for 4 days probably stimulated gastrointestinal function or rendered the environment in the digestive tract more favourable to the absorption of cyclosporin. Furthermore, the blood cyclosporin concentrations 0, 6 and 12 h after simultaneous oral administration of cyclosporin and AST-120 (group 1) were significantly higher compared with those of group 2. Simultaneous oral administration of AST-120 and cyclosporin might change the environment surrounding the cyclosporin in the digestive tract so that absorption of cyclosporin might be enhanced.

In this study, the blood cyclosporin concentration after oral administration of the solution of cyclosporin in olive oil alone was significantly higher than that after oral administration of cyclosporin alone. Augmentation of the blood cyclosporin concentration in groups receiving combined oral administration of cyclosporin and AST-120 might be because the capacity for gastrointestinal absorption of cyclosporin stimulated by AST-120 might become significantly higher than the adsorptive capacity of AST-120 for cyclosporin. In the groups receiving combined oral administration of the solution of cyclosporin in olive oil and AST-120, however, AST-120 had no significant effect on blood cyclosporin concentration. This might be because the absorption volume of cyclosporin in this form is too large to be influenced by the augmented cyclosporin absorption volume in the gastrointestinal tract simulated by AST-120. Miyamoto et al (1993), however, reported that the blood cyclosporin concentration temporarily increased in a renal transplant patient orally administered cyclosporin and AST-120.

In summary, the blood cyclosporin concentration did not fluctuate after combined oral administration of AST-120 and the solution of cyclosporin in olive oil, one of the forms used clinically. This suggests that oral administration of AST-120 does not affect the absorption of this form of cyclosporin, and that it is possible to administer both drugs orally, simultaneously, without altering the dose of cyclosporin. However, oral administration of AST-120 in combination with oral administration of cyclosporin will lead to elevation of blood cyclosporin concentrations. Clinically, the blood cyclosporin concentration was temporarily increased in a renal transplant patient after oral administration of cyclosporin and AST-120. Very careful monitoring of blood cyclosporin concentrations is, therefore, needed after combined oral administration of cyclosporin and AST-120.

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