Effect of Streptozotocin-Induced Diabetes on Cyclosporin A Disposition in Rats

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We studied the effect of diabetes on the pharmacokinetics of cyclosporin A (CyA) after intravenous and oral administration of CyA using the plasma and lymph of streptozotocin (STZ)-induced diabetic rat. There were no significant differences in the systemic and lymphatic availabilities after intravenous administration of CyA in diabetic rats compared with those of the controls. On the other hand, systemic and lymphatic availabilities after oral administration of CyA were significantly different in diabetic rats compared to those in the controls. These results suggest that the pharmacokinetics of CyA, particularly absorption, were altered in diabetic rats. Gastrointestinal transit in diabetic rats was also studied. The gastric emptying rate in diabetic rats was enhanced compared with that of the controls, but small intestinal transit was reduced in diabetic rats, suggesting that a change in gastrointestinal transit in diabetic rats may influence the absorption of CyA.

The increased absorption of CyA from the digestive tract of diabetic rats altered not only the systemic availability but also the lymphatic availability, suggesting that altered systemic availability may cause adverse effects and that altered lymphatic availability may influence the immunosuppressive effects.

Key words cyclosporin A; diabetes; rat; streptozotocin; absorption; gastrointestinal transit

Many diabetic patients develop serious complications, including cardiovascular disorders, neuropathy, nephropathy and retinopathy.1) Intake of medication is, therefore, frequent, and in many cases involves highly potent drugs or agents with narrow therapeutic ranges. Moreover, the absorption, disposition and action of the drugs used may be altered by the disease itself, e.g., a delay in the absorption of tolazamide and a decrease in the extent of absorption of ampicillin have been reported.2,3) Della-Coletta and Eller found that the absorption of tolazamide was 26% slower in diabetic patients with asymptomatic autonomic neuropathy than in healthy subjects.2) Adithan et al. reported a 26% decrease in the extent of absorption of orally administered ampicillin compared with that of nondiabetic controls.3) Cytokinin A (CyA), a potent immunosuppressive agent, has been increasingly used in the last decade in a variety of organ transplants.4)–6) CyA has also been shown to be effective in treating several autoimmune diseases i.e., adjuvant arthritis in rats,7) rheumatoid arthritis,8) and Type 1 insulin-dependent diabetes in both animals9) and humans.10) It is widely accepted that there is a relationship between the concentration of CyA in blood and both immunosuppressive and adverse effects.11)–13) Due to interpatient variability in the pharmacokinetics of CyA, interindividual variation in plasma levels following the same dose of CyA is large,14) implying the need for an individual dosage adjustment. Although a recent article reviewed many studies on CyA pharmacokinetics,15) there have been few reports on the pharmacokinetics of CyA in diabetes.16)–19) In this study, we examined the effect of diabetes on the pharmacokinetics of CyA after intravenous and oral administration in streptozotocin (STZ)-induced diabetic rats. The immunosuppressive activity of CyA is related to a selective action against T lymphocytes, which play a central role in the induction of immune responsiveness.20) Therefore, the immunosuppressive activity of CyA may be related to the CyA concentration in the lymphatic system. Based on this assumption, we studied thoracic lymphatic CyA levels in STZ-induced diabetic rats. Furthermore, we measured CyA levels in the intestinal lymph, since it has an important role in the transfer of CyA into the thoracic lymph duct after being absorbed from the gastrointestinal tract.21) We also examined gastrointestinal transit, using an oral radioactive marker, in the diabetic rats to evaluate the gastric emptying rate (GER) and small intestinal transit.22)

MATERIALS AND METHODS

Materials CyA and cyclosporin D (CyD, used as an internal standard for HPLC) were kindly supplied by Sandoz, Ltd. (Basle, Switzerland). STZ was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). All other reagents were the best available commercial products of analytical grade.

Animals Male Wistar rats, 9 weeks of age, were used. Diabetes was induced by a single intravenous injection of STZ (35 mg/kg), which had been dissolved immediately before use in 0.05 M citrate buffer, pH 4.5. Seven days later, the rats with fasting plasma glucose levels greater than 250 mg/dl were selected for this experiment. The control group received only the citrate buffer. Blood glucose level was determined by the glucose oxidase method (Boehringer Mannheim GmbH, Mannheim, Germany).

CyA Administration and Blood Collection Each rat was anesthetized with ether, and the left jugular vein was exposed and cannulated with PE-50 polyethylene tubing (i.d., 0.58 mm; o.d., 0.965 mm; Clay Adams, Parsippany, N.J., U.S.A.). Cannulae were exteriorized between the scapulae, then sealed until the time of the experiment. After the rats had recovered from anesthesia, CyA dissolved in propylene glycol was slowly administered, either intravenously (10 mg/kg) via the jugular vein or orally (10 mg/kg) using a gastric sonde. Blood samples (200 μl) were obtained from the jugular vein at 0.017, 0.083, 0.17, 0.33, 0.5, 1, 2,

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3, 5 and 7 h after the intravenous administration, and at 0.5, 1, 2, 3, 4, 5, 6 and 7 h after the oral administration. At each sampling time, 200 μl of saline was injected through the jugular vein to prevent volume depletion. Blood samples were immediately centrifuged for 5 min (10000 rpm), and the plasma was frozen in a freezer until analysis.

**CyA Administration and Thoracic Lymph Collection**

Each rat was anesthetized with ether, and the thoracic lymph duct was cannulated with PE-50 polyethylene tubing rinsed with dilute heparin. After the operation, the rats were placed in Bollman restraining cages with free access to water. After the rats had recovered from anesthesia, CyA dissolved in propylene glycol was administered intravenously (10 mg/kg) through the jugular vein or orally (10 mg/kg) using a gastric sonde. Continuous output of the lymph from the thoracic lymph duct was collected at 0.5, 1, 2, 3, 4, 5, 6 and 7 h after the administration. Lymph samples were immediately centrifuged for 5 min (10000 rpm), and frozen in a freezer until analysis.

**CyA Administration and Intestinal Lymph Collection**

Each rat was anesthetized with ether, and the intestinal lymph duct was cannulated with PE-50 polyethylene tubing rinsed with dilute heparin. After the operation, the rats were placed in Bollman restraining cages with free access to water. After the rats had recovered from anesthesia, CyA dissolved in propylene glycol was injected (10 mg/kg) by a syringe into the lumen of the duodenum. After enteral administration, the continuous output of the lymph from the intestinal lymph duct was collected in the sampling tubes at 0.5, 1, 2, 3, 4, 5, 6 and 7 h after the administration. Lymph samples were immediately centrifuged for 5 min (10000 rpm) and frozen in a freezer until analysis.

**Analytical Method**

The plasma and lymph concentrations of CyA were determined according to the HPLC procedure described by Takada et al. Chromatographic determination of CyA was performed using a HPLC system with a model 600A solvent delivery system (Waters, Milford, U.S.A.). The analytical column was an RP-18 Lichrosorb column (4.0 × 250 mm, 5 μm particle size, Merck, Germany) and the ultraviolet detector was a Waters 480. The column and precolumn were maintained at 70°C by a column heater. The mobile phase was composed of acetonitrile-water (70:30, v/v), and the flow rate was maintained at 1 ml/min. CyA and CyD used as internal standards were detected at 205 nm. Under these conditions, the retention time was 12 min for CyA and 15 min for CyD. The concentrations of CyA and CyD were calculated by the peak height measurements.

**Data Analysis**

Pharmacokinetic evaluations were carried out by non-compartmental analysis of the plasma concentration–time data based on the statistical moment theory. The moments, the area under the plasma concentration–time curve (AUC), the mean residence time (MRT), and the mean absorption time (MAT) were calculated by the trapezoidal method with a monoexponential extrapolation of the terminal phase. The bioavailability of CyA after oral administration of CyA was calculated using AUC values after the intravenous administration of CyA. The MAT of CyA was calculated using MRT, and MRT values.

**Determination of Gastrointestinal Transit**

Gastrointestinal transit was determined by orally administering Na3CrO4 (1.2 MBq/2 ml/kg) dissolved in propylene glycol. The rat was sacrificed by cervical dislocation at 1 or 2 h after administration of a radioactive marker. The stomach, small intestine, cecum and colon were carefully dissected. The small intestine was then placed on a ruled template and divided into 20 equal segments, and the colon was divided into 2 equal segments. The individual segments were placed successively in counting vials and the gamma emissions were recorded for 1 min using a gamma emission counter (Aloka ARC-300, Tokyo, Japan).

**RESULTS**

**Rat Body Weight and Blood Glucose Levels**

Body weight and blood glucose levels of both diabetic and control rats changed significantly during the seven-day period following a single intravenous injection of STZ (Table 1). The rats were fasted overnight (15 to 18 h) prior to the experiment, but water was allowed ad libitum.

**Pharmacokinetics of CyA after Intravenous and Oral Administration**

The plasma concentration–time curves of CyA after intravenous administration (10 mg/kg) and oral administration (10 mg/kg) of CyA are shown in Fig. 1 and Fig. 2, respectively. The pharmacokinetic parameters are summarized in Table 2. There were no significant differences in CyA pharmacokinetics after the intravenous administration of CyA in the diabetic rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>222.0 ± 5.2</td>
<td>67.1 ± 1.1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>174.8 ± 1.8</td>
<td>304.6 ± 8.6</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. of 12 rats. Significantly different from control, *p* < 0.001.

**Fig. 1.** Effect of Diabetes on Plasma Concentration of CyA after Intravenous Administration of CyA (10 mg/kg) in Rats

○, control; ●, diabetes. Each result represents the mean ± S.E. of 4 rats.
Fig. 2. Effect of Diabetes on Plasma Concentration of CyA after Oral Administration of CyA (10 mg/kg) in Rats

○, control; ●, diabetes. Each result represents the mean ± S.E. of 5 rats. Significantly different from the control, a) p < 0.05, b) p < 0.01.

Table 2. Effect of Diabetes on Pharmacokinetic Parameters of CyA (10 mg/kg) after Intravenous and Oral Administration in Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC∞ (µg h/ml)</td>
<td>12.7 ± 0.6</td>
<td>16.4 ± 2.5</td>
</tr>
<tr>
<td>MRT∞ (h)</td>
<td>2.1 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>AUC∞ (µg h/ml)</td>
<td>4.4 ± 0.4</td>
<td>7.3 ± 0.8*</td>
</tr>
<tr>
<td>MRT∞ (h)</td>
<td>3.4 ± 0.3</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>34.9</td>
<td>44.5</td>
</tr>
<tr>
<td>MAT (h)</td>
<td>1.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The AUC value was calculated from time zero to 7 h for i.v. and p.o. Each result represents the mean ± S.E. of 5 rats. Significantly different from control, a) p < 0.05.

compared with those of the control rats. In contrast, after the oral administration of CyA, the peak level (C_max) was 0.83 ± 0.07 µg/ml and the peak time (T_max) was 2 h in the controls. On the other hand, in diabetic rats, the C_max was increased to 1.58 ± 0.20 µg/ml, and the T_max was reduced to 1 h. The AUC∞ value in diabetic rats was approximately 65.5% greater than that in the controls. Furthermore, MAT in the diabetic rats was reduced to 1.03 h from 1.32 h in the controls. The oral bioavailability of CyA in the diabetic rats was 44.49% while that in the controls was 34.86%.

CyA in the Thoracic Lymph after Intravenous and Oral Administrations Figure 3 and 4 show the thoracic lymph appearance rate–time curves of CyA in the thoracic lymph after intravenous (10 mg/kg) and oral administrations (10 mg/kg) of CyA. There were no significant differences in the appearance rate of CyA in the thoracic lymph after intravenous administration of CyA in diabetic rats compared with that of controls. In contrast, after oral CyA administration, the CyA level of diabetic rats was higher than that of the controls, particularly early after the oral administration. The lymph flow was increased in diabetic rats (233 ± 52 vs. 196 ± 60 ml/h, p < 0.05), but the number of lymphocytes was unchanged compared with that of controls.

CyA in the Intestinal Lymph after Enteral Administration The rate–time curves of CyA in the intestinal lymph after

Fig. 3. Effect of Diabetes on Appearance Rate of CyA in Thoracic Lymph after Intravenous Administration of CyA (10 mg/kg) in Rats

○, control; ●, diabetes. Each result represents the mean ± S.E. of 4 rats.

Fig. 4. Effect of Diabetes on Appearance Rate of CyA in Thoracic Lymph after Oral Administration of CyA (10 mg/kg) in Rats

○, control; ●, diabetes. Each result represents the mean ± S.E. of 5 rats. Significantly different from the control, a) p < 0.05.

Fig. 5. Effect of Diabetes on Appearance Rate of CyA in Intestinal Lymph after Enteral Administration of CyA (10 mg/kg) in Rats

○, control; ●, diabetes. Each result represents the mean ± S.E. of 5 rats. Significantly different from the control, a) p < 0.05, b) p < 0.01, c) p < 0.001.
enteral administration of CyA (10 mg/kg) are shown in Fig. 5. After enteral CyA administration, the time of the peak in diabetic rats was reduced from 3 h to 1 h compared to the controls, but the level of the peak in diabetic rats was almost the same as that of the controls. On the other hand, the total CyA level in diabetic rats was decreased compared to that of the controls.

Gastrointestinal Transit Gastrointestinal transit was determined at several time intervals after the oral administration of Na$_2$CrO$_4$ dissolved in propylene glycol (Fig. 6). After an additional 1 h, the majority of the radioactive markers (Cr dissolved in propylene glycol) in diabetic rats had transferred to the small intestine, but in the controls about 30% remained in the stomach. Two hours after administration, the majority of the radioactive markers remained in the small intestine of diabetic rats, but in the controls about 35% had entered the cecum. The results show that the GER in diabetic rats was enhanced compared to that of the controls, but the small intestinal transit in diabetic rats was reduced.

DISCUSSION

We measured the CyA concentration in the central circulation to evaluate the systemic availability of CyA after intravenous or oral administration of CyA. The kinetic profile of CyA in plasma was not significantly different compared to that in whole blood. There were no significant differences in the pharmacokinetic parameters of $AUC_{in}$, $MRT_p$, and half-life ($T_{1/2}$) after intravenous administration of CyA in diabetic rats compared with those of controls. Therefore, the distribution and elimination of CyA in diabetic rats may not be significantly different from those of the controls. On the other hand, after oral administration of CyA, $C_{max}$ was increased about two-fold, $T_{max}$ was reduced from 2 h to 1 h, the $AUC_{po}$ was increased by approximately 65.5%, bioavailability was increased by about 30% and MAT was reduced in diabetic rats compared with those of the controls, respectively. Although the CyA pharmacokinetics in diabetic rats after intravenous administration were not significantly different from the controls, the absorption of CyA after oral administration in diabetic rats was increased compared with that of the controls. These results suggest that diabetes can influence the absorption of CyA.

The immunosuppressive activity of CyA is related to a selective action against T lymphocytes, which play a central role in the induction of immune responsiveness. Therefore, the immunosuppressive activity of CyA may be related to the CyA concentration in the lymphatic system. Because the appearance of CyA in the thoracic lymph after intravenous administration was not significant, it would suggest that the blood-to-lymph transfer in diabetic rats was not significant compared with that of the controls. On the other hand, since the appearance of CyA in the thoracic lymph after oral administration was increased in diabetic rats, it suggests that the transfer of CyA into the thoracic lymph in diabetic rats was enhanced compared with that of controls. There was a close correlation between the pharmacokinetics of CyA on the plasma concentration and the thoracic lymph concentration of CyA in diabetic rats. In this study, we measured the intestinal lymph level after enteral administration of CyA as a reference to the tract-to-lymph transfer in diabetic rats. The absorption rate of CyA into the intestinal lymph was increased, but the total intestinal lymph level was reduced in diabetic rats. These results suggest that the pharmacokinetics of CyA, particularly absorption, were altered in diabetic rats. We previously reported that the small intestinal absorption of CyA in STZ-induced diabetic rats is increased by the in situ recirculating perfusion method and in vitro by the everted sac method. Accordingly the absorption of CyA after oral administration into systemic circulation and the thoracic lymph may
be related to its enhancement in diabetic rats.

We examined the gastrointestinal transit in diabetic rats because CyA is absorbed in the small intestine, and gastric emptying time appears to be a rate-limiting factor in the absorption. The GER in diabetic rats was enhanced compared with that in controls, but the small intestinal transit was reduced in diabetic rats. These results suggest that the enhancement of the GER may reduce the $T_{max}$ of CyA, and the reduction in small intestinal transit may increase the $C_{max}$ and $AUC$ of CyA in diabetic rats.

In the present study, the absorption of CyA from the small intestine in diabetic rats increased its systemic and lymphatic availability, suggesting that the changes in systemic and lymphatic availabilities in diabetic rats may alter the immunosuppressive effects and adverse effects.

REFERENCES


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