Effect of Non-Insulin Dependent Diabetes on Cyclosporin A Disposition in Goto-Kakizaki (GK) Rats

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We previously reported that the pharmacokinetics of cyclosporin A (CyA), particularly absorption, were altered in diabetic rats treated with streptozotocin. In the present study, the effect of diabetes on pharmacokinetics of CyA after intravenous and oral administration of CyA using the blood and lymph and the gastrointestinal transit were examined in Goto-Kakizaki (GK) rats, a genetic model of non-obese non-insulin-dependent diabetes mellitus (NIDDM), and compared to non-diabetic Wistar rats. Although the systemic and lymphatic availability after intravenous administration of CyA to the GK rats was not significantly different from those of the control Wistar rats, those availability after oral administration of CyA to GK rats was markedly reduced in comparison. These results suggest that the pharmacokinetics of CyA, particularly absorption, was altered in GK rats. Studies on the gastrointestinal transit in GK rats showed that the gastric emptying rate was lower than that of Wistar rats, suggesting that a change in gastrointestinal transit in GK rats may influence the absorption of CyA. The gastric emptying rate in GK rats altered not only the systemic availability but also the lymphatic availability, suggesting that the altered systemic availability may cause adverse effects and that altered lymphatic availability may influence the immunosuppressive effects.

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

We examined the effect of diabetes on pharmacokinetics of Cyclosporin A (CyA) after intravenous and oral administration to Goto-Kakizaki (GK) rats, a genetic model of non-obese non-insulin-dependent diabetes mellitus (NIDDM). We previously reported that the systemic and lymphatic availabilities of CyA from the digestive tract and the small intestinal absorption of CyA were increased in diabetic rat treated with streptozotocin (STZ rat).1,3) The GK rat, one of the best characterized animal models for genetic susceptibility to NIDDM in non-obese individuals, was created by selective breeding of an outbred colony of Wistar rats, with selection for high glucose levels in an oral glucose tolerance test.3–6) Immunosuppressive activity of CyA is related to a selective action against T lymphocytes, which play a central role in the induction of immune responsiveness.7) Therefore, the immunosuppressive activity of CyA may be related to the CyA concentration in the lymphatic system. Based on this assumption, we studied the thoracic lymphatic CyA levels in GK rats. We also examined gastrointestinal transit in GK rats, using an oral radioactive marker, to evaluate the gastric emptying rate (GER) and small intestinal transit.8

MATERIALS AND METHODS

Materials CyA was kindly supplied by Sandoz, Ltd. (Basel, Switzerland). All other reagents were the best available commercial products of analytical grade.

Animals Diabetic GK rats were obtained from the Experimental Animal Center, Tohoku University School of Medicine. Non-diabetic Wistar rats were used as controls. All animals were 9 weeks of age, with a mean body weight of 232 ± 4 g for the GK rats and 221 ± 6 g for the Wistar rats. Fasting blood glucose concentration was 94.7 ± 3.3 mg/dl in the GK rats and 78.7 ± 4.2 mg/dl in the

Wistar rats (p < 0.05). The rats were fasted overnight prior to the experiment, but water was allowed ad libitum.

CyA Administration and Blood Collection Each rat was anesthetized with ether, and the left jugular vein was exposed and cannulated with polyethylene tubing. After the animal had recovered from anesthesia, CyA dissolved in propylene glycol was slowly administered, either intravenously (10 mg/ml/kg) via the jugular vein or orally (10 mg/2 ml/kg). Blood samples (100 µl) were obtained from the jugular vein after the administration. At each sampling time, 100 µl of saline was injected through the same vein to prevent volume depletion. Blood samples were 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 polyethylene tubing rinsed with dilute heparin. After the operation, the rats were placed in Bollman restraining cages. When they had recovered from anesthesia, CyA dissolved in propylene glycol was administered intravenously (10 mg/ml/kg) through the jugular vein or orally (10 mg/2 ml/kg). Continuous output of the lymph from the thoracic lymph duct was collected after the administration. Lymph samples were frozen in a freezer until analysis.

Analytical Method Concentrations of CyA in blood and lymph were measured by a selective monoclonal antibody-based radioimmunoassay (INCSTAR CYCLOTrac® SP kit).

Data Analysis Pharmacokinetic evaluations were carried out by non-compartmental analysis of the blood 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

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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 \( MRT_{iv} \) and \( MRT_{po} \) values.

**Determination of Gastrointestinal Transit** Gastrointestinal transit was determined by orally administering \( Na_2^{51}CrO_4 \) 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 dissected. The small intestine was then divided into 20 equal segments, and the colon was divided into 2 equal segments. The individual segments were placed in counting vials and the gamma emissions were recorded using a gamma emission counter (Aloka ARC-300, Tokyo, Japan).

**RESULTS**

**Pharmacokinetics of CyA after Intravenous and Oral Administrations** There were no significant differences in CyA pharmacokinetics between GK and Wistar rats after the intravenous administration of CyA. However, after oral administration of CyA, there was a significant reduction in the peak level (\( C_{max} \)) and the peak time (\( T_{max} \)) in the GK rats compared to those of Wistar rats (Fig. 1). \( AUC_{po} \) of CyA was significantly reduced in GK rats compared to the other group; hence, the oral bioavailability of CyA was more reduced in the GK rats. \( MAT \) was 1.3 h and 0.5 h, respectively (Table 1).

**CyA in the Thoracic Lymph after Intravenous and Oral Administrations** There was no significant difference in the appearance rate of CyA in the thoracic lymph after intravenous administration of CyA in GK rats compared with that of Wistar rats, in contrast, after oral CyA administration, the CyA level of GK rats was lower than that of the Wistar group (Fig. 2). The lymph flow and the number of lymphocytes in GK rats were unchanged, as well as that of Wistar rats.

**Gastrointestinal Transit** After an additional 1 h, the radioactive marker remaining in the stomach of GK rats was about 45% while that of Wistar rats was about 30% (Fig. 3). After 2 h, 90% of radioactive marker in Wistar rats was located in the distal part of the ileum and the cecum. On the other hand, about 25% of the radioactive marker was still left in the stomach and about 60% in the ileum of GK rats.

**DISCUSSION**

The present study assessed the effect of diabetes on the pharmacokinetics of CyA in GK rats by examining the blood and lymph after intravenous or oral administration of CyA. Immunosuppressive activity of CyA is related to a selective action against T lymphocytes, which plays a central role in the induction of immune responsiveness. Therefore, the immunosuppressive activity of this substance may be related to its concentration in the lymphatic system. Although the systemic and lymphatic availabilities after intravenous administration of CyA in GK rats were not significantly different from Wistar rats, those after oral administration of CyA in GK rats was markedly reduced compared to those of Wistar rats. Therefore, the distribution, elimination and blood-to-lymph transfer of

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**Table 1. Pharmacokinetic Parameters of CyA (10 mg/kg) after Intravenous and Oral Administration in GK Rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wistar</th>
<th>GK</th>
</tr>
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<tbody>
<tr>
<td>( AUC_{iv} ) (( \mu g \cdot h/ml ))</td>
<td>21.9 ± 4.1</td>
<td>20.6 ± 0.8</td>
</tr>
<tr>
<td>( MRT_{iv} ) (h)</td>
<td>2.9 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>( AUC_{po} ) (( \mu g \cdot h/ml ))</td>
<td>11.3 ± 1.9</td>
<td>1.8 ± 0.8*</td>
</tr>
<tr>
<td>( MRT_{po} ) (h)</td>
<td>3.4 ± 0.1</td>
<td>4.2 ± 0.3*</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>51.5</td>
<td>8.5</td>
</tr>
<tr>
<td>( MAT ) (h)</td>
<td>0.5</td>
<td>1.3</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 6 rats. Significantly different from control, \( a) p<0.001 \), \( b) p<0.001 \).

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Fig. 1. Effect of Diabetes on Blood Concentration of CyA after Oral Administration of CyA (10 mg/kg) to GK Rats

Key: □, Wistar; ▲, GK. Each result represents the mean ± S.D. of 5 rats. Significantly different from the control, \( a) p<0.005 \), \( b) p<0.001 \).

Fig. 2. Effect of Diabetes on Appearance Rate of CyA in Thoracic Lymph after Oral Administration of CyA (10 mg/kg) to GK Rats

Key: □, Wistar; ▲, GK. Each result represents the mean ± S.D. of 5 rats. Significantly different from the control, \( a) p<0.05 \), \( b) p<0.001 \).
CyA in GK rats may not be significantly different from those of Wistar rats. On the other hand, after oral administration of CyA, $C_{\text{max}}$, $AUC_{\text{po}}$, bioavailability and transfer of CyA into the thoracic lymph were more reduced in GK than in Wistar rats. There was a close correlation between the pharmacokinetics of CyA on the blood concentration and the thoracic lymph concentration of CyA in GK rats. These results suggest that the pharmacokinetics of CyA, particularly absorption, were altered in these rats. We also examined the gastrointestinal transit in diabetic rats because CyA is absorbed in the small intestine, and the gastric emptying time appears to be a rate-limiting factor in the absorption.\textsuperscript{10} Clinical disorders of the gastrointestinal tract, such as gastroparesis, may occur in diabetic patients who have had the disease for several years.\textsuperscript{1,12} Such complications are usually attributed to the development of diabetic autonomic neuropathy. In the GK rats, GER and the small intestinal transit were reduced more than those in Wistar rats. These results suggest that the reduction in GER and in the small intestinal transit may reduce $C_{\text{max}}$, $T_{\text{max}}$, $AUC_{\text{po}}$ and bioavailability of CyA in the GK rats. We concluded that the blood and lymph concentrations of CyA in both the GK and the STZ rats may be influenced in part by the alteration of gastrointestinal motility and absorption by the intestine. The gastrointestinal transit in GK rats in this study reduced the systemic and lymphatic availabilities, suggesting that the changes in these availabilities in diabetic rats may alter the immunosuppressive effects and cause adverse effects.

REFERENCES