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Study of Pharmacokinetic Properties of Cyclosporine A after Subcutaneous Injection in Cynomolgus Monkey

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Abstract: Intramuscular or intravenous injection of Cyclosporine A (CyA) has been performed in some transplantation animal studies, including non-human primates. However, the pharmacokinetics after subcutaneous (s.c.) injection of CyA in cynomolgus monkey has not been reported. In this study CyA was administered subcutaneously in cynomolgus monkeys at three doses and investigated the pharmacokinetics. Six female cynomolgus monkeys were used in the study. CyA was administered once daily at a prescribed time for 7 days in the back of each monkey at doses of 2.5, 5, or 10 mg kg⁻¹ in different administration periods. The blood trough level of CyA was measured on consecutive days. On days 0, 1, and 4, the level was measured 2, 4, 6, 8 and 12 h after CyA administration. The mean trough concentrations on day 4 in the 6 animals at doses of 2.5, 5, and 10 mg kg⁻¹ were 162±56, 356±97 and 652±137 ng mL⁻¹, respectively, showing a dose-dependent increase. The average Tₚ₅₀ range at each concentration on days 0 and 4 was 5-11 h and was 5-6 h on day 4 at steady state, with Cmax tending to be not high. The T₁/₂ showed general prolongations that were 14-19 h on day 4. Average pharmacokinetic data on day 4 showed dose-dependent and almost linear increases in AUC which were 9822±3473, 24323±9520 and 43418±12591 ng hr mL⁻¹ at dose of 2.5, 5, and 10 mg kg⁻¹, respectively. The pharmacokinetics of CyA after s.c. injection in cynomolgus monkeys showed a slow absorption phase, a Cmax that is not high and a prolonged elimination phase. The linearity of the AUC with dose and the high AUC and trough level in the early days after first administration suggest high bioavailability and sufficient efficacy after s.c. injection.

Key words: Cyclosporine A, cynomolgus monkey, pharmacokinetics, subcutaneous injection, uterus transplantation

INTRODUCTION

Recent improvements in transplantation, anastomosis and tissue preservation techniques have brought great benefits to many patients. In organ transplantation, transplantation surgery and postoperative management (especially of immunosuppressive drugs) are both important. Control of rejection in many organs has become possible through improved understanding of the mechanism of immune rejection and development of immunosuppressive drugs but administration of these drugs should be carefully managed because rejection has a major impact on the prognosis of patients.

In recent years, uterus transplantation in patients with uterine factor infertility has become an option in the field of obstetrics gynecology and preclinical studies with various animals have been performed (Kisu et al., 2013). Introduction of new medical techniques requires data collection in preclinical studies in large animals, including primates and such studies provide important findings for

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clinical use (Milliez, 2009). Preclinical studies of allogeneic uterus transplantation have been performed using immunosuppressive drugs but the number of studies in primates is still small (Kisu et al., 2013).

Primates such as baboon and cynomolgus monkey are used in many preclinical studies because they are anatomically and physiologically similar to humans. Explorative allogeneic uterus transplantation using immunosuppressive drugs, including calcineurin inhibitors was performed. Since postoperative control of oral immunosuppressive drugs was difficult, there are cases with rejection of the uterus (Kisu et al., 2014). Strict control of drip infusion is required for immunosuppressive drugs administered after surgery in cynomolgus monkey to avoid acute rejection but postoperative control of drip infusion cannot be performed in many facilities because of difficult management in monkeys and drip infusion-related problems have been known. Blood concentration was controlled after surgery by oral administration, rather than drip infusion, due to regulations in an experimental facility. However, we were unable to maintain the blood concentration of the immunosuppressive drug at the target trough level due to lowered compliance with oral administration in the monkeys because of the highly invasive surgery and malabsorption from the intestinal tract (Kisu et al., 2014). These findings suggest that control of immunosuppressive drugs is an important task in preclinical studies careful and accurate administration of these drugs is required.

Injections into subcutaneous (s.c.) or muscle tissue are commonly used routes of drug administration in cases in which disease or the pharmacokinetic properties of the drug preclude oral dosing. Intramuscular (i.m.) injection of Cyclosporine A (CyA) has been performed in some transplantation animal studies, including non-human primates (Schuerman et al., 2001; Leonard et al., 1996; Oginnake et al., 1987; Pernock et al., 1981). It was planned to control blood levels using s.c. administration of CyA, rather than i.m. injection, to avoid pain and muscle damages caused by continuous injection. However, there have been no reports on CyA pharmacokinetics after s.c. injection in cynomolgus monkey. Therefore, in this study, CyA subcutaneously was administered in cynomolgus monkeys at three doses and investigated the pharmacokinetics.

MATERIALS AND METHODS

Animals: Six female cynomolgus monkeys (Macaca fascicularis), aged 9-11 years and with body weights of 3-4 kg were used in the study. The study was performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Research Council. The study protocol was approved by the animal care and Use Committee of the Research Center for Animal Life Science, Shiga University of Medical Science, Japan (Permit Number: 13-003).

Administration of cyclosporine A: CyA (Sandimmun for i.v. Infusion; Novartis Pharm K.K.) was administered s.c. once daily at a prescribed time for 7 days in the back of each monkey at doses of 2.5, 5 or 10 mg kg⁻¹ in different administration periods. One course included daily dosing for one week and a second week for drug cessation. Before the next course at a different dosage, the blood level of CyA was confirmed to be lower than the assay sensitivity.

Blood sampling and general monitoring: The blood trough level of CyA was measured on consecutive days in the first week. In week 2 (after completion of administration), this measurement was performed 3 times. On days 0, 1 and 4 in week 1, the level was measured 2, 4, 6, 8 and 12 h after CyA administration, in addition to the trough level measurement before administration. All blood sampling were performed using Na-EDTA-treated vacutainer tubes. Whole blood samples (0.5 mL) for CyA were taken to the analytical laboratory. Several hematological and biochemical tests were also performed in each course in addition to the daily observation of general conditions.

Determination of cyclosporine: The CyA concentration in whole blood samples were determined in duplicate with a radioimmunoassay kit using a monoclonal antibody (detection limit 20 ng mL⁻¹) produced by DiaSorin Inc. (Stillwater, MN, USA). The CyA was assessed by whole blood concentration, determined by a monoclonal, parent-compound-specific radioimmunoassay (RIA; DiaSorin CYCLO-Trac™ whole blood RIA kit, Stillwater, Minnesota, MN; cross-reactivity with CyA metabolites <2.0%, linear from 20-1600 ng mL⁻¹)). In an accuracy test with this kit for measurement of CyA concentrations, the difference from the predetermined value was within ±15 and ±4% for reference serum with CyA concentrations of 63-93 ng mL⁻¹ and 370-490 ng mL⁻¹, respectively. In a within-run reproducibility test in which 5 simultaneous measurements were performed, the coefficients of variance of the measured values were <16 and <14% for reference serum with CyA concentrations of 69-93 ng mL⁻¹ and 370-490 ng mL⁻¹, respectively. Pharmacokinetic parameters were calculated by non-compartmental analysis using Phoenix WinNonlin 6.3 software (Pharsight Corp., Mountain View, CA, USA).
time to peak concentration (Tmax), peak concentration (Cmax), half-time (T1/2), area under the concentration-time curve (AUC) and clearance (CL).

Statistical analysis: Student's t-test was used to compare means of the continuous variables. One way analysis of variance was used to compare means of continuous samples in two or more groups. The linearity of two continuous variables was analyzed by fitting general linear models. Significance levels in all tests were 5% (two-sided). Statistical calculations were performed with SPSS (version 22).

RESULTS

Pharmacokinetics: All injections of CyA were successfully performed with no leakage from the skin. Changes in trough level in 6 animals after CyA injection s.c. at 3 doses are shown in Fig. 1. The mean trough concentrations on day 4 in the 6 animals at doses of 2.5, 5 and 10 mg kg⁻¹ were 162±56, 356±97 and 652±137 ng mL⁻¹, respectively, showing a dose-dependent increase (p<0.05, r = 0.904). Some animals achieved steady state levels in 24 h after first administration but some other animals showed increasing levels even after one week. At all doses, serum CyA was lower than the detection sensitivity at one week after the last administration in most animals, suggesting that the drug was fully eliminated.

Mean pharmacokinetic parameters on days 0 and 4 are shown in Table 1. Individual and average CyA concentrations determined on day 4 after daily s.c. injection are presented in Fig. 2-3, respectively. The average Tmax range at each concentration on days 0 and 4 was 5-11 h and was approx. 5-6 h on day 4 at steady state. Tmax averaged over all doses was shorter on day 4 (5.6 h) than on day 0 (8.7 h) (p<0.05). Absorption of CyA after s.c. injection was slower than that after intravenous (i.v.) injection, with Cmax tending to be lower than that after i.v. injection and T1/2 showing a general prolongation

![Image](image_url)

Fig. 1(a-c): CyA concentration after s.c. administration at doses of (a) 2.5, (b) 5 and (c) 10 mg kg⁻¹

<table>
<thead>
<tr>
<th>Days and dose (mg kg⁻¹)</th>
<th>Tmax (h)</th>
<th>Cmax (ng mL⁻¹)</th>
<th>T1/2 (h)</th>
<th>AUCγ.24 (ng h mL⁻¹)</th>
<th>AUC∞ (ng h mL⁻¹)</th>
<th>CL (mL kg⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.3±0.82</td>
<td>398.3±128.91</td>
<td>14.7±8.77</td>
<td>6004.6±1366.18</td>
<td>9129.2±2170.61</td>
<td>287.6±70.35</td>
</tr>
<tr>
<td>5.0</td>
<td>11.3±1.63</td>
<td>555.0±162.08</td>
<td>19.0±12.35</td>
<td>9720.0±2569.72</td>
<td>1834.1±5884.98</td>
<td>292.6±76.12</td>
</tr>
<tr>
<td>10.0</td>
<td>8.0±2.53</td>
<td>1066.6±390.26</td>
<td>40.4±30.38</td>
<td>1896.0±6405.87</td>
<td>5105.3±1852.44</td>
<td>219.6±79.88</td>
</tr>
<tr>
<td>4</td>
<td>5.0±2.10</td>
<td>415.0±122.76</td>
<td>14.0±12.50</td>
<td>6605.1±1154.27</td>
<td>9822.5±473.04</td>
<td>279.7±90.58</td>
</tr>
<tr>
<td>5.0</td>
<td>5.0±1.10</td>
<td>823.3±179.85</td>
<td>19.4±9.370</td>
<td>13296.7±2298.02</td>
<td>2432.3±9319.69</td>
<td>231.5±83.72</td>
</tr>
<tr>
<td>10.0</td>
<td>5.6±1.97</td>
<td>1466.6±121.11</td>
<td>17.4±9.340</td>
<td>2567.1±1588.43</td>
<td>4341.8±1291.19</td>
<td>245.7±64.52</td>
</tr>
</tbody>
</table>

Data is Mean±SD, n = 6 for each treatment
Fig. 2(a-c): Blood concentration-time profiles of CyA determined on day four after daily s.c. administration at doses of (a) 2.5, (b) 5 and (c) 10 mg kg\(^{-1}\).

that was especially significant (40 h) on day 0 at a dose of 10 mg kg\(^{-1}\) (p<0.05). Despite the variation of daily changes among animals (Fig. 2), average pharmacokinetic data on day 4 showed dose-dependent and almost linear increases in Cmax (p<0.05, r = 0.955) and AUC∞ (p<0.05, r = 0.845) (Fig. 3, Table 1). Clearance (CL) was similar at all doses (p = 0.458).

**General observation:** The study was performed in individual animals for approximately 5 months. During this period, a decrease of weight occurred in 5 animals (-0.46±0.48 kg, Mean±SD). A bite wound was observed in the forearm of one animal but there were no other side effects, including no tissue reactions at the injection site. Hematological tests showed mild anemia due to frequent blood sampling in most animals in each course. In biochemical tests, renal functions were normal in all animals but some monkeys at doses of 10 mg kg\(^{-1}\) showed mild liver disorder. This data normalized after completion of each course.

**DISCUSSION**

In this study, the pharmacokinetics of CyA after subcutaneous injection in cynomolgus monkeys were examined. In organ transplantation, it is especially important to inhibit rejection in the acute phase and careful control of immunosuppressive drug levels is
required from immediately after surgery (Levy et al., 2002; Hibi et al., 2011). The oral bioavailability of CyA is as low as 20-30% (Pachciński et al., 1987; Aweeka et al., 1994; Sato et al., 2009; Ku et al., 1998). In a preliminary study of allogeneic uterus transplantation in cynomolgus monkeys, it was found that low compliance and poor absorption in oral administration of an immunosuppressive drug after surgery which caused difficulty with maintenance of the blood concentration (Kisu et al., 2014). Thus, it was considered alternative routes of administration. Subcutaneous injection was selected because i.v. injection is difficult due to regulations in experimental animal facilities and i.m. injection can cause pain and crush injury of muscles due to long-term daily administration. The pharmacokinetics of CyA after oral, i.v. and i.m. administration in primates such as baboon and rhesus monkey are known (Schaarman et al., 2001; Novitzky et al., 1985; Kurlansky et al., 1986) but there are no data for pharmacokinetics after s.c. injection of CyA in cynomolgus monkey. Therefore, in this study, The pharmacokinetics after s.c. injection of CyA in cynomolgus monkey was examined, with the goal of finding an appropriate CyA dosage for future preclinical studies of uterus transplantation in this animal.

As shown in Fig. 1 and 2, there were large variations in changes of trough level and daily changes of CyA concentrations among and within animals. Since the trough level reached close to steady state with a single administration and the AUC on day 0 was similar to that on day 4, high exposure was obtained in the early days after first administration. Thus, it is likely that the bioavailability is high after s.c. injection, suggesting that drug efficacy will be obtained in the early days after first administration. In rats, the bioavailability of CyA injection s.c. has been found to be as high as approximately 70% on day 3 (De Waal et al., 1992).

In other animal studies, pharmacokinetics of CyA injection s.c. showed late absorption (prolonged T_max), low peak blood concentration (low C_max) and a long elimination phase (prolonged T_1/2) (Shah et al., 1992). Similar results were found in the current study. Regarding the absorption phase, T_max after single administration (day 0) in this study was longer compared to data for oral delivery and i.m. injection in other studies in primates (Schaarman et al., 2001). This may be because subcutaneous tissues have fewer blood vessels than muscular tissues and because the CyA injection solution which first accumulates in subcutaneous tissues, is gradually absorbed in a lymphogenous manner due to the solution containing polyoxyethylated castor oil.

C_max in animal 953 after administration at 2.5 mg kg⁻¹ on day 4 was higher than that in other animals (Fig. 2). This may be because the drug was injected into the muscle during s.c. injection (i.v. injection is unlikely because the absorption phase would have been observed at an earlier time). The CyA blood concentration reached steady state at approximately 3-4 days based on T_1/2 (Winter, 2004). However, the absorption phase after daily s.c. injection was generally longer than that anticipated before the study and for this reason there were fewer measurement points during the elimination phase, resulting in reduced accuracy in the calculation of T_1/2.

The value for T_1/2 was 40 h at a dose of 10 mg kg⁻¹ on day 0 which was longer than that for other doses and times
and this high value may have been due to a lack of measurement points in the elimination phase. Regarding exposure, AUC∞ at doses of 5 and 10 mg kg⁻¹ were 2.0 and 3.6 times higher on day 0 and 2.5 and 4.4 times higher on day 4, respectively, compared to those at 2.5 mg kg⁻¹ (Table 1). These data show a dose-dependent and almost linear increase in AUC∞. Moreover, AUC∞ on day 0 was similar to that on day 4, suggesting sufficient highly exposure of the drug from early days after first administration. CL was almost stable regardless of dosage and almost equivalent to the data for CyA i.v. injection to humans (317±51 mL min⁻¹) (Ducharme et al., 1995).

Schuurman et al. (2001) performed CyA i.m. injection in rhesus monkey at the same dose as that used in our study. A comparison of pharmacokinetic data on day 4 in the two studies showed that Tmax and Cmax after s.c. injection were 1.0-2.0 times and 1.2-1.5 times higher, respectively, compared to i.m. injection. The increased Tmax may be due to slower absorption after s.c. injection, while the increased Cmax indicates that bioavailability might be higher with s.c. injection. AUC∞ values after s.c. injection at doses of 2.5, 5 and 10 mg were 1.36, 1.30 and 1.50 times higher than the respective values after i.m. injection. The efficacy of CyA depends on AUC and the higher exposure after s.c. injection suggests that this administration route might provide greater efficacy of CyA compared to i.m. injection at the same dose, although these comparison are between different species in primates.

A decrease in weight was observed in many animals. This might have been due to frequent blood sampling, appetite loss during administration of the immunosuppressive drug and stress caused by blood sampling and drug administration.

CONCLUSION

In conclusion, the pharmacokinetics study of CyA after subcutaneous injection in cynomolgus monkeys showed a slow absorption phase, a Cmax that is not high and a prolonged elimination phase. The linearity of the AUC with dose and the high AUC and trough level in the early days after first administration suggest high bioavailability and sufficient efficacy after subcutaneous injection as seen for intravenous and intramuscular injections.

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