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Pharmacokinetic changes of cyclosporine after intravenous and oral administration to rats with uranyl nitrate-induced acute renal failure

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Abstract

The effects of renal failure on the pharmacokinetics of cyclosporine were investigated after intravenous, 30 mg/kg, and oral, 100 mg/kg, administration of the drug using a rat model of uranyl nitrate-induced acute renal failure (U-ARF). After intravenous administration to rats with U-ARF, the volume of distribution at steady state (1.97 vs. 2.56 l/kg) was significantly smaller, and the area under the blood concentration–time curve (348 vs. 296 μg h/ml) tended to be greater and total body clearance (0.0851 vs. 0.102 l/h per kg) tended to be slower than those in control rats. After oral administration, the pharmacokinetic parameters were not significantly different between the control rats and rats with U-ARF, suggesting that U-ARF did not considerably affect the pharmacokinetics of cyclosporine after oral administration.

Keywords: Cyclosporine; Pharmacokinetics; Rats; Acute renal failures; Uranyl nitrate

1. Introduction

Drugs are eliminated from the body by metabolism (mainly in the liver) and/or excretion (mainly via the kidney by glomerular filtration and/or renal tubular secretion). It has been reported (Kim et al., 1998 and references therein) that the total body, renal, and nonrenal clearances of drugs that were eliminated mainly by metabolism or mainly by renal excretion were altered in animals with uranyl nitrate-induced acute renal failure (U-ARF). Therefore, it could be expected that the pharmacokinetics and hence the pharmacodynamics of drugs could be altered in the renal failure.

Cyclosporine, an immune depressant, is essentially completely metabolized in the liver, mainly by CYP 3A and less than 1.0% of the drug is excreted in urine and bile as unchanged drug (Lindholm, 1991). After oral administration of cyclosporine to human subjects, it was absorbed predominantly in the small intestine (Drewe et al.,

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1992). The extent of absolute oral bioavailability of cyclosporine (F) in human subjects was low, approximately 20–50%, and this could be mainly due to intestinal first-pass effects (Wu et al., 1995). Although the pharmacokinetics of cyclosporine in human subjects (Fahr, 1993 and references therein) and in animals (Awni and Sawchuk, 1985; Shibata et al., 1993; Lindberg and Karlsson, 1994 and references therein) were extensively studied, the pharmacokinetic changes of the drug in rats with U-ARF seemed not to be published to date. U-ARF was chosen in the present study since renal dysfunction is a common problem in cyclosporine-treated patients due to nephrotoxicity of the drug or its metabolites and also of course if a renal transport has been performed.

The purpose of this study is to investigate the effect of renal failure on the pharmacokinetics of cyclosporine after intravenous and oral administration of the drug using a rat model of U-ARF.

2. Materials and methods

2.1. Chemicals

Cyclosporine (Sandimmune® for injection, 50 mg/ml, 5 ml vial, and Sandimmune Neoral® for oral administration, 25 mg capsule) were obtained from Sandoz Pharmaceutical (Basle, Switzerland). Uranyl nitrate was purchased from BDH Chemicals (Poole, UK). Other chemicals were of reagent grade or HPLC grade and therefore used without further purification.

2.2. Animals

Male Sprague–Dawley rats of 8 weeks old were purchased from Dai-Han Laboratory of Animal Development (Seoul, South Korea). They were housed in a specific pathogen-free room (College of Pharmacy, Ewha Womans University, Seoul, Korea) with 12-h light and 12-h dark cycle and temperature of 22 ± 2°C. The rats were randomly divided into two groups; control rats and rats with U-ARF.

2.3. Induction of acute renal failure in rats by uranyl nitrate injection

Uranyl nitrate (uranyl nitrate powder was dissolved in 0.9% NaCl injectable solution to make 0.5%), 1 ml/kg (5 mg/kg), was injected once via the tail vein of rat to induce ARF (Kim et al., 1996, 1998). The same volume of 0.9% NaCl injectable solution was injected to control rats. They were fed food (Cheiljedang Corporation, Seoul, South Korea) and water ad libitum. The plasma levels of urea nitrogen and creatinine increased significantly from third day to fifth day after intravenous administration of uranyl nitrate (Kim et al., 1996), therefore, the present rat experiments were performed on the fifth day.

2.4. Pretreatment of rats

For preliminary study, serum was collected for the measurement of urea nitrogen and creatinine concentrations, and the whole kidney and liver were excised, rinsed or perfused with 0.9% NaCl injectable solution, blotted dry with paper tissue, and weighed on the fifth day after injection of uranyl nitrate (rats with U-ARF, $n = 6$) or 0.9% NaCl injectable solution (control rats, $n = 6$).

In the early morning on the fifth day after injection of uranyl nitrate (rats with U-ARF) or 0.9% NaCl injectable solution (control rats) via the tail vein, the left femoral artery and the left femoral vein were cannulated with a polyethylene tube (PE 50, Clay Adams, Parsippany, NJ) under light ether anesthesia. The exposed areas were surgically sutured. Each rat was allowed 2 h to recover from anesthesia before the study began. Each rat was held in supine position during the entire pharmacokinetic studies by tying four feet on a plate.

2.5. Intravenous study

Pharmacokinetic study was carried out in a separate group of rats. Cyclosporine (Sandimmune® intravenous solution was diluted with 5% dextrose-in-water for injection to make a cyclosporine concentration of 30 mg/ml), 30 mg/kg was administered over 5-s via the femoral vein of

control rats ($n = 6$) and rats with U-ARF ($n = 6$). Total injection volume was 1.0 ml/kg. Blood samples (0.2 ml) were collected via the femoral artery into ethylenediamine tetraacetate tube (Vacutainer, Franklin Lakes, NJ) at 0 (to serve as a control), 2, 5, 10, 15, 30, 60, 90, 120 min, and 4, 8, 12, 24, 36, 48 h after intravenous administration. The same volume (0.2 ml) of fresh blood from other untreated rats was replaced just after each blood sampling. Approximately 0.2 ml of heparinized 0.9% NaCl injectable solution (50 U/ml) was used to flush the cannula after each blood sampling to prevent blood clotting. Blood samples were stored in the −20°C freezer until radioimmunoassay of cyclosporine (Cyclo-Trac®, 1995).

2.6. Oral study

The whole contents in Neoral® capsule were suspended in water for injection to make a cyclosporine concentration of 66.7 mg/ml. Cyclosporine, 100 mg/kg, was administered orally using a feeding tube (Solco Company, Seoul, South Korea) to control rats ($n = 5$) and rats with U-ARF ($n = 6$). Total oral volume was 1.5 ml/kg. Blood samples (0.20 ml) were collected at 0 (to serve as a control), 5, 15, 30, 45, 60, 90, and 120 min, and 4, 8, 12, 24, 36, and 48 h after oral administration. Other procedures were similar to those in the intravenous study.

2.7. Analysis of cyclosporine

The concentrations of cyclosporine in whole blood were measured by radioimmunoassay (Cyclo-Trac®, 1995) with Cyclo-Trac® SP-Whole Blood Kit (Incstar, Stillwater, MN). To a 200-μl aliquot of whole blood sample, an 800-μl aliquot of methanol was added. After vortex-mixing, the mixture was centrifuged at 2600 rpm for 5 min. To a 50-μl aliquot of the supernatant, a 100-μl aliquot of [^{125}I]Cyclo-Trac SP (Incstar) and a 1-ml aliquot of Anti-Cyclo-Trac SP Immuno Sep. (Incstar) were added. After vortex-mixing, the mixture was incubated for 1 h at 20–25°C. After centrifugation at 2600 rpm for 20 min, the radioactivity of cyclosporine in the precipitant was counted for 1 min using γ-scintillation counter (Packard, Cobra-Autogamma, Downers Glove, IL).

2.8. Measurement of urea nitrogen and creatinine concentrations in serum

The concentrations of urea nitrogen and creatinine in serum were measured by Hitachi 747 Automatic Analyzer (Tokyo, Japan).

2.9. Pharmacokinetic analysis

The total area under the whole blood concentration–time curve from time zero to time infinity (AUC) was calculated by the trapezoidal rule-extrapolation method (Kim et al., 1993); this method employed the logarithmic trapezoidal rule for the calculation of the area during the declining blood-level phase (Chiou, 1978) and the linear trapezoidal rule for the rising blood-level phase. The area from the last data point to time infinity was estimated by dividing the last measured blood concentration by the terminal rate constant.

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters; the time-averaged total body clearance (Cl), area under the first moment of the whole blood concentration–time curve (AUMC), mean residence time (MRT), and apparent volume of distribution at steady state (V_{ss}).

The mean values of Cl (Chiou, 1980), V_{ss} (Chiou, 1979), and terminal half-life (Eatman et al., 1977) were calculated by the harmonic mean method.

2.10. Statistical analysis

A P value of less than 0.05 was considered to be statistically significant using unpaired t-test. All data are expressed as mean ± standard deviation (S.D.).

3. Results and discussion

3.1. Induction of U-ARF in rats

In rats with U-ARF, impaired kidney function was observed; the serum levels of urea nitrogen (911% increase) and creatinine (923% increase) increased significantly, and kidney weight (0.783

vs. 0.716% of body weight) increased considerably ($P < 0.0703$) compared to control rats (Table 1). Similar results were also reported (Kim et al., 1996, 1998 and references therein). Note that body weight gain decreased significantly by pretreatment with uranyl nitrate (from 273 to 243 g), while it increased significantly in control rats (from 268 to 295 g) (Table 1).

3.2. Pharmacokinetics of cyclosporine after intravenous administration to control rats and rats with U-ARF

The whole blood concentration–time profiles of cyclosporine after intravenous administration of the drug, 30 mg/kg, to control rats and rats with U-ARF are shown in Fig. 1, and the relevant pharmacokinetic parameters are listed in Table 2. After intravenous administration, the mean arterial whole blood levels of cyclosporine declined rapidly for up to 4–6 h and declined slowly thereafter for all rats studied (Fig. 1). Some pharmacokinetic parameters of cyclosporine seemed to be changed in rats with U-ARF (Table 2). In rats with U-ARF, the V_{ss} of cyclosporine was significantly smaller (23% decrease) than that in control rats (Table 2). The smaller V_{ss} was also reported with diltiazem in rats with U-ARF, and this could be due to decreased free fraction of diltiazem in plasma (Lee et al., 1992). However, the volume of distribution of azosemide (Park et al., 1998), DA-1131 (Kim et al., 1998), and phenytoin (eliminated mainly by metabolism, Itoh et al., 1988) was larger in rats with U-ARF than those in control rats. The larger V_{ss} of azosemide and phenytoin in rats with U-ARF was due to increased free fraction of the drugs in plasma (serum), however, plasma protein binding change was not the main reason for DA-1131. In rats with U-ARF, the blood concentrations of cyclosporine tended to be higher (Fig. 1) and this resulted in a considerably greater AUC (18% increase, $P < 0.0524$) than that in control rats (Table 2). The considerably greater AUC of cyclosporine in rats with U-ARF was due to considerably slower Cl of cyclosporine (17% decrease, $P < 0.1156$, Table 2); this could be due to a slower metabolism of cyclosporine in rats with U-ARF. However, the change in Cl of cyclosporine was not dramatic in rats with U-ARF, and this could be due to the fact that hepatic dysfunction was not considerable in rats with U-ARF. It was reported that no significant findings were observed by liver microscopy (Kim et al., 1998) and plasma GOT and GPT levels increased significantly (Kim et al., 1996) in rats with U-ARF. It was reported (Shibata et al., 1993) that the Cl of cyclosporine also reduced after intravenous administration of the drug to rats with glycerol-induced acute renal failure: the Cl of cyclosporine was negatively related to erythrocyte-to-plasma distribution ratio (E/P). They concluded (Shibata et al., 1993) that an alteration in the E/P will be a valuable indication for predicting the Cl of cyclosporine at various disease states (CCl_4-induced acute hapatic failure rats, glycerol-induced acute renal failure rats, anemic rats, and aged rats). The Cl of propranolol (Terao and Shen, 1984), theophylline (Kim, 1991), amiodarone (Fruncillo et al., 1986), diltiazem (Lee et al., 1992), azosemide (Park et al., 1998), DA-125 (Kim et al., 1996), and adriamycin (Lee et al.,

Table 1
Mean ($\pm$ standard deviation, S.D.) physiological parameters in serum and organ weight of control rats and rats with uranyl nitrate-induced acute renal failure (U-ARF) measured on the fifth day after the tail vein injection of 0.9% NaCl injectable solution (control rats) or uranyl nitrate (rats with U-ARF)

Parameters	Control rats ($n = 6$)	Rats with U-ARF ($n = 6$)
Body weight (BW, g)[a]	268 ± 18.4	273 ± 13.1
Body weight (g)[b]	295 ± 15.9	243 ± 21.7*
Serum urea nitrogen (mg %)	35.5 ± 9.48	359 ± 94.8*
Serum creatinine (mg %)	0.880 ± 0.247	9.00 ± 2.72*
Kidney weight (% of BW)	0.716 ± 0.0416	0.783 ± 0.0872
Liver weight (% of BW)	3.28 ± 0.182	3.00 ± 0.257

[a] Measured just before the injection of 0.9% NaCl injectable solution (control rats) or uranyl nitrate (rats with U-ARF).
[b] Measured on the fifth day after injection of 0.9% NaCl injectable solution (control rats) or uranyl nitrate (rats with U-ARF).
* $P < 0.05$.

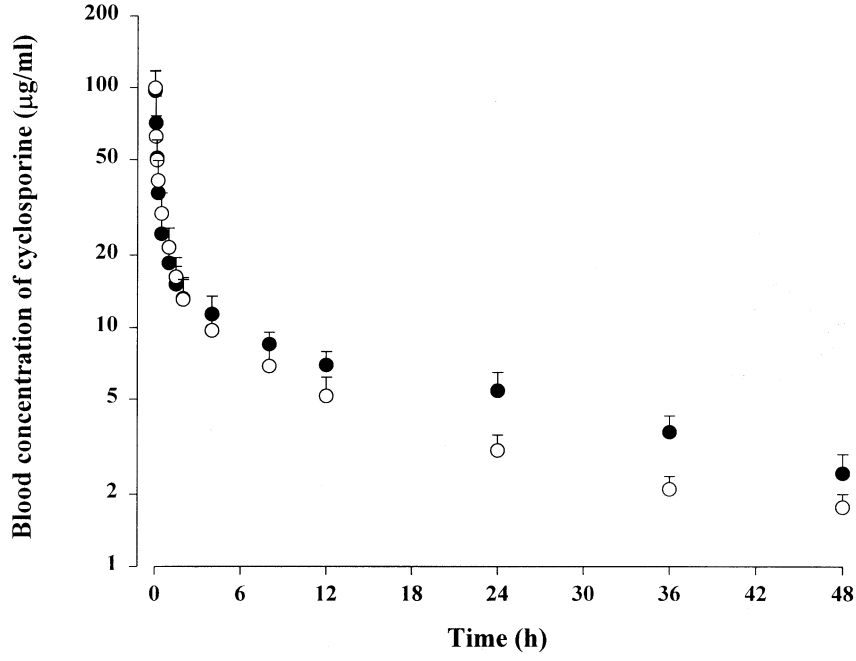

Fig. 1. Mean arterial whole blood concentration–time profiles of cyclosporine after intravenous administration of the drug, 30 mg/kg, to control rats (○, $n=6$) and rats with uranyl nitrate-induced acute renal failure (U-ARF, ●, $n=6$). Bars represent standard deviation (S.D.).

1996), which are eliminated mainly via metabolism, was also reported to be slower in rats with U-ARF than those in control rats.

3.3. Pharmacokinetics of cyclosporine after oral administration to control rats and rats with U-ARF

The whole blood concentration–time profiles of cyclosporine after oral administration of the drug, 100 mg/kg, to control rats and rats with U-ARF are shown in Fig. 2, and the relevant pharmacokinetic parameters are listed in Table 3. After oral administration, the mean whole blood levels of cyclosporine increased for up to 4 h and declined thereafter in a monoexponential fashion for all rats studied (Fig. 2). The pharmacokinetic parameters of cyclosporine listed in Table 3 were very similar (not significantly different) between control rats and rats with U-ARF suggesting that U-ARF did not considerably affect the pharmacokinetics of cyclosporine after oral administration.

The extent of absolute oral bioavailability (F) in control rats and rats with U-ARF at 100 mg/kg was estimated for comparison based on AUC values after intravenous and oral administration; the values were very similar, 23.8 and 23.4% for oral administration of the drug, to control rats and rats with U-ARF, respectively. The similar F

Table 2
Mean ($\pm$ standard deviation, S.D.) pharmacokinetic parameters of cyclosporine after intravenous administration of the drug, 30 mg/kg, to control rats and rats with uranyl nitrate-induced acute renal failure (rats with U-ARF)

Parameters	Control rats ($n=6$)	Rats with U-ARF ($n=6$)
Body weight (g)	271 ± 13.2	243 ± 21.7
Terminal half-life (h)	21.2 ± 3.76	17.5 ± 3.58
AUC (μg h/ml)	296 ± 38.7	348 ± 59.0
MRT (h)	26.6 ± 4.81	23.7 ± 5.58
Cl (l/h per kg)	0.102 ± 0.0146	0.0851 ± 0.0162
V_{ss} (l/kg)	2.56 ± 0.612	1.97 ± 0.299*

* $P<0.05$.

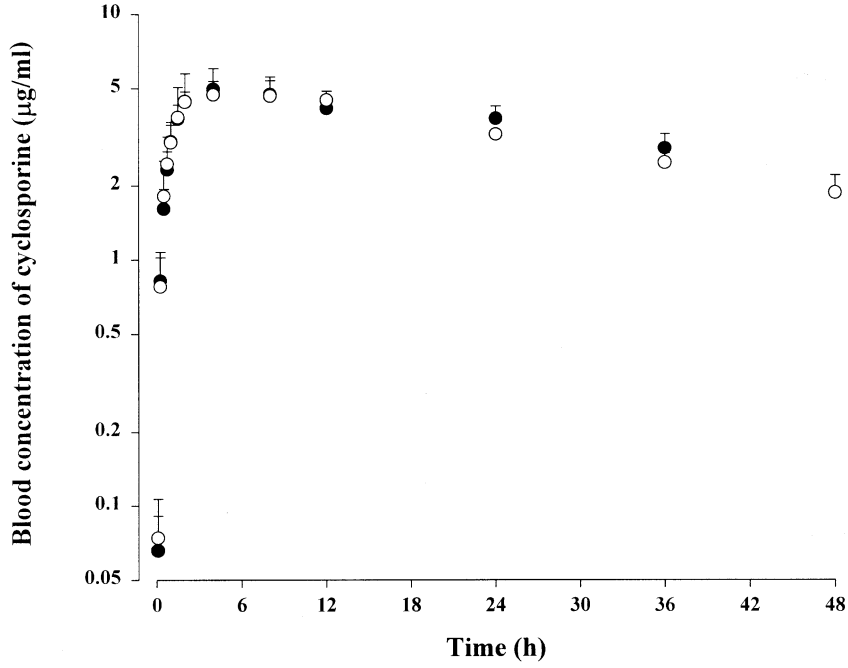

Fig. 2. Mean arterial whole blood concentration–time profiles of cyclosporine after oral administration of the drug, 100 mg/kg, to control rats (○, $n = 5$) and rats with uranyl nitrate-induced acute renal failure (U-ARF, ●, $n = 6$). Bars represent standard deviation (S.D.).

Table 3
Mean ($\pm$ standard deviation, S.D.) pharmacokinetic parameters of cyclosporine after oral administration of the drug, 100 mg/kg, to control rats and rats with uranyl nitrate-induced acute renal failure (rats with U-ARF)

Parameters	Control rats ($n = 5$)	Rats with U-ARF ($n = 6$)
Body weight (g)	256 ± 9.62	254 ± 7.52
Terminal half-life (h)	28.0 ± 3.10	30.3 ± 11.4
AUC (μg h/ml)	235 ± 49.0	271 ± 81.3
C_{max} (μg/ml)	5.25 ± 1.01	5.05 ± 0.492
t_{max} (h)	4.44 ± 2.19	5.33 ± 2.06

values, 24%, after intravenous, 6 mg/kg, and oral, 10 mg/kg, administration of cyclosporine (Hedayati et al., 1992), and approximately 30% after intravenous, 3 mg/kg, and oral, 10 and 30 mg/kg, administration of [^{3}H]cyclosporine (Wagner et al., 1987) to control rats have also been reported.

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