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Rapid communication

Dose-independent pharmacokinetics of clindamycin after intravenous and oral administration to rats: Contribution of gastric first-pass effect to low bioavailability

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Abstract

The pharmacokinetic parameters of clindamycin were evaluated after intravenous (at doses of 50, 100, and 200 mg/kg) and oral (at doses of 75, 150, and 300 mg/kg) administration of the drug to rats. The first-pass effect of clindamycin was also evaluated after intraportal, intragastric, and intraduodenal administration of the drug at a dose of 150 mg/kg to rats. After both intravenous and oral administration of clindamycin, the pharmacokinetic parameters of the drug were dose-independent. Hence, the extent of absolute oral bioavailability (F) was also independent of oral doses. After oral administration of clindamycin (150 mg/kg), 7.68% of oral dose was not absorbed up to 24 h and F value was 28.2%. The gastric first-pass effect of clindamycin was 60.7% of oral dose. The first-pass effects of clindamycin in the lung, heart, intestine, and liver were almost negligible, if any, in rats. The low F of clindamycin in rats was mainly due to considerable gastric first-pass effect. Clindamycin was stable in rat gastric juice and various buffer solutions having pHs ranging from 1 to 13. The plasma-to-blood cells partition ratio of clindmaycin was 7.59 in rat blood. The plasma protein binding of clindamycin in rats was 67.5%.

Keywords: Clindamycin; Dose-independent pharmacokinetics; Intravenous, oral, intraportal, intragastric, and intraduodenal administration; Gastric first-pass effect; Rats

1. Introduction

Clindamycin (7-chlorolincomycin), a semisynthetic derivate of lincomycin, has been extensively used in the therapy of obstetric and gynecologic infections over 20 years. It is more active than erythromycin or clarithromycin against anaerobic bacteria, especially *B. fragilis*. In humans, absorption of clindamycin is rapid and virtually complete (90%) following oral administration and widely distributed throughout the body (DeHaan et al., 1972). The major metabolites of clindamycin excreted in the urine and feces are clindamycin sulfoxide and *N*-demethylclindamycin (Gatti et al., 1998). After single intravenous (its phosphate salt) and oral (its hydrochloride salt) administration of clindamycin at a dose of 600 mg to 16 healthy volunteers, the time-averaged total body clearance (CL), terminal half-life, apparent volume of distribution at steady state (V_{ss}), and extent of absolute oral bioavailability (F) of the drug were 0.27 ± 0.06 l/h/kg, 2.1 ± 0.2 h, 0.79 ± 0.13 l/kg, and 53 ± 14%, respectively (Gatti et al., 1993). Although the pharmacokinetics of clindamycin in humans were published (Gatti et al., 1993, 1998, and references therein), the reason for the incomplete F seemed not to be studied. Hence, in the present study, the reason for the incomplete F of clindamycin was evaluated using rats as an animal model.

The purposes of this study are to report the (1) dose-independent pharmacokinetic parameters of clindamycin after intravenous infusion (at doses of 50, 100, and 200 mg/kg) and

Abbreviations: AUC, total area under the plasma concentration–time curve from time zero to time infinity; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; MRT, mean residence time; V_{ss}, apparent volume of distribution at steady state; F, extent of absolute oral bioavailability; C_{max}, peak plasma concentration; T_{max}, time to reach a C_{max}; $Ae_{0-24\,h}$, percent of dose excreted in 24 h urine; $GI_{24\,h}$, percent of dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h

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oral administration (at doses of 75, 150, and 300 mg/kg) of the drug to rats, and (2) first-pass (gastric, intestinal, and hepatic) effect of clindamycin after intravenous, intraportal, intragastric, and intraduodenal administration of the drug at a dose of 150 mg/kg to rats to find the reason for the incomplete F of clindamycin.

2. Materials and methods

2.1. Chemicals

Clindamycin phosphate-injectable solution (300 mg as clindamycin phosphate/2 ml ampoule) and tetramethylammonium chloride (TMA) were purchased from Samjin Pharmaceutical Company (Seoul, South Korea) and TCI (Tokyo, Japan), respectively. Propranolol (an internal standard of high-performance liquid chromatographic, HPLC, analysis of clindamycin) was a product from Sigma–Aldrich Corporation (St. Louis, MO). Various buffer solutions having pHs ranging from 1 to 13 were purchased from Shinyo Pure Chemicals (Osaka, Japan). Other chemicals were of reagent grade or HPLC grade.

2.2. Rats

Male Sprague–Dawley rats of 7–9 weeks of age (weighing 225–310 g) were purchased from Samtako Bio Korea (Osan, South Korea). Rats were maintained in a clean room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea) at a temperature of between $23 \pm 2\,^{\circ}C$ with 12 h light (07:00–19:00 h) and 12 h dark (19:00–07:00 h) cycles, and a relative humidity of $55 \pm 5\%$. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and with food (Sam Yang Company, Pyeongtaek, South Korea) and water ad libitum.

2.3. Intravenous study

In the early morning, the jugular vein (for drug administration) and the carotid artery (for blood sampling) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) while each rat was under light ether anesthesia (Kim et al., 1993). Both cannulae were exteriorized to the dorsal side of the neck, where each cannula was terminated with a long silastic tube (Dow Corning, Midland, MI). Both silastic tubes were inserted into a wire sheath to allow free movement of the rat. Each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from the anesthesia within 4–5 h before the study was begun. They were not restrained during the study.

Clindamycin phosphate-injectable solution (diluted in distilled water) at doses of 50 ($n = 7$), 100 ($n = 8$), and 200 ($n = 7$) mg/kg as clindamycin base was infused (total infusion volume of 2 ml/kg) over 1 min via the jugular vein of rats. An approximately 0.22 ml aliquot of blood sample was collected via the carotid artery at 0 (to serve as a control), 1 (at the end of the infusion), 5, 15, 30, 45, 60, 75, 90, 120, 150, 180, and 210 min after the start of the intravenous administration of clindamycin. Heparinized 0.9% NaCl-injectable solution (20 units/ml), 0.3 ml, was used to flush the cannula immediately after each blood sampling to prevent blood clotting. Blood samples were centrifuged immediately and a 100 μl aliquot of each plasma sample was stored in a $-70\,^{\circ}C$ freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of clindamycin. At the end of 24 h, each metabolic cage was rinsed with 15 ml of distilled water and the rinsings were combined with 0–24 h urine sample. After measuring the exact volume of combined urine sample, two 100 μl aliquots of the combined urine sample were stored in a $-70\,^{\circ}C$ freezer until HPLC analysis of clindamycin. At the same time (24 h), as much blood as possible was collected via the carotid artery and each rat was sacrificed through cervical dislocation. And then the entire gastrointestinal tract (including its contents and feces) of each rat was removed, transferred into a beaker that contained 100 ml of methanol (to facilitate the extraction of clindamycin), and cut into small pieces with scissors. After shaking manually and stirring with a glass rod for 1 min, two 100 μl aliquots of the supernatant were collected from each beaker and stored in a $-70\,^{\circ}C$ freezer until HPLC analysis of clindamycin.

2.4. Oral study

Clindamycin phosphate-injectable solution (the same solution that was used in the intravenous study) at doses of 75 ($n = 7$), 150 ($n = 6$), and 300 ($n = 7$) mg/kg as clindamycin base was administered orally (total oral volume of 5 ml/kg) to rats using a feeding tube. Blood sampling time schedules were 0, 5, 15, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min after oral administration of clindamycin. Other procedures were similar to those of the intravenous study.

2.5. Measurement of hepatic first-pass effect of clindamycin in rats

The carotid artery and the jugular vein of each rat were cannulated under light ether anesthesia (Kim et al., 1993). At the same time, the vein from the caecum was cannulated and the cannula was pushed forward about 4.0 cm toward the liver through the portal vein to minimize impaired blood flowing into the portal vein (Murakami et al., 2003). Clindamycin phosphate-injectable solution (the same solution that was used in the intravenous study) at a dose of 150 mg/kg as clindamycin base was infused (total infusion volume of 2 ml/kg) over 30 min into the jugular vein and the portal vein for intravenous ($n = 5$) and intraportal ($n = 4$) administration, respectively, after 4–5 h recovery from light ether anesthesia with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, Southnatick, MA). At the same time, an equal volume (2 ml/kg) of 0.9% NaCl-injectable solution was also infused over 30 min via the portal vein for intravenous study and via the jugular vein for intraportal study. An approximately 0.22 ml aliquot

of blood sample was collected via the carotid artery at 0, 15, 30 (at the end of the infusion), 31, 35, 45, 60, 75, 90, 120, 150, 180, 210, and 240 min after the start of the infusion of clindamycin. After centrifugation, a 100 μl aliquot of plasma sample was kept in a −70 °C freezer until the HPLC analysis of clindamycin.

2.6. Measurement of gastric and intestinal first-pass effects of clindamycin in rats

Rats were fasted overnight with free access to water. The carotid artery and the vein from the caecum of each rat were cannulated (Kim et al., 1993; Murakami et al., 2003). For intraportal infusion ($n = 4$), clindamycin phosphate-injectable solution (the same solution that was used in the intravenous study) as at a dose of 150 mg/kg as clindamycin base was infused (2 ml/kg) via the portal vein for 30 min, and 2 ml/kg of 0.9% NaCl-injectable solution was instilled into the stomach and duodenum, respectively, using a 23 gauge needle. For intraduodenal instillation ($n = 4$), 2 ml/kg of 0.9% NaCl-injectable solution was instilled into the stomach and infused via the portal vein for 30 min, respectively, and clindamycin at a dose of 150 mg/kg was instilled (2 ml/kg) into the duodenum. For intragastric instillation ($n = 5$), 2 ml/kg of 0.9% NaCl-solution was instilled into the duodenum and infused via the portal vein for 30 min, respectively, and clindamycin at a dose of 150 mg/kg was instilled (2 ml/kg) into the stomach. Other procedures were similar to those described to measure the hepatic first-pass effect of clindamycin.

2.7. Stability of clindamycin

The procedures were similar to the reported methods (Yu et al., 2003). Clindamycin phosphate stock solution in distilled water was spiked into each test tube that contained rat plasma, urine, and gastric juice (pH of 2.0), and various buffer solutions with pHs ranging from 1 to 13 to produce a clindamycin base concentration of 5 μg/ml. Each sample was incubated in a water-bath shaker kept at a temperature of 37 ± 2 °C and at a rate of 50 oscillations per min (opm) for 24 h, except for 4 h for rat gastric juice.

2.8. In vitro distribution kinetics of clindamycin between plasma and blood cells of rat blood

The procedures were similar to those reported previously (Lee et al., 1981). One millilitre of heparinized blood (freshly withdrawn via the carotid artery from seven unanesthetized rats; the rat blood was pooled together) was pipetted into each glass test tube (22 tubes for each concentration). The initial clindamycin concentrations in rat blood were 1, 5, and 10 μg/ml, respectively. Each sample was incubated in a water-bath shaker kept at a temperature of 37 ± 2 °C and at a rate of 50 opm. At 0, 1, 3, 5, 7, 10, 15, 30, 60, 90, and 120 min, blood sample was centrifuged and plasma sample was collected. Whole blood concentrations of clindamycin were also measured by adding 2 volumes of distilled water to facilitate the hemolysis and to increase the reproducibility of HPLC assay of clindamycin (Lee et al., 1981).

2.9. HPLC analysis of clindamycin

The concentrations of clindamycin in the above samples were analyzed using the reported HPLC method (Cho et al., 2005). A 100 μl aliquot of sample was deproteinized with a 20 μl aliquot of 10% trichloroacetic acid, and a 20 μl aliquot of distilled water that contained a 20 μg/ml of propranolol (an internal standard) was added. After centrifugation at 15,000 × g for 20 min, the supernatant was transferred to a new tube and a 25 μl aliquot of a 0.4 M sodium hydroxide solution was added, and then the mixture was extracted with 0.5 ml of dichloromethane. After vortex-mixing for 30 s and centrifugation at 15,000 × g for 10 min, the upper aqueous layer was discarded, and the organic layer was evaporated under a gentle stream of nitrogen gas at room temperature. The residue was reconstituted in a 100 μl aliquot of the mobile phase and a 50 μl aliquot was injected directly onto the reversed-phase (250 mm ℓ × 3.9 mm i.d.; particle size, 5 μm; Waters, Milford, MA) HPLC column. The mobile phase, 3.8 mM TMA:acetonitrile (40:60, v/v; adjusted pH to 3.5 with 85% phosphoric acid), was run at a flow rate of 1.0 ml/min, and the column effluent was monitored using an ultraviolet detector set at 204 nm at room temperature. The retention times of clindamycin and the internal standard (propranolol) were approximately 9.3 and 12.1 min, respectively. The detection limit of clindamycin in plasma and urine were 0.35 and 0.5 μg/ml, respectively. The coefficients of variation for intra- and inter-day were below 10.0%.

2.10. Pharmacokinetic analysis

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

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters using the noncompartment analysis (WinNonlin®; professional edition version 2.1, Pharsight, Mountain View, CA); the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, terminal half-life, first moment of AUC (AUMC), mean residence time (MRT), V_{ss}, and F. The F value was calculated based on the AUC after intravenous administration of clindamycin at a dose of 100 mg/kg, since the AUC values were dose-independent at three intravenous doses (Table 1). The peak plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were read directly from the experimental data.

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

Table 1
Pharmacokinetic parameters of clindamycin after intravenous administration of the drug at various doses to rats

Parameter	50 mg/kg ($n=7$)	100 mg/kg ($n=8$)	200 mg/kg ($n=7$)
Body weight (g)	284 ± 8.63	278 ± 6.18	279 ± 14.1
AUC[a] (μg min/ml)	1240 ± 160	2230 ± 207	4770 ± 343
Terminal half-life (min)	49.8 ± 8.13	54.2 ± 3.75	49.1 ± 3.40
MRT (min)	36.0 ± 6.98	35.7 ± 3.85	43.4 ± 4.21
CL (ml/min/kg)	40.0 ± 5.82	44.8 ± 4.14	41.9 ± 3.22
CL_R (ml/min/kg)	1.43 ± 0.201	1.79 ± 0.309	1.53 ± 0.805
CL_{NR} (ml/min/kg)	37.1 ± 5.86	41.1 ± 3.89	38.3 ± 2.35
V_{ss} (ml/kg)	1386 ± 474	1588 ± 207	1813 ± 129
$Ae_{0-24\,h}$ (% of dose)	3.63 ± 0.69	4.11 ± 0.63	4.25 ± 1.46
$GI_{24\,h}$ (% of dose)	7.33 ± 2.44	8.23 ± 4.13	6.42 ± 2.60

Each value represents the mean ± S.D.
[a] Dose-normalized (based on 50 mg/kg) values were compared when statistical analysis was performed.

2.11. Statistical analysis

A P-value of less than 0.05 was considered to be statistically significant using a t-test between the two means for the unpaired data, or a Duncan's multiple range test of Social Package of Statistical Sciences (SPSS) posteriori analysis of variance (ANOVA) among the three means for the unpaired data. All data are expressed as mean ± S.D.

3. Results

3.1. Pharmacokinetics of clindamycin after intravenous administration of the drug to rats

After intravenous administration of clindamycin at doses of 50, 100, and 200 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Fig. 1(A), and some relevant pharmacokinetic parameters are listed in Table 1. Note that the dose-normalized (based on 50 mg/kg) AUC values of clindamycin were comparable (not significantly different) among three doses studied. The slope between log AUC and log dose of clindamycin was close to 1.0 (the value was 0.995). Moreover, other pharmacokinetic parameters of clindamycin listed in Table 1 were also not significantly different among three intravenous doses studied, indicating that the pharmacokinetic parameters of clindamycin are independent of three intravenous doses studied.

3.2. Pharmacokinetics of clindamycin after oral administration of the drug to rats

After oral administration of clindamycin at doses of 75, 150, and 300 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Fig. 1(B), and some relevant pharmacokinetic parameters are listed in Table 2. After oral administration of clindamycin, the absorption of the drug from the rat gastrointestinal tract was rapid; clindamycin was detected in plasma from the first blood sampling time (5 min) and rapidly reached T_{max} (15 min) for all three oral doses studied. Note that the dose-normalized (based on 75 mg/kg) AUC values of clindamycin were also comparable

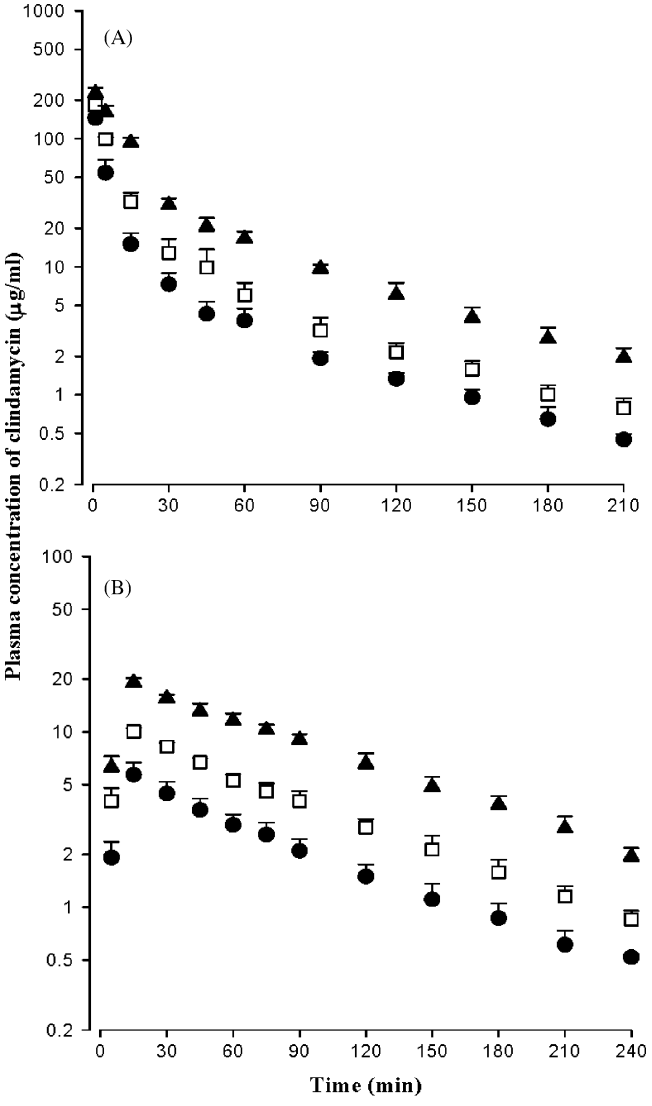

Fig. 1. Mean arterial plasma concentration–time profiles of clindamycin after 1 min intravenous infusion at doses of 50 (●; $n=7$), 100 (□; $n=8$), and 200 (▲; $n=7$) mg/kg (A), and oral administration at doses of 75 (●; $n=7$), 150 (□; $n=6$), and 300 (▲; $n=l$) mg/kg (B) to rats. Vertical bars represent S.D.

Table 2
Pharmacokinetic parameters of clindamycin after oral administration of the drug at various doses to rats

Parameter	75 mg/kg ($n=7$)	150 mg/kg ($n=6$)	300 mg/kg ($n=7$)
Body weight (g)	269 ± 33.3	244 ± 3.76	259 ± 7.76
AUC[a] (μg min/ml)	504 ± 77.2	944 ± 73.6	1990 ± 170
Terminal half-life (min)	64.8 ± 5.75	67.7 ± 6.70	63.9 ± 6.29
C_{max}[a] (μg/ml)	5.69 ± 0.985	9.80 ± 0.747	19.1 ± 1.16[b]
T_{max} (min)	15.0 ± 0.00	15.0 ± 0.00	15.0 ± 0.00
CL_R (ml/min/kg)	1.13 ± 1.10	1.26 ± 0.802	1.15 ± 0.517
$Ae_{0-24\,h}$ (% of dose)	1.06 ± 0.630	0.974 ± 0.387	0.814 ± 0.410
$GI_{24\,h}$ (% of dose)	13.7 ± 4.84	10.0 ± 3.27	9.18 ± 2.96
F (%)	30.1	28.2	29.7

Each value represents the mean ± S.D.
[a] Dose-normalized (based on 75 mg/kg) values were compared when statistical analysis was performed.
[b] 300 mg/kg was significantly different ($P<0.05$) from 75 mg/kg.

(not significantly different) among the three doses studied. The slope between log AUC and log dose of clindamycin was close to 1.0 (the value was 0.998). Hence, the F values were also independent of doses; the values were 30.1, 28.2, and 29.7% for oral doses of 75, 150, and 300 mg/kg, respectively. Moreover, other pharmacokinetic parameters of clindamycin listed in Table 2 were also not significantly different among three oral doses studied, except C_{max}, indicating that the pharmacokinetic parameters of clindamycin are also independent of three oral doses studied.

3.3. Measurement of hepatic first-pass effect of clindamycin in rats

After intravenous and intraportal administration of clindamycin at a dose of 150 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Fig. 2(A). The AUC of clindamycin after intraportal administration of the drug was comparable to that after intravenous administration of the drug (3740 ± 1150 and 4140 ± 1230 μg min/ml), suggesting that the hepatic first-pass effect of clindamycin after absorption into the portal vein was almost negligible, if any, in rats.

3.4. Measurement of gastric and intestinal first-pass effects of clindamycin in rats

After intraportal, intragastric, and intraduodenal administration of clindamycin at a dose of 150 mg/kg to rats, the mean arterial plasma concentration–time profiles of the drug are shown in Fig. 2(B). The AUC values of clindamycin after intraportal, intragastric, and intraduodenal administration of the drug were 3720 ± 905, 1350 ± 307, and 3150 ± 1010 μg min/ml, respectively. The AUC values were comparable between intraportal and intraduodenal administration, suggesting that intestinal first-pass effect of clindamycin is almost negligible, if any, in rats. However, the AUC after intragastric administration was considerably smaller (63.7% decrease) than that after intraportal administration, suggesting that the gastric first-pass effect of clindamycin was 63.7% in rats.

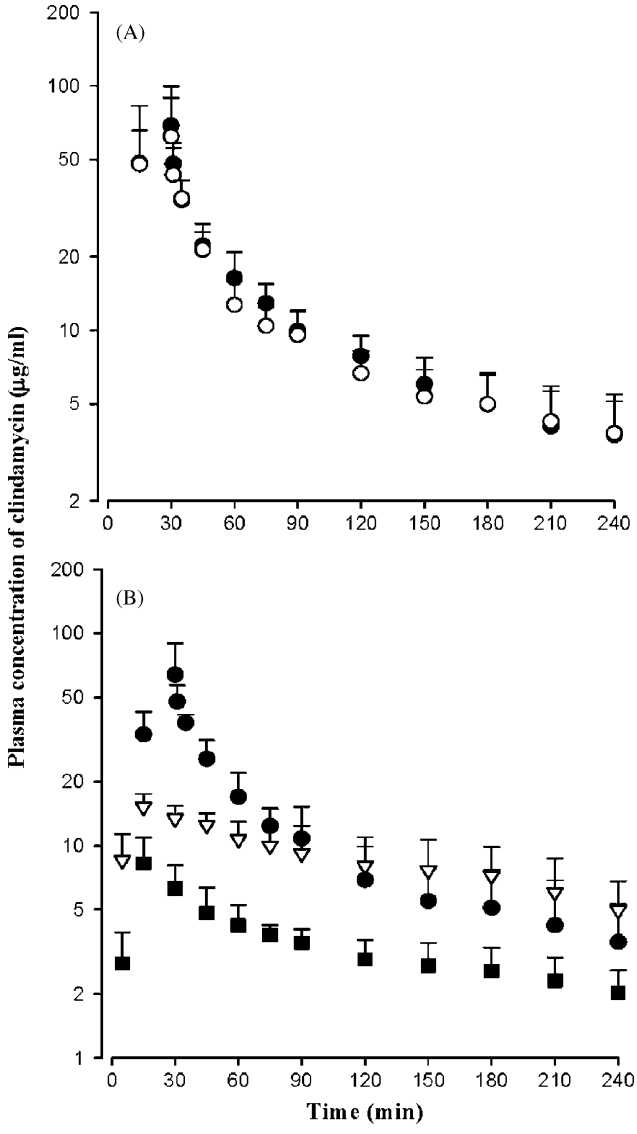

Fig. 2. Mean arterial plasma concentration–time profiles of clindamycin after intravenous (●; $n=5$) and intraportal (O; $n=4$) administration (A), and intraportal (●; $n=4$), intragastric (■; $n=5$), and intraduodenal (▽; $n=4$) administration (B) at a dose of 150 mg/kg to rats. Vertical bars represent S.D.

3.5. Stability of clindamycin

Clindamycin was stable up to 24 h incubation in rat plasma, urine, and various buffer solutions having pH ranging from 1 to 13 and up to a 4 h incubation in the gastric juice; more than 97.3% of the spiked amounts of clindamycin were recovered. The above data suggest that chemical and enzymatic (in rat gastric juice) degradation of clindamycin is almost negligible, if any, in rats. For the exact measurement of the amount of clindamycin remaining in the whole gastrointestinal tract (including its contents and feces) at 24 h as an unchanged drug ($GI_{24\,h}$) after intravenous (Table 1) and oral (Table 2) administration of the drug, the stability test is required.

3.6. In vitro distribution kinetics of clindamycin between plasma and blood cells of rat blood

The whole blood and plasma concentrations of clindamycin were almost constant up to 2 h incubation for initial blood clindamycin concentrations of 1–10 μg/ml (data not shown). This suggests that clindamycin reached equilibrium rapidly (within 30 s mixing manually) between plasma and blood cells of rat blood. The equilibrium plasma-to-blood cells partition ratios of clindamycin were independent of initial blood clindamycin concentrations ranging from 1 to 10 μg/ml; the mean value was 7.59 (range, 7.45–7.83).

4. Discussion

In pharmacokinetic studies, accurately measured plasma drug concentrations are usually assumed to be equal to their in vivo plasma concentrations. Such an assumption may be valid for drugs that have very rapid or instantaneous rate of equilibration between plasma and blood cells (Lee et al., 1981). If this equilibration process is slow or irregular, then the length of time elapsed between collection and centrifugation of a blood sample might have a profound effect on the measured drug concentration ("the blood storage effect"); the sooner the centrifugation, the higher will be the plasma concentration measured (Chiou, 1979b). This factor might have contributed in part to the 'reported' inconsistencies in the time to achieve the peak plasma level after intravenous administration, to the 'calculated' time-dependent renal clearances, and to the 'unsmooth' plasma level profiles reported in the literature (Chiou, 1979b). Moreover, it was reported that the bound fractions of adriamycin (Lee and Chiou, 1989a) and propranolol (Lee and Chiou, 1989b) to red blood cells act as barriers for their elimination. Hence, the experiments on the distribution kinetics of clindamycin between plasma and blood cells of rat blood were performed. The mean equilibrium plasma-to-blood cells partition ratios of clindamycin was 7.59, suggesting that the binding of clindamycin to rat blood cells was not considerable.

The CL_R of clindamycin was estimated as free (unbound to plasma protein) fractions of the drug in plasma based on the CL_R (Table 1) and rat plasma protein binding of the drug. The protein binding of clindamycin at 5 μg/ml to fresh rat plasma ($n = 4$) was 67.5 ± 2.66% using the reported equilibrium dialysis technique (Shim et al., 2000). The values thus estimated were 4.40, 5.51, and 4.71 ml/min/kg for intravenous doses of 50, 100, and 200 mg/kg, respectively. The 4.40–5.51 ml/min/kg were close to the reported glomerular filtration rate of 5.24 ml/min/kg in rats (Davies and Morris, 1993). The above data indicate that clindamycin is mainly excreted in urine via the glomerular filtration. Based on the CL_R of clindamycin (Table 1) and reported renal blood flow rate of 36.8 ml/min/kg (Davies and Morris, 1993) and hematocrit of approximately 45% (Mitruka and Rawnsley, 1981) in rats, the renal extraction ratio (CL_R of clindamycin/renal plasma flow rate; only for urinary excretion of unchanged clindamycin) of clindamycin were estimated. The values thus estimated were 7.07, 8.84, and 7.56% for 50, 100, and 200 mg/kg, respectively. The above data suggest that clindamycin is a poor renal extraction ratio drug in rats.

The AUC values of clindamycin were dose-independent after intravenous administration at doses of 50–200 mg/kg (Table 1) and oral administration at doses of 75–300 mg/kg (Table 2) to rats. Hence, the dose of 150 mg/kg was arbitrarily chosen to measure the first-pass effects of clindamycin in rats.

The F value of clindamycin was low; 28.2% at an oral dose of 150 mg/kg (Table 2). After oral administration of clindamycin at a dose of 150 mg/kg, the total amount of clindamycin recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h as an unchanged drug ($GI_{24\,h}$) was 10.0% of oral dose (Table 2). It is possible that this unchanged clindamycin, 10.0%, might be partly attributed to the gastrointestinal excretion of the absorbed drug. Based on the linear pharmacokinetics (Tables 1 and 2), the mean "true" fraction of oral dose unabsorbed (F_{unabs}) at a dose of 150 mg/kg in this study could be estimated by the following equation (Lee and Chiou, 1983):

$$0.1 = F_{unabs} + (0.282 \times 0.0823) \quad (1)$$

in which 0.282 and 0.0823 are the F and mean fraction of intravenous dose (100 mg/kg) of clindamycin recovered from the entire gastrointestinal tract at 24 h as an unchanged drug, $GI_{24\,h}$ (Table 1), respectively. The F_{unabs} thus calculated was 7.68%, indicating that the contribution of gastrointestinal excretion of the absorbed drug to the total drug recovered from the entire gastrointestinal tract at 24 h following oral administration at a dose of 150 mg/kg was almost negligible, 2.32%. Since 7.68% of orally administered clindamycin at a dose of 150 mg/kg was not absorbed from the gastrointestinal tract up to 24 h, 64.1% (100 − 7.68 − 28.2%) of orally administered clindamycin at a dose of 150 mg/kg could be eliminated by the first-pass effect.

After intravenous administration of clindamycin, the CL values of 40.0–44.8 ml/min/kg based on plasma data (Table 1) were considerably smaller than the reported cardiac output of 295 ml/min/kg in rats based on blood data (Davies and Morris, 1993) and hematocrit of approximately 45% in rats (Mitruka and Rawnsley, 1981). This suggests that the first-pass effects of clindamycin in the lung and heart could be almost negligible, if any, in rats.

After intraportal and intraduodenal administration of clindamycin at a dose of 150 mg/kg to rats, the AUC values of clindamycin were comparable between two routes of admin-

istration, suggesting that the intestinal first-pass effect of clindamycin was almost negligible, if any, in rats. However, the AUC after intragastric administration was 36.3% of that after intraportal administration, suggesting that the gastric first-pass effect of clindamycin could be 63.7% of oral dose in rats. The approximately 63.7% is equivalent to 60.7% of oral dose, considering the unabsorbed fraction, 7.68%. Therefore, it could be concluded that 31.6% (100 − 60.7 − 7.68%) of orally administered clindamycin at a dose of 150 mg/kg could be absorbed into the portal vein.

After intraportal administration of clindamycin at a dose of 150 mg/kg to rats, the AUC of clindamycin was comparable to that after intravenous administration at a dose of 150 mg/kg, suggesting that the hepatic first-pass effect of clindamycin was almost negligible, if any, in rats. Hence the 31.6% is close to the *F* of 28.2% at oral dose of 150 mg/kg (Table 2).

It was reported that based on human liver and intestinal microsomes, clindamycin was primarily oxidazed to form clindamycin sulfoxide (greater than 90% of the total clindamycin consumed) via the hepatic cytochrome P450 (CYP) 3A4 and *N*-demethylclindamycin was a minor metabolite in liver microsomes (Wynalda et al., 2003). It was also reported that based on Sprague–Dawley rat liver microsomes, clindamycin sulfoxide was the predominant product, and based on in vivo rat studies, 53% was identified as unchanged clindamycin and clindamycin sulfoxide and *N*-demethylclindamycin were 31 and 15%, respectively, in urine (Sun, 1973). More studies are required in rats why the gastric first-pass effect of clindamycin (what enzyme systems are involved for the mechanism of the drug in the stomach) is much greater than those of hepatic first-pass effect.

The considerable gastric first-pass effect of furosemide, 2-(allylthio)pyrazine (once synthesized as a new chemopreventive agent), YJA-20379-8 (a new reversible proton pump inhibitor), and KR-60436 (a new proton pump inhibitor) in rats, chlorpheniramine in rabbits, and ethanol in humans was reported (Yu et al., 2003 and references therein).

In conclusion, after oral administration of clindamycin at a dose of 150 mg/kg to rats, the unabsorbed fraction was approximately 7.68% of dose, *F* value was 28.2%, and gastric first-pass effect was approximately 60.7% of dose. The low *F* of clindamycin in rats was mainly due to considerable gastric first-pass effect.

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