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Pharmacokinetics of a new antitumor 3-arylisoquinoline derivative, CWJ-a-5

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Abstract

1-(4-Methylpiperazinyl)-3-phenylisoquinoline hydrochloride (CWJ-a-5) is newly developed from benzo[c]phenanthridine alkaloids and derivative and has exhibited potent antitumor activities, in vitro and in vivo. The pharmacokinetics of this novel antitumor 3-arylisoquinoline derivative was studied after intravenous (iv), oral (po) and hepatoportal (pv) administration in rats. A simple high performance liquid chromatographic method was developed to determine the concentrations of CWJ-a-5 in plasma, bile and urine. Plasma concentration profiles of CWJ-a-5 were best fitted by the two-compartment model after iv administration and showed a linear pharmacokinetic behavior up to 20 mg/kg doses. The half-life of CWJ-a-5 in the post-distributive phase ($t_{1/2\beta}$), total-body plasma clearance (CL_t), and volume of distribution at steady-state (Vd_{ss}) were 86.9 min, 5.72 l/h per kilogram and 9.79 l/kg, respectively, after iv administration of 10 mg/kg. Biliary and urinary excretion of CWJ-a-5 was $<1\%$ after iv injection of 10 mg/kg. The bioavailability of CWJ-a-5 after po and pv administration (50 and 10 mg/kg, respectively) was 52.9 and 72.2%, respectively. Gastrointestinal bioavailability was calculated to be 73.3%. The apparent partition coefficient (log P) of CWJ-a-5 between n-octanol and water was 2.64. Plasma protein binding of CWJ-a-5 measured by the ultrafiltration method was $>95\%$.

Keywords: Pharmacokinetics; Isoquinoline; Antitumor; CWJ-a-5; Preformulation

1. Introduction

Much attention has been focused on developing new cancer chemotherapeutic agents from natural products (Mackay et al., 1997), among which benzo[c]phenanthridine alkaloids have been documented to possess great antitumor activities

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(Janin et al., 1993). In our previous studies of antitumor agents related to the above alkaloids, it was found that a 3-arylisoquinoline derivative exhibited very strong antitumor activity against human tumor cell lines (Cho et al., 1996, 1997). Recently, a series of 3-arylisoquinoline derivatives was synthesized and the cytotoxicity against various human melanoma tumor cells was evaluated (Cho et al., 1997, 1998b, 1999). A three-dimensional quantitative structure–activity relationship was also investigated using the comparative molecular field analysis (CoMFA) (Cho et al., 1998a). Among the compounds tested, 1-(4-methylpiperazinyl)-3-phenylisoquinoline (CWJ-a-5, Fig. 1) exhibited potent antitumor activity against five human cancer cell lines, A 549 (lung), SKOV-3 (ovarian), SK-MEL-2 (melanoma), XF 498 (CNS) and HCT 15 (colon). Moreover, CWJ-a-5 in an in vivo assay in mice inoculated with various cancer cell lines showed a significant increase of survival period compared to the control (Cho et al., 1998b). The potent activity together with low toxicity (unpublished results) makes this compound a good candidate for clinical studies.

In order to develop CWJ-a-5 into a clinical agent, the pharmacokinetic behavior of this compound needs to be investigated. Methods as well as results of plasma concentration profiles of CWJ-a-5 after oral (po), hepatoportal venous (pv) and intravenous (iv) administration, and their absolute bioavailability will be described herein. Studies on the excretion of the parent chemical via urine and bile after iv administration, plasma protein binding and plasma-to-blood partition of CWJ-a-5 in vitro, and apparent partition coefficient between *n*-octanol and buffer solution of the compound will also be included in this preformulation study.

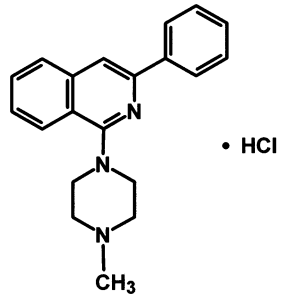

Fig. 1. The chemical structure of CWJ-a-5.

2. Materials and methods

2.1. Materials

The hydrochloride salt of CWJ-a-5 was synthesized in the Medicinal Chemistry Laboratory of Chonnam National University (Kwangju, South Korea) as previously described (Cho et al., 1998a,b, 1999). HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Solvents for HPLC were passed through a 0.22 μm filter and thoroughly degassed in an ultrasonic bath before use. All other reagents were analytical grade and used without further purification.

2.2. Administration of CWJ-a-5 via various routes

Male Sprague–Dawley rats (230–280 g) were used in all experiments. The rats were fixed in a supine position during the experiment. Under light ether anesthesia, femoral arteries of the rats were cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams) for blood sampling. For hepatoportal venous (pv) administration of CWJ-a-5, the pyloric vein was cannulated according to literature with slight modification (Suh et al., 1991). Briefly, the tip of an injection needle was bent 120° and attached to a PE-50 catheter which was connected to a 1-ml syringe. After the abdomen was opened through a midline incision, the tip of an injection needle was inserted into the hepatic portal vein through the pyloric vein. The needle was fixed with surgical glue (Aron Alpha, Sankyo Co., Tokyo) and then the incision was carefully sutured. CWJ-a-5 was injected through the syringe into the portal vein. Oral (po) administration was performed by inserting a round-tip needle, which was connected to a 1-ml syringe. Intravenous (iv) administration was performed with a 26-gauge needle via the tail vein.

After complete recovery (1 h) from the anesthesia, CWJ-a-5 was administered at doses of 5, 10

or 20 mg/kg for the iv study, 50 mg/kg for the po study and 10 mg/kg for the pv study. CWJ-a-5 was dissolved in sterilized physiological saline to yield appropriate concentrations. Blood samples (0.3 ml) were withdrawn from the femoral artery with PE-50 catheter prior to administration at appropriate time intervals for 180 min. Plasma samples were obtained by immediately centrifuging the blood samples at $7000 \times g$ for 5 min and stored at -20°C until analysis.

2.3. Excretion of CWJ-a-5 after intravenous administration

For the bile collection, the rat was placed in the supine position and the peritoneal cavity was exposed under light ether anesthesia. The common bile duct was cannulated using polyethylene tubing (PE-10), after which the incision was sutured. When the rat was fully recovered from the anesthesia and the flow of bile juice was constant, CWJ-a-5 solution was administered via the tail vein at a dose of 10 mg/kg.

For urine collection, CWJ-a-5 (10 mg/kg) was administered via the tail vein and the rat was placed individually in metabolic cages. Total volumes of bile and urine were measured after collecting for 18 and 24 h, respectively. Samples were immediately frozen at -20°C until analysis.

2.4. HPLC analysis

The plasma samples (0.1 ml) were added with equal volume of acetonitrile and vortexed for 2 min. The mixture was then centrifuged at $7000 \times g$ for 5 min. The supernatants were injected into the HPLC system for analysis.

The bile and urine samples (0.5 ml) were extracted with 3 ml of dichloromethane, mixed in a vortex mixer for 2 min and centrifuged for 10 min at 4000 rpm. The organic phases (2.5 ml) were transferred into cap tubes and were completely evaporated in a 60°C water bath. CWJ-a-5 was dissolved again in 0.3 ml of acetonitrile and was injected into HPLC.

Concentration of CWJ-a-5 was determined using a HPLC system equipped with a binary pump system (Gilson Model 305 and 306) and automatic injector (Gilson Model 234). A Gilson C18 SynChropak column (6.5 μm, 250×4.6 mm) was used for analysis at ambient temperature. The mobile phase consisted of methanol and 10 mM phosphate buffer (pH 8.0) solution (85:15 for plasma samples and 77:23 for bile and urine samples, respectively) at a flow rate of 1.0 ml/min. The variable wavelength ultraviolet detector (Gilson Model 118) was set at 254 nm. Injections of 20 μl were made for all solutions to be analyzed.

The recoveries of CWJ-a-5 from rat plasma (0.1–200 μg/ml), urine and bile (0.1–10 μg/ml) samples were determined. The values were compared with corresponding values of standards in water to provide the recovery values. Quadruplicate assays were carried out on the same samples at different times of the same day to determine intra-day variance. Inter-day variance was determined by assaying the spiked samples in quadruplicates on days 1, 8, 16 and 20 after spiking. Coefficients of variation (CVs) were calculated from these values.

2.5. Data analysis

The plasma concentration profiles of CWJ-a-5 after iv, po and pv administration were fitted to the conventional two-compartment model using the WinNonlin® program (Scientific Consulting, Inc., Cary, NC). The fractions of CWJ-a-5 transported (availability = 1 − extraction ratio) across the liver (F_H), and gastrointestinal tract (F_{GI}) were calculated from the following equations (Gillette and Pang, 1977; Sugiyama, 1985; Echizen and Eichelbaum, 1986) using the AUC data after various routes of administration:

$$AUC_{pv}Dose_{iv}/AUC_{iv}Dose_{pv} = F_H \quad (1)$$

$$AUC_{po}Dose_{pv}/AUC_{pv}Dose_{po} = F_{GI} \quad (2)$$

In this model, CWJ-a-5 was presumed not to be cleared in the lung and not to be subject to enterohepatic recirculation.

The fraction of intact CWJ-a-5 excreted in the urine and bile was calculated as the total amount of drug recovered in the urine or bile divided by the iv administered dose (10 mg/kg).

The differences between the pharmacokinetic parameters of CWJ-a-5 after iv administration of various doses were compared by ANOVA at the $P < 0.05$ level.

2.6. Stability of CWJ-a-5 in plasma

Plasma samples (1.0 ml) were spiked with CWJ-a-5 to make 20 μg/ml and were then placed in a 37°C shaking water bath. At predetermined time intervals (0, 2, 4, 6 and 8 h), 100 μl of plasma was withdrawn and the concentration of CWJ-a-5 was determined by HPLC. The initial concentration of CWJ-a-5 was considered as 100% and the remaining concentration of CWJ-a-5 versus time profiles was plotted to determine the degradation rate constants.

2.7. Solubility of CWJ-a-5 in various media

The solubilities of CWJ-a-5 in buffer solutions (pH 2.08 and 7.12) and in distilled water were measured at 25°C. An excess of the compound was added to each medium and mixed by vortexing. The solution was immersed in a shaking water bath at 25°C and allowed to equilibrate for 72 h. The saturated solutions were then filtered through Minisart RC 4 filters (0.45 μm, Sartorius, Germany). Concentrations of CWJ-a-5 were analyzed by HPLC after appropriate dilution.

2.8. Apparent partition coefficient

The partition coefficient of CWJ-a-5 between *n*-octanol and the buffer solution (pH 2.08 and 7.12) was determined according to the method of Dearden and Bresnen (1988). *n*-Octanol was mutually saturated with water or buffer solutions (pH 2.08 and 7.12) by gentle mechanical stirring for 12 h, after which each phase was separated. Methanolic solution of CWJ-a-5 (5 mg/ml) was placed in a glass tube (50 μl) and 2.5 ml of each saturated solvent was added to the tube after completely evaporating the methanol. After shaking the stoppered tube for 24 h at 20 inversions per minute, the phases were separated by centrifugation at 3000 rpm for 20 min. Concentration of CWJ-a-5 in each phase was determined by HPLC after appropriate dilution with methanol.

2.9. Plasma-to-blood partition

All procedures were carried out immediately after blood collection via heart puncture. To 1.0 ml of whole blood of rats, 10 μl of CWJ-a-5 solution (1, 2, 5, 10 and 20 mg/ml) was added and mixed in the heparinized Vacujet tubes (Green Cross Med. Co., Seoul, South Korea). The resultant concentrations of CWJ-a-5 in the whole blood were 10, 20, 50, 100 and 200 μg/ml. The mixtures were incubated at 37°C for 30 min. Plasma was separated by centrifuging the blood samples at $7000 \times g$ for 5 min and analyzed for CWJ-a-5 by HPLC, as described above.

2.10. Plasma protein binding

Plasma protein binding of CWJ-a-5 was determined by an ultrafiltration technique using a micropartition system, MPS-1 (Amicon Corp., Danvers, MA) (Katsuki et al., 1996). Plasma aliquots (1.0 ml) containing various concentrations of CWJ-a-5 (0.2, 0.5 and 1.0 mg/ml) were incubated for 30 min at 37°C. Then, the aliquots were ultrafiltered at $1000 \times g$ for 20 min at 4°C. The filtrates were then injected directly into a HPLC column. The fraction of drug unbound was determined by the following equation:

$$f_u = C_u / C_t \quad (3)$$

where f_u is the fraction of drug unbound in plasma and C_u and C_t are the unbound and total concentrations of the drug in plasma, respectively. In a control study using isotonic phosphate buffer (pH 7.4) solution, binding of CWJ-a-5 to the filter was insignificant.

3. Results

3.1. HPLC analysis of CWJ-a-5

Fig. 2(A) shows the typical HPLC chromatogram of the blank plasma. Fig. 2(B) shows the chromatogram of plasma containing 1.5 μg/ml of CWJ-a-5. The peak of CWJ-a-5 was detected at around 11.9 min and no discernible peaks were observed within the time frame in which CWJ-a-5

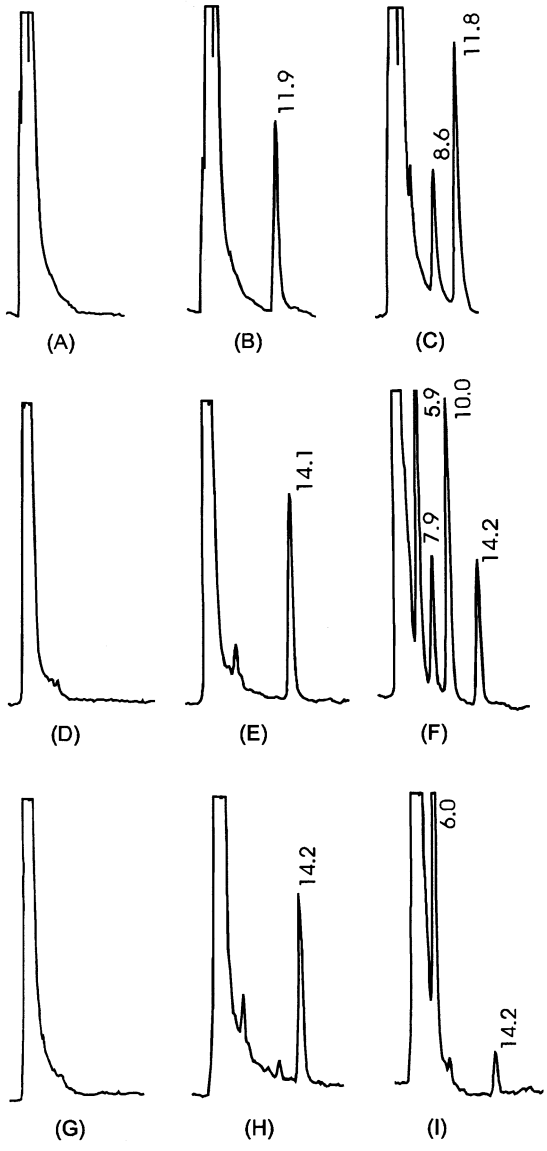

Fig. 2. The HPLC chromatograms of CWJ-a-5: (A) blank plasma; (B) plasma spiked with CWJ-a-5 (1.5 μg/ml); (C) plasma after 20 min of iv injection (20 mg/kg); (D) blank bile; (E) bile spiked with CWJ-a-5 (500 ng/ml); (F) bile after 18 h of iv injection (10 mg/kg); (G) blank urine; (H) urine spiked with CWJ-a-5 (500 ng/ml); and (I) urine after 24 h of iv injection (10 mg/kg).

was detected. A typical chromatogram of CWJ-a-5 in rat plasma at 20 min after iv administration (20 mg/kg) is shown in Fig. 2(C). CWJ-a-5 was appropriately separated from the other substances in the plasma. The peak detected at a retention time of 8.6 min represents an unknown metabolite of CWJ-a-5. The recovery of CWJ-a-5 from rat plasma was $>75\%$ and the detection limit was <0.1 μg/ml. The reproducibility of the method was determined by both intra- and inter-day variability. The intra-day variations of CWJ-a-5 at concentrations of 0.5, 1.0, 1.5 and 10 μg/ml were 4.88, 4.73, 3.05 and 2.92%, respectively. The inter-day variations at the same concentrations were 5.27, 6.21, 4.30 and 4.21%, respectively.

Fig. 2(D,G) show the chromatograms of blank bile and urine, respectively. Fig. 2(E,H) show the chromatograms of bile and urine containing 500 ng/ml of CWJ-a-5. CWJ-a-5 was detected at around 14.2 min without any significant interference. Typical chromatograms of CWJ-a-5 in bile and urine after iv administration (10 mg/kg) are shown in Fig. 2(F,I). CWJ-a-5 was appropriately separated from the other substances in the samples. The peaks detected at retention times of 5.9, 7.9 and 10.0 min represent the unknown metabolites of CWJ-a-5. The recovery of CWJ-a-5 from urine and bile was $>85\%$ and the detection limit was <0.1 μg/ml.

3.2. Pharmacokinetics of CWJ-a-5

Plasma concentration profiles of CWJ-a-5 after iv administration of CWJ-a-5 at different doses (5, 10 and 20 mg/kg) were plotted as a function of time in Fig. 3. Various pharmacokinetic parameters calculated by a two-compartment model using WinNonlin® program are summarized in Table 1. There was no significant difference in parameters among doses of 5–20 mg/kg, indicating that the pharmacokinetics of CWJ-a-5 are linear in the dose range examined. Mean values of CL_t, Vd_{ss} and $t_{1/2\beta}$ of CWJ-a-5 in the dose range were 4.95 (± 0.99) l/h per kilogram, 9.77 (± 0.42) l/kg and 98.78 (± 22.00) min, respectively. AUC proportionally increased as the iv dose increased up to 20 mg/kg with the correlation coefficient (r^2) of 0.983 (Fig. 4).

The plasma concentration profiles of CWJ-a-5 after iv and pv administration of 10 mg/kg and po administration of 50 mg/kg are shown in Fig. 5.

The relevant bioavailability parameters are summarized in Table 2. Bioavailabilities were calculated by comparing the AUC ratio of each route to that of the iv route.

The plasma concentration profile after pv administration was nearly parallel to that of iv administration. The terminal slopes of the curves following pv and po administration were identical to that of iv administration. The peak plasma concentration (C_{max}) of CWJ-a-5 after po administration of 50 mg/kg was 2.3 μg/ml and the time to reach the peak (T_{max}) was 48.4 min (Table 2). From the relationships shown by Eqs. (1) and (2), the availability of CWJ-a-5 across the liver (F_H) and GI tract (F_{GI}) were calculated to be 0.72 ($\pm$0.10) and 0.73 ($\pm$0.10), respectively.

3.3. Excretion of CWJ-a-5 after intravenous administration

The average amount ($\pm$S.E., $n=7$) of intact CWJ-a-5 excreted via bile and urine was 0.19 ($\pm$0.02)% and 0.12 ($\pm$0.02)%, respectively. No glucuronide metabolite of unchanged CWJ-a-5 was detected in the urine and bile samples, as determined indirectly by hydrolysis of urine and bile with β-glucuronidase (Hui et al., 1998) (data not shown).

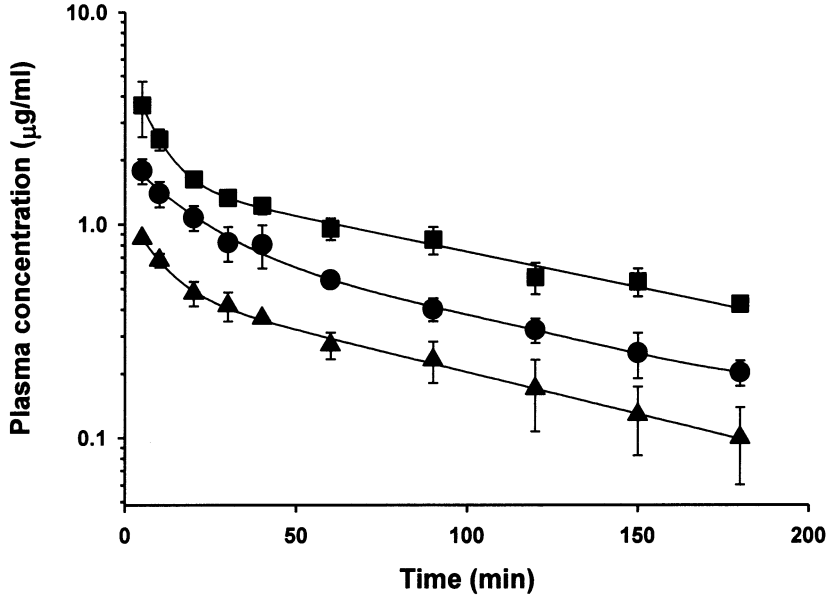

Fig. 3. Plasma concentration profiles of CWJ-a-5 after iv administration of ▲, 5; ●, 10; and ■, 20 mg/kg in rats. Each point represents the mean $\pm$ S.E. of four to six experiments.

Table 1
Pharmacokinetic parameters of CWJ-a-5 after intravenous administration of various doses to rats[a]

Parameters	5 mg/kg	10 mg/kg	20 mg/kg
$t_{1/2\beta}$ (min)	124.18 ($\pm$37.40)	86.91 ($\pm$18.55)	85.25 ($\pm$9.94)
Vd_{ss} (L/kg)	9.34 ($\pm$1.61)	9.79 ($\pm$1.95)	10.18 ($\pm$1.01)
CL_t (l/h/kg)	3.83 ($\pm$0.56)	5.72 ($\pm$1.15)	5.30 ($\pm$0.28)
AUC (μg·min/ml)	84.67 ($\pm$13.53)	115.39 ($\pm$16.18)	228.79 ($\pm$12.73)
MRT (min)	165.12 ($\pm$48.72)	112.03 ($\pm$26.12)	117.42 ($\pm$15.92)

[a] Each value represents the mean ($\pm$S.E.) of four to six experiments.

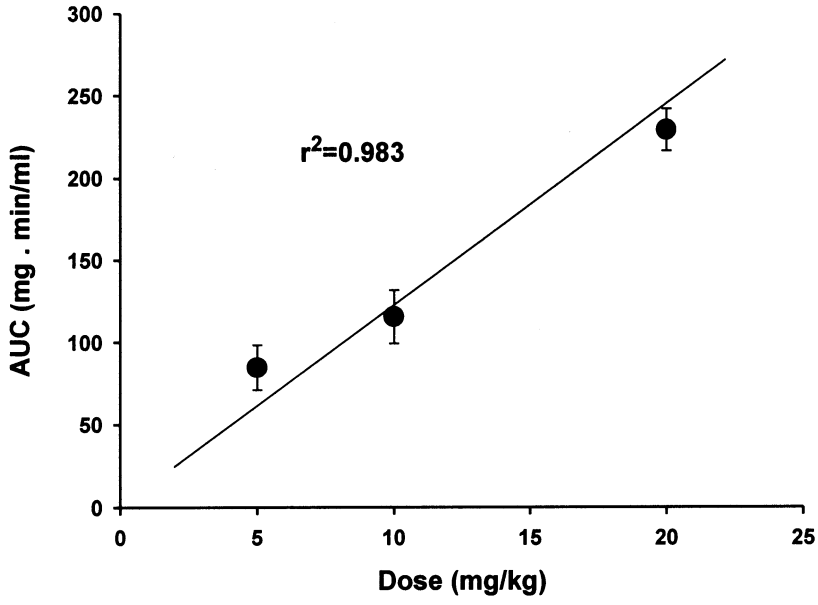

Fig. 4. Relationship between iv injection dose and area under the curve (AUC) value.

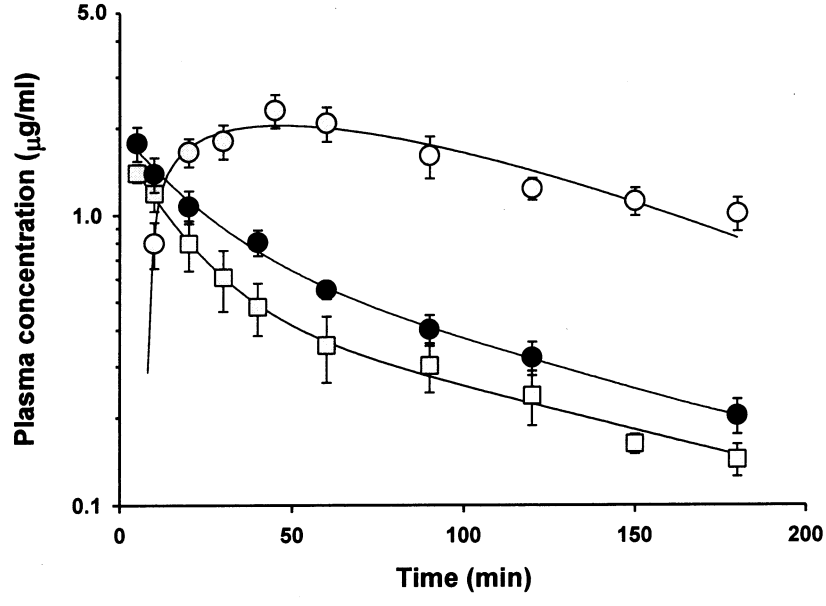

Fig. 5. Plasma concentration profiles of CWJ-a-5 after ●, iv; □, pv administration of 10 mg/kg; and ○, po administration of 50 mg/kg in rats. Each point represents the mean ± S.E. of four experiments.

3.4. Solubility and apparent partition coefficient

Table 3 shows the solubility of CWJ-a-5 in various media when equilibrated for 72 h at 25°C. CWJ-a-5 was most soluble in a pH 2.08 buffer solution. However, it was barely soluble in a pH

7.12 buffer. The partition coefficients (log P) of CWJ-a-5 between n-octanol and buffer solutions are also shown in Table 3. CWJ-a-5 was lipophilic with n-octanol/water partition coefficient of 2.64 ($\pm$0.02). A higher log P value and lower solubility at a higher pH suggest that a larger fraction of CWJ-a-5 exists in a free base form at pH 7.12, while a larger fraction exists in a hydrochloride salt form at pH 2.08. A high log P at the physiological pH also implies high tissue distribution and blood cell partitioning of CWJ-a-5 after administration.

3.5. Plasma-to-blood partition

CWJ-a-5 partitioning reached equilibrium within 30 min with negligible hemolysis during incubation. Mean ($\pm$S.D.) partition of CWJ-a-5 to red blood cells was constant over the blood concentration range of 10–200 μg/ml, i.e. 51.04 ($\pm$0.97), 49.91 ($\pm$0.89), 51.16 ($\pm$0.32), 50.59 ($\pm$1.40) and 50.29 ($\pm$0.36)% of CWJ-a-5 were found in the blood cells when determined three times at the blood concentrations of 10, 20, 50, 100 and 200 μg/ml, respectively. The average ratio of CWJ-a-5 present in the blood cells among total CWJ-a-5 was calculated to be 50.59 ($\pm$0.45)%.

Table 2
Bioavailability parameters of CWJ-a-5 after oral (po) and hepatoportal vein (pv) administration at a dose of 50 and 10 mg/kg, respectively[a]

Parameters	po	pv
C_{max} (μg/ml)	2.28 ($\pm$0.19)	–
T_{max} (min)	48.35 ($\pm$4.44)	–
MRT (min)	–	114.24 ($\pm$2.80)
AUC (μg·min/ml)	334.73 ($\pm$44.00)	91.33 ($\pm$13.04)
Bioavailability (%)	52.91 ($\pm$6.96)	72.19 ($\pm$10.31)

[a] Each value represents the mean ($\pm$S.E.) of four experiments.

Table 3
Solubility and apparent partition coefficient (log P) of CWJ-a-5 between n-octanol and various media at 25°C[a]

Medium	Solubility (mg/ml)	log P
pH 2.08 buffer	194.65 ($\pm$2.52)	1.31 ($\pm$0.09)
pH 7.12 buffer	0.10 ($\pm$0.03)	3.04 ($\pm$0.09)
Water	171.08 ($\pm$15.07)	2.64 ($\pm$0.02)

[a] Each value represents the mean ($\pm$S.D.) of three experiments.

3.6. Plasma protein binding

Plasma protein binding of CWJ-a-5 was determined by the ultrafiltration method. The bound fractions of CWJ-a-5 were calculated to be 99.43 ($\pm$0.14), 98.77 ($\pm$0.56) and 97.82 ($\pm$0.83)% for concentrations of 0.2, 0.5 and 1.0 mg/ml, respectively, when expressed as the mean ($\pm$S.D.) of three experiments. There was no concentration dependency in the binding and 97.31% of CWJ-a-5 was found to exist in the bound form up to 1.0 mg/ml concentration.

4. Discussion

The plasma concentration profiles of CWJ-a-5 after iv administration indicated a linear pharmacokinetics up to 20 mg/kg dose. The average CL_t (4.95 l/h per kilogram) of CWJ-a-5 was larger than the hepatic blood flow (2.9 l/h per kilogram, Jarugula et al., 1994) of the rat, suggesting a significant amount of extrahepatic elimination of this compound. However, CWJ-a-5 was very stable in rat plasma, since the degradation of this compound in blood at 37°C was almost negligible for at least 8 h (data not shown). Although the binding of CWJ-a-5 to plasma protein was very high (>95%), total body clearance was relatively large. Since the amount of parent CWJ-a-5 excreted via bile and urine was almost negligible (<1%), it is very likely that the unbound form of this compound is rapidly metabolized before excretion via the urine and bile. HPLC chromatograms indicated several unknown metabolite peaks in the bile and urine samples after iv administration of CWJ-a-5 (Fig. 2). However, hydrolysis of the urine and bile samples with β-glucuronidase (data not shown) indicated no glucuronide

metabolite of unchanged CWJ-a-5 present. The retention times of these peaks suggest they are more polar than the parent compound. Thus, further studies are necessary to verify whether they are hydroxy (Poat et al., 1978; Assandri et al., 1981; Mayr et al., 1998), *N*-desmethyl, *N*-oxide or carboxylic acid (Mutlib et al., 1996; Kassahun et al., 1997; Luffer-Atlas et al., 1997) metabolites, which are the known metabolite forms of isoquinoline and piperazine compounds.

The volume of distribution (Vd_{ss}) of CWJ-a-5 was substantially large (9.77 ± 0.42 l/kg), which suggests extensive distribution of CWJ-a-5 into the extravascular region. In spite of very large protein binding ($>95\%$) of CWJ-a-5, partitioning to red blood cells was $>50\%$. From the partition coefficient of CWJ-a-5 between *n*-octanol and pH 7.12 buffer solution ($\log P = 3.04$), it can also be speculated that large tissue distribution and partition to blood cells of CWJ-a-5 could be attributed to the high partition coefficient of CWJ-a-5 at the physiological pH. A tissue distribution study is under way in this laboratory to fully elucidate the reason for the large Vd_{ss}.

Bioavailability of pv CWJ-a-5 (72%) reflected extraction (28%) by the liver. The lower bioavailability after po administration (53%) than after pv administration may be attributed to the poor absorption and/or the loss of this compound during the absorption through the gastrointestinal tract. GI tract bioavailability of CWJ-a-5 calculated by Eq. (2) was 73%.

In summary, CWJ-a-5 is a promising drug candidate due to its potent activity and low toxicity. It is notable that the oral bioavailability of this compound is reasonably high, indicating the possibility of it being developed into an oral formulation. Also, these pharmacokinetic data should serve as useful information in future clinical studies of CWJ-a-5.

Acknowledgements

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References

Assandri, A., Perazzi, A., Martinelli, E., Ferrari, P., Ripamonti, A., Tuan, G., Galliani, G., 1981. Influence of metabolism on the activity of a new anti-fertility agent, 2-(3-ethoxyphenyl)-5,6-dihydro-*s*-triazolo[5,1-a]

× isoquinoline (DL 204-1T), in the rat and the hamster. Arzneimettelforschung 31, 2104–2111.

Cho, W.J., Yoo, S.J., Chung, B.H., Choi, B.G., Cheon, S.H., Whang, S.H., Kim, S.K., Kang, B.H., Lee, C.O., 1996. Synthesis of benzo[c]phenanthridine derivatives and their in vitro antitumor activities. Arch. Pharm. Res. 19, 321–325.

Cho, W.J., Yoo, S.J., Park, M.J., Chung, B.H., Lee, C.O., 1997. Synthesis and antitumor activity of 3-arylisoquinoline derivatives. Arch. Pharm. Res. 20, 264–268.

Cho, W.J., Kim, E.K., Park, M.J., Choi, S.U., Lee, C.O., Cheon, S.H., Choi, B.G., Chung, B.H., 1998a. Synthesis and comparative molecular field analysis (CoMFA) of antitumor 3-arylisoquinoline derivatives. Bioorg. Med. Chem. 6, 2449–2458.

Cho, W.J., Park, M.J., Chung, B.H., Lee, C.O., 1998b. Synthesis and biological evaluation of 3-arylisoquinolines as antitumor agents. Bioorg. Med. Chem. Lett. 8, 41–46.

Cho, W.J., Park, M.J., Imanishi, T., Chung, B.H., 1999. A novel synthesis of benzo[c]phenanthridine skeleton and biological evaluation of isoquinoline derivatives. Chem. Pharm. Bull. 47, 900–902.

Dearden, J.C., Bresnen, G.M., 1988. The measurement of partition coefficients. Quant. Struct. — Act. Relat. 7, 133–144.

Echizen, H., Eichelbaum, M., 1986. Clinical pharmacokinetics of verapamil, nifedipine and diltiazem. Clin. Pharmacokinet. 11, 425–449.

Gillette, J.R., Pang, K.S., 1977. Theoretical aspects of pharmacokinetic drug interaction. Clin. Pharmacol. Ther. 22, 623–639.

Hui, X., Hewitt, P.G., Poblete, N., Maibach, H.I., Shainhouse, J.Z., Wester, R.C., 1998. In vivo bioavailability and metabolism of topical diclofenac lotion in human volunteers. Pharm. Res. 15, 1589–1595.

Janin, Y.L., Croisy, A., Riou, J.F., Bisagni, E., 1993. Synthesis and evaluation of new 6-amino-substituted benzo[c]phenanthridine derivatives. J. Med. Chem. 36, 3686–3692.

Jarugula, V.R., Schinazi, R.F., Fulcrand, G., Kattan, Y.E., Liotta, D.C., Boudinot, F.D., 1994. Pharmacokinetics of 5-carboranyl-2′-deoxyuridine in rats. J. Pharm. Sci. 83, 1697–1699.

Kassahun, K., Mattiuz, E., Nyhart, E., Jr, Obermeyer, B., Gillespie, T., Murphy, A., Goodwin, R.M., Tupper, D., Callaghan, J.T., Lemberger, L., 1997. Disposition and biotransformation of the antipsychotic agent olansapine in humans. Drug Metab. Dispos. 25, 81–93.

Katsuki, H., Yagi, H., Arimori, K., Nakamura, C., Nakano, M., Katafuchi, S., Fujioka, Y., Fujiyama, S., 1996. Determination of R(+)- and S(−)-lansoprazole using chiral

stationary-phase liquid chromatography and their enantioselective pharmacokinetics in humans. Pharm. Res. 13, 611–615.

Luffer-Atlas, D., Vincent, S.H., Painter, S.K., Arison, B.H., Stearns, R.A., Chiu, S.H., 1997. Orally active inhibitors of human leukocyte elastase. III. Identification and characterization of metabolites of L-694 458 by liquid chromatography-tandem mass spectrometry. Drug Metab. Dispos. 25, 940–952.

Mackay, S.P., Meth-Cohn, O., Waich, R.D., 1997. In: Katritzky, A.R. (Ed.), Advances in Heterocyclic Chemistry, vol. 67. Academic Press, Orlando, FL, pp. 345–389.

Mayr, C.A., Sami, S.M., Remers, W.A., Dorr, R.T., 1998. Identification and characterization of in vitro metabolites of 2-[2′-(dimethylamino)ethyl]-1,2-dihydro-3H-dibenz[de,h]-isoquinoline-1,3-dione. Drug Metab. Dispos. 26, 105–109.

Mutlib, A.E., Jurcak, J., Hirb, N., 1996. Metabolism of an atypical antipsychotic agent, 3-[4-[4-(6-fluorobenzo[b]thien-3-yl)-1-piperazinyl]butyl]-2,5,5-trimethyl-4-thiazolidinone (HP236). Drug Metab. Dispos. 24, 1139–1150.

Poat, J.A., Woodruff, G.N., Watling, K.J., 1978. Direct effect of a nomifensine derivative on dopamine receptors. J. Pharm. Pharmacol. 30, 495–497.

Sugiyama, Y., 1985. Physiological pharmacokinetics. In: Hanano, M., Uremura, K., Iga, T. (Eds.), Applied Pharmacokinetics — Theory and Experiments. Soft Science, Tokyo, pp. 408–409.

Suh, N.J., Shim, C.K., Lee, M.H., Kim, S.K., Chang, I.M., 1991. Pharmacokinetic study of an iridoid glucoside: Aucubin. Pharm. Res. 8, 1059–1063.