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Pharmacokinetics of 11-Hydroxyaclacinomycin X (ID-6105), a Novel Anthracycline, after i.v. Bolus Multiple Administration in Rats

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We investigated the pharmacokinetics of 11-hydroxyaclacinomycin X (ID-6105), a novel anthracycline, after intravenous (i.v.) bolus administration at a multiple dose every 24 h for 5 days in rats. To analyze ID-6105 levels in biological samples, we used an HPLC-based method which was validated in a pharmacokinetic study by suitable criteria. The concentrations of ID-6105 after the multiple administration for 5 days were not significantly different from the results after the single administration. The $t_{1/2\alpha}$, $t_{1/2\beta}$, V_{dss}, and CL_t after the multiple administration were not significantly different from the values after the single administration. Moreover, the concentrations of ID-6105 1 min at day 1-5 after i.v. bolus multiple administration did not show the significant difference. Of the various tissues, ID-6105 mainly distributed to the kidney, lung, spleen, adrenal gland, and liver after i.v. bolus multiple administration. ID-6105 concentrations in the kidney or lung 2 h after i.v. bolus administration were comparable to the plasma concentration shortly after i.v. bolus administration. However, the ID-6105 concentrations in various tissues 48 h after i.v. bolus administration decreased to low levels. ID-6105 was excreted largely in the bile after i.v. bolus multiple administration at the dose of 3 mg/kg. The amounts of ID-6105 found in the bile by 12 h or in the urine by 48 h after the administration were calculated to be 14.1% or 4.55% of the initial dose, respectively, indicating that ID-6105 is mostly excreted in the bile. In conclusion, ID-6105 was rapidly cleared from the blood and transferred to tissues, suggesting that ID-6105 might not be accumulated in the blood following i.v. bolus multiple dosages of 3 mg/kg every 24 h for 5 days. By 48 h after i.v. bolus administration, ID-6105 concentrations in various tissues had decreased to very low levels. The majority of ID-6105 appears to be excreted in the bile.

Key words: 11-Hydroxyaclacinomycin X (ID-6105), Pharmacokinetics, Multiple administration, Tissue distribution, Excretion

INTRODUCTION

Anthracycline antibiotics such as daunorubicin, doxorubicin, aclacinomycin and retracenomycin are clinically and commercially important anticancer agents (Arcamone *et al.*, 1969; Oki *et al.*, 1979). Their cardio-toxic nature limits their therapeutic utility (Myers *et al.*, 1998). This detrimental side effect has led to an intensive search for new members of anthracycline either by screening of blocked mutants (Johdo *et al.*, 1991; Yoshimoto *et al.*, 1986; Nakagawa *et al.*, 1986) or by a hybrid biosynthetic approach (Hwang *et al.*, 1995; Niemi *et al.*, 1995). In the course of study on the biosynthesis, a new anthracycline antibiotic, designated as 11-hydroxyaclacinomycin X (ID-6105), was isolated from the culture broth of *Streptomyces galilaeus* ATCC 31133 (Hong *et al.*, 1994; Kim *et al.*, 1996a, 1996b). ID-6105 was identified as 7-(*O*-rhodosaminyl-deoxyfucosyl-rednosyl)-aklavinone (Fig. 1).

In an *in vitro* cytotoxicity study of ID-6105 in the human

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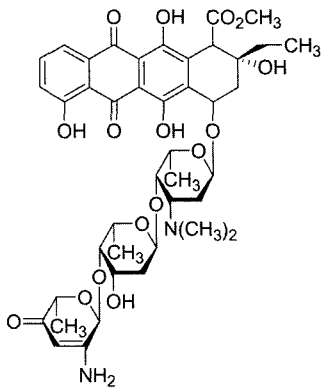

Fig. 1. Structural formula of 11-hydroxyaclacinomycin X (ID-6105)

cancer cell lines, this compound showed potent activity against MOLT-4 leukemia cells and SK-MEL-2 melanoma cells (Hong *et al.*, 1994; Kim *et al.*, 1996a, 1996b). In the preliminary studies of our laboratory, ID-6105 was more cytotoxic than doxorubicin and aclacinomycin A against 14 tumor cell lines originated from humans and mice. Furthermore, the *in vitro* efficacy of ID-6105 was superior to other conventional chemotherapeutic agents in biopsied gastric cancer cells. Also importantly, the cardiotoxicity of ID-6105 was remarkably lower than that of doxorubicin. This potent cytotoxicity and selectivity for human tumor cell lines suggests that ID-6105 may be a promising candidate for a novel anthracycline cancer chemotherapeutic agent (Hong *et al.*, 1994; Kim *et al.*, 1996a, 1996b).

The anti-tumor properties of ID-6101 are currently being investigated by clinical trials. However, accurate pharmacokinetic analyses of ID-6105 have not yet been reported. We, therefore, have investigated the pharmacokinetics of ID-6105 after intravenous (i.v.) bolus administration at the multiple administration every 24 h for 5 days to rats. ID-6105 kinetic parameters at both day 1 and day 5 after i.v. bolus multiple administrations were determined. Furthermore, the elimination of ID-6105 by the biliary and urinary systems and ID-6105 tissue distribution were determined. To analyze ID-6105 levels in biological samples, we used the high performance liquid chromatography (HPLC)-based method which has been developed and validated in our laboratory.

MATERIALS AND METHODS

Chemicals and reagents

11-Hydroxyaclacinomycin X (ID-6105) and aclacinomycin A were obtained from IL-Dong Pharmaceutical Co. Ltd. (Kyongki, Korea). HPLC grade solvents used in HPLC analysis were filtered and degassed just prior to use. All other chemicals used in this study were of an analytical reagent grade.

Animals

Adult male Sprague Dawley rats weighing 230-250 g (Sam Tac Co. Ltd., Kyunggi, Korea) were used for the pharmacokinetic studies. They were housed in individual metabolic cages during and after administration of ID-6105. The animals were maintained under a 12 h light/dark cycle with free access to water.

HPLC analysis of ID-6105 levels in biological samples

ID-6105 levels were assayed by reverse phase HPLC on a Kromasil C_{18} column (Eka Chemicals AB, 4.6 mm x 250 mm, 5 μm) that was interfaced with a Jasco HPLC system. This system consisted of a model PU-980 pump, a model AS-950-10 autoinjector, an UV-VIS detector, and a LC-Net II control borwin integrator (Jasco Co. Ltd., Japan). The mobile phase was a mixture of acetonitrile and doubly deionized water (38:62, v/v %). The flow rate was 1 mL/min. The ID-6105 in elutes was monitored fluorometrically at an excitation wavelength (λ_{ex}) of 250 nm and an emission wavelength (λ_{em}) of 550 nm.

The lower limit of quantification (LOQ) was defined as the lowest concentration yielding a precision of less than 20% (coefficients of variation, CV) and an accuracy of between 80 and 120% of the theoretical value. The linearity of the assay was assessed by preparing quality control samples containing ID-6105 at concentrations ranging from 0.02 to 100 μg/mL (0.02, 0.1, 0.2, 1, 10, 100 μg/mL) and then plotting the actual versus the measured concentrations. The ID-6105 samples were prepared in plasma, urine, bile, feces and liver homogenates. The resulting straight line regression equations were treated statistically (weighting factor: 1/concentration) and are presented with their correlation coefficients. The precision and accuracy of the method were determined by preparing quality control samples, five for each of the ID-6105 concentrations that range from 0.02 to 100 μg/mL, and then assaying these samples on the same day (repeatability) and on five consecutive days (reproducibility). The ID-6105 samples were prepared in plasma, urine, bile, feces and liver homogenates. ID-6105 at concentrations of 0.02, 1, 10, and 100 μg/mL was prepared in rat plasma, urine, bile, feces and liver homogenates, respectively, and then assayed 1, 2, 15, 18, 24, 48, and 72 h later.

Administration of ID-6105 and analysis of ID-6105 plasma levels

Under light pentobarbital sodium anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing (Intramedic, Clay Adams, U.S.A.) for ID-6105 administration and blood sampling, respectively. ID-6105 was administered into the femoral vein at a multiple dose of 3 mg/kg every 24 h for 5 days. Blood (200 μL) was

collected into heparinized tubes from the femoral artery 1, 2, 5, 15, 30, 60, 120, 240, 360, 480 and 720 min after i.v. bolus dose at day 1 or day 5. The blood samples were centrifuged for 15 min at 1500 g and the plasma was harvested. Immediately after the collection of the plasma samples, aclacinomycin-A (10 μL, 100 μg/mL) was added to each plasma test tube as an internal standard. Methanol (3 mL) was then added to precipitate the proteins and extract the compounds of interest. These mixtures were vortexed for 15 min and centrifuged for 15 min at 1500 g. The supernatants were withdrawn, dried under a stream of dry nitrogen and reconstituted in 150 μL mobile phase for quantitative HPLC analyses.

Analysis of biliary, urinary, and fecal excretion of ID-6105

Under light pentobarbital sodium anesthesia, the femoral vein was cannulated with PE-50 polyethylene tubing for IH-901 administration. A catheter (PE-10, Intramedic, Clay Adams, U.S.A.) was then implanted into the bile duct *via* a small abdominal incision. Blank bile was obtained just before the administration of 3 mg/kg ID-6105 into three sets of rats *via* the femoral vein. Bile was collected 0-1, 1-2, 2-4, 4-6, 6-8, 8-12, 12-24, and 24-48 h after the dose was administered at day 5. Urine or feces was collected with the use of metabolic cages over the 72 h after ID-6105 administration at day 5 and stored at -70 until analysis. The ID-6105 levels in the bile, urine, and feces were determined as described above.

Determination of the tissue distribution of ID-6105

The rats were decapitated 2, 8, 24, and 48 h after i.v. bolus administration of ID-6105 at day 5 (3 mg/kg), respectively. The liver, kidney, adrenal gland, stomach, small intestine, large intestine, spleen, heart, lung, trachea, thymus, brain, testis, muscle, and skin were immediately removed, blotted onto filter papers, and weighed. The tissues were minced in an ice-cold 50 mM tris-HCl buffer (containing 0.25 M sucrose, pH 7.4) and homogenized with a glass Potter-Elvehjem-type homogenizer with a Teflon pestle. After extracting 100 μL of 20% homogenate with 3 mL methanol, the concentration of ID-6105 in the supernatant was measured as described above.

Pharmacokinetic analysis

ID-6105 plasma concentration profiles after i.v. bolus administration were analyzed by fitting the data to the following biexponential equation according to the nonlinear least-squares method (MULTI) (Yamaoka *et al.*, 1981): $C_p = A \cdot \exp^{(-\alpha t)} + B \cdot \exp^{(-\beta t)}$. The pharmacokinetic parameters were subsequently calculated as follows: $k_{21}=(A\beta+B\alpha)/(A+B)$, $k_{el}=\alpha\beta/k_{21}$, $k_{12}=(\alpha+\beta)-(k_{21}+k_{el})$, $t_{1/2\alpha}= 0.693/\alpha$, and $t_{1/2\beta}=0.693/\beta$, where k_{12} and k_{21} represent the rate constants of transport between the central and peripheral compartments, respectively, k_{el} represents the elimination rate constant, and $t_{1/2\alpha}$ and $t_{1/2\beta}$ represent the plasma half lives at the α and β phases, respectively. Non-compartmental methods were also used to determine pharmacokinetic parameters. The area under the plasma concentration-time curve from time zero to infinity (AUC) was calculated from the equation $AUC = AUC_t + C_t/\beta$, where C_t is the last quantifiable concentration. The area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration (AUC_t) was calculated by linear trapezoidal approximation. The following parameters were also calculated by standard methods: steady-state volume of distribution (Vd_{ss}), the total plasma clearance (CL_t), and the mean residence time (MRT).

Statistical analysis

Two means were compared by the unpaired Student's t-test. One-way analysis of variance was used to test for significant differences between groups. Statistical significance was defined as $p < 0.05$.

RESULTS

Validation of the HPLC method

Fig. 2 illustrates typical chromatograms of ID-6105 and the internal standard (IS) in plasma. The blank sample shows no peaks that interfere with the ID-6105 and IS signals. For the plasma, the mean regression equations were y=0.911x-0.0152 (r^2=0.999), where y is the peak area ratio and x is the concentration. These equations show significant linearity (P<0.001) over the concentration

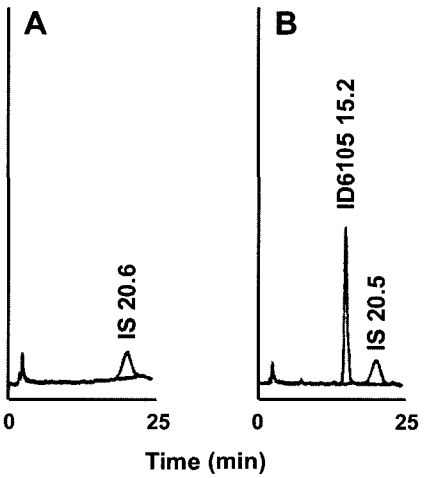

Fig. 2. Representative chromatograms of ID-6105 in plasma. A and B represents a blank sample and a biological sample (100 μL), respectively. Ten μL of 100 μg/mL aclacinomycin-A has been added as an internal standard (IS).

range of 0.02-100 μg/mL. The mean regression equations for bile, urine, feces, and tissue homogenates were not significantly different from the equation for plasma.

In between-day results, variations of both the precision and the accuracy never exceeded 15%. The same acceptance criteria were fulfilled for the within-day results, which demonstrate the repeatability of the method. The mean absolute recovery of ID-6105 in plasma was over 93.7%. ID-6105 in rat plasma, bile, urine, feces, and liver homogenate also proved to be stable over three days at 4 as no significant degradation was observed. Thus, the integrity of samples obtained at various time points and stored at 4 was demonstrated.

Pharmacokinetic characteristics of ID-6105 after i.v. bolus administration

Fig. 3 shows the concentrations of ID-6105 over time in rat plasma after i.v. bolus single administration or i.v. bolus multiple administration every 24 h for 5 days at a dose of 3 mg/kg. ID-6105 rapidly disappeared from the plasma by 15 min (α phase) after i.v. administration, followed by late disappearance in the β phase. The concentrations of ID-6105 after the multiple administration were not significantly different from the results after the single administration. The concentrations of ID-6105 1 min at day 1-5 after i.v. bolus multiple administration did not show the significant difference (Table I).

The pharmacokinetic parameters of ID-6105 after single or multiple administration are summarized in Table II. The mean plasma half-lives of ID-6105 at the α and β phases did not show the significant difference between the single and multiple administration. The $t_{1/2\alpha}$ and $t_{1/2\beta}$ after the single administration were 0.736 and 120 min, respectively.

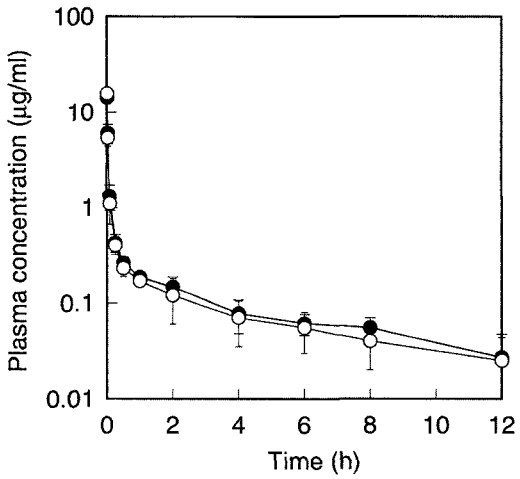

Fig. 3. ID-6105 concentration in rat plasma over time after i.v. bolus single administration at a dose of 3 mg/kg (○) or after i.v. bolus multiple administration at a dose of 3 mg/kg every 24 h for 5 days (●). Each point represents the mean ± S.E. of three rats.

Table I. Plasma concentration-time data of ID-6105 at 1 min after i.v. bolus administration at the multiple dose of 3 mg/kg every 24 h for 5 days in rats[a]

Day	Plasma concentration of ID-6105 (μg/mL)
1	14.8 ± 1.33
2	14.0 ± 0.928
3	13.9 ± 0.428
4	15.1 ± 0.565
5	14.1 ± 1.49

[a]Mean ± S.E. (n=3).

Table II. Pharmacokinetic parameters of ID-6105 following i.v. bolus administration at the multiple dose of 3 mg/kg every 24 h for 5 days in rats[a]

Parameter	Day 1	Day 5
A (μg/mL)	29.8 ± 2.36	32.7 ± 3.41
B (μg/mL)	0.312 ± 0.0362	0.338 ± 0.0240
α (min^{-1})	0.942 ± 0.0870	0.877 ± 0.174
β (min^{-1})	0.0058± 0.0004	0.0060± 0.0010
K_{12} (min^{-1})	0.580 ± 0.0820	0.507 ± 0.100
K_{21} (min^{-1})	0.016 ± 0.0046	0.015 ± 0.0010
K_{el} (min^{-1})	0.353 ± 0.0482	0.361 ± 0.0760
$T_{1/2\alpha}$ (min)	0.736 ± 0.0711	0.875 ± 0.145
$T_{1/2\beta}$ (min)	120 ± 9.47	118 ± 5.50
AUC (μg· min mL^{-1})	97.0 ± 6.83	102 ± 8.73
MRT (min)	189 ± 15.2	195 ± 18.3
Vdss (mL/kg)	5850 ±638	5870 ±798
CLt (mL/min)	30.9 ± 3.05	30.1 ± 2.71

[a]Mean ± S.E. (n=3).

The corresponding values after the multiple administration were 0.875 and 118 min, respectively. The pharmacokinetic parameters were also determined by non-compartmental methods. The steady-state volume of distribution (V_{dss}), the mean residence time (MRT), and the total plasma clearance (CL_t) after the multiple administration were not significantly different from the values after the single administration.

Biliary, urinary and fecal excretion of ID-6105 after i.v. administration to Rats

Fig. 4 shows the cumulative amount of ID-6105 excreted in the bile after i.v. bolus multiple administration every 24 h for 5 days at a dose of 3 mg/kg. ID-6105 was not detected in bile samples of 12-48 h after i.v. bolus administration. The cumulative amounts of ID-6105 excreted in the bile 12 h after administering 3 mg/kg were 0.421 mg/kg. This value represents 14.1% of the ID-6105 that was administered.

The urinary excretion of ID-6105 was maintained for up

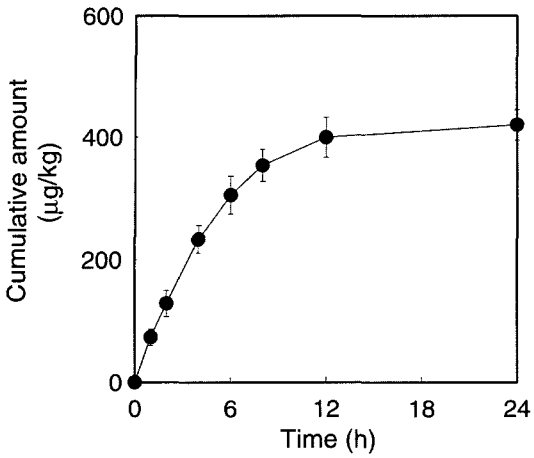

Fig. 4. Cumulative biliary excretion of ID-6105 after i.v. bolus multiple administration at a dose of 3 mg/kg every 24 h for 5 days. Each point represents the mean ± S.E. of three rats.

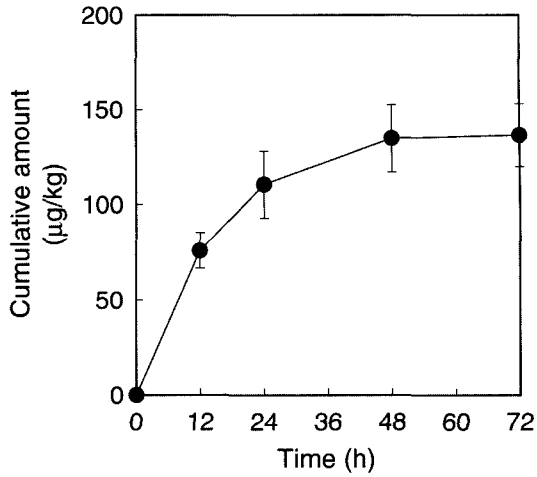

Fig. 5. Cumulative urinary excretion of ID-6105 after after i.v. bolus multiple administration at a dose of 3 mg/kg every 24 h for 5 days. Each point represents the mean ± S.E. of three rats.

to 48 h after i.v. bolus administration to rats at a dose of 3 mg/kg (Fig. 5). The cumulative amounts of ID-6105 excreted in the urine 48 h after administering 3 mg/kg were 136 mg/kg, representing 4.557% of the ID-6105 that was administered. These findings indicated that i.v. administered ID-6105 was mainly excreted in the bile. On the other hand, ID-6105 was not detected in the feces after i.v. bolus multiple administration for 5 days in rats at a dose of 3 mg/kg.

Tissue distribution of ID-6105 in rats

The distribution of ID-6105 in various tissues 2, 8, 24, and 48 h after i.v. bolus multiple administration of 3 mg/kg is summarized in Table III. ID-6105 mainly went to the kidney, lung, spleen, adrenal gland, and liver after i.v. bolus multiple administration. Moreover, the ID-6105 concentration in the kidney or lung 2 h after i.v. bolus administration was about 18 and 24 μg/g tissue, which is comparable to the concentration in the plasma shortly after i.v. bolus administration of 3 mg/kg (Fig. 3). The ID-6105 con-

Table III. Tissue distribution of ID-6105 at 5 days after i.v. bolus administration at the multiple dose of 3 mg/kg every 24 h for 5 days in rats [a]

Tissue	Day 5			
	2 h	8 h	24 h	48 h
Liver	10.5 ± 1.68	1.91 ± 0.198	0.42 ± 0.120	0.42 ± 0.076
Stomach	8.19 ± 0.750	3.65 ± 0.144	2.11 ± 0.273	1.38 ± 0.147
Adrenal gland	18.4 ± 1.46	6.82 ± 1.87	2.26 ± 0.440	1.56 ± 0.263
Spleen	17.3 ± 3.29	15.8 ± 1.62	9.57 ± 1.04	5.15 ± 0.454
Kidney	24.1 ± 3.96	6.10 ± 0.322	2.26 ± 0.169	1.32 ± 0.184
Small intestine	7.67 ± 0.632	3.74 ± 0.758	1.56 ± 0.083	0.71 ± 0.104
Large intestine	2.70 ± 0.332	4.23 ± 0.693	2.19 ± 0.359	1.11 ± 0.237
Testis	0.23 ± 0.024	0.22 ± 0.125	0.11 ± 0.066	0.07 ± 0.070
Thymus	2.81 ± 0.582	3.44 ± 0.746	4.14 ± 0.649	1.16 ± 0.690
Trachea	3.19 ± 0.390	1.78 ± 0.251	1.65 ± 0.349	1.18 ± 0.140
Lung	18.3 ± 2.26	5.58 ± 1.44	2.27 ± 0.209	1.35 ± 0.097
Heart	6.46 ± 0.543	2.64 ± 0.708	0.85 ± 0.136	0.22 ± 0.115
Muscle	1.59 ± 0.208	1.40 ± 0.190	0.60 ± 0.065	0.30 ± 0.152
Skin	1.86 ± 0.431	1.55 ± 0.049	0.75 ± 0.086	0.29 ± 0.156
Brain	N.D.[b]	N.D.	N.D.	N.D.

[a]ig/g Tissue (Mean ± S.E., n=3).
[b]N.D.: Not detected (Below the Quantifiable Limit).

centrations in various tissues 48 h after i.v. bolus administration were below 2 μg/g tissue except spleen. The ID-6105 concentration in the testis was below 0.1 μg/g tissue, while ID-6105 was not detected in the brain.

DISCUSSION

The anti-tumor properties of ID-6105 are currently being investigated in clinical trials by a number of pharmaceutical companies and thus, determination of its pharmacokinetic characteristics will be highly useful. Consequently, we investigated the pharmacokinetics of ID-6105 after i.v. bolus multiple administration every 24 h for 5 days at a dose of 3 mg/kg. The plasma concentration of ID-6105 decreased to below the quantifiable limit at 12 h after i.v. bolus administration, and was simulated by the two-compartment model to achieve the best fit (Fig. 3, Table II). The concentrations of ID-6105 after the multiple administration were not significantly different from the results after the single administration (Fig. 3). The $t_{1/2\alpha}$, $t_{1/2\beta}$, V_{dss}, and CL_t after the multiple administration were not significantly different from the values after the single administration. Moreover, the concentrations of ID-6105 1 min at day 1-5 after i.v. bolus multiple administration did not show the significant difference (Table I). These findings indicated that ID-6105 might not be accumulated in the blood following i.v. bolus multiple dosages of 3 mg/kg every 24 h for 5 days. Over 99 % of ID-6105 in the plasma was estimated to be disappeared by 10 h (approximate 5 times of half life) after i.v. bolus administration. Thus, most of ID-6105 appears to be cleared before the next administration of ID-6105 every 24 h.

It has been reported that aclacinomycin A was rapidly cleared from the blood and transferred to tissues. But, low levels of the drug remained in the blood 10 h after its i.v. administration (Iguchi *et al.*, 1981a, 1981b). Tissue levels of aclacinomycin A given to mice were highest in the lungs and spleen. Higher distribution was also observed in the liver and kidney 2 h after administration of aclacinomycin A. In the present study, ID-6105 mainly distributed to the kidney and lung after i.v. bolus administration as was observed with aclacinimycin A (Table III). ID-6105 concentrations in the kidney or lung 2 h after i.v. bolus administration were comparable to the plasma concentration shortly after i.v. bolus administration (Fig. 3). The ID-6105 concentrations in various tissues 48 h after i.v. bolus administration decreased to low levels (Table III).

ID-6105 was excreted largely in the bile after i.v. bolus multiple administration at the dose of 3 mg/kg. The amounts of ID-6105 found in the bile by 12 h or in the urine by 48 h after the administration were calculated to be 14.1% or 4.55% of the initial dose, respectively (Fig. 4, 5), which were similar to values for aclacinomycin A (Iguchi *et al.*, 1981b). These results indicated that ID-6105 is mostly excreted in the bile. However, the mechanism by which ID-6105 is selectively excreted into the bile requires further study.

In conclusion, ID-6105 was rapidly cleared from the blood and transferred to tissues. The pharmacokinetic parameters such as $t_{1/2\alpha}$, $t_{1/2\beta}$, V_{dss}, and CL_t after the multiple administration were not significantly different from the values after the single administration, suggesting that ID-6105 might not be accumulated in the blood following i.v. bolus multiple dosages of 3 mg/kg every 24 h for 5 days. By 48 h after i.v. bolus administration, ID-6105 concentrations in various tissues had decreased to very low levels. The majority of ID-6105 appears to be excreted in the bile.

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