Cancer Chemother Pharmacol (1990) 27: 20–26

Cancer Chemotherapy and Pharmacology

Prediction of ACNU plasma concentration-time profiles in humans by animal scale-up

Yoshihiro Mitsuhashi[1], Yuichi Sugiyama[2], Shogo Ozawa[3,*], Takashi Nitanai[4], Kunihiro Sasahara[4], Kan-Ichi Nakamura[5], Minoru Tanaka[5], Takuzo Nishimura[1], Makoto Inaba[3], and Tomowo Kobayashi[1]

[1] Bioscience Research Laboratories, [4] Product Development Laboratories, [5] Analytical and Metabolic Research Laboratories, Sankyo Co., Ltd., 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo 140, Japan

[2] Faculty of Pharmaceutical Sciences, University of Tokyo, 3-1, Bunkyo-ku 7-chome, Tokyo 113, Japan

[3] Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 37-1, Kami-Ikebukuro 1-chome, Toshima-ku, Tokyo 170, Japan

Received 12 March 1990/Accepted 6 June 1990

Summary. Plasma concentration-time profiles of nimustine hydrochloride, 1-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU), in the mouse, rat, rabbit, and dog were determined by high-performance liquid chromatographic analysis. The pharmacokinetic parameters for these four animal species and previously reported clinical data were analyzed for investigation of interspecies correlation. Log-log plots of body weight (W; kg) vs total plasma clearance (CLtot,p; ml/min) and steady-state distribution volume (Vd, ss; l) for the four animal species were linear, with high correlation coefficients (r 0.996 for both parameters), despite the fact that the nonrenal clearance was >97% in these species. Linear regression on the plots excluding human data yielded allometric equations ($CL_{tot,p} = 50.6\ W^{0.957}$; $Vd,ss = 1.29\ W^{1.03}$) that were extrapolated to predict ACNU pharmacokinetic parameters in humans. For both parameters, however, there were 3-fold differences between the predicted and observed parametric values. To investigate these discrepancies, we measured serum protein binding of ACNU in these animal species and in humans. The values of CLtot,p and Vd,ss were converted into those of $CL^{u}_{tot,p}$ and $Vd,^{u}_{ss}$, which correspond to the parameters for unbound ACNU. In this case, correlation coefficients of the log-log plots excluding human data ($CL^{u}_{tot,p} = 71.7\ W^{0.891}$; $Vd,^{u}_{ss} = 1.82\ W^{0.966}$) were also high ($r \geqq 0.991$). The extrapolated values vs those observed in a 70-kg human were the following: $CL^{u}_{tot,p}$, 3,160 vs 2,290 ml/min; $Vd,^{u}_{ss}$, 110 vs 106 l. Thus, the animal data were successfully extrapolated to yield better predictions of human pharmacokinetic parameters if the analysis was based on the unbound plasma concentration of ACNU. In addition, the predicted plasma concentration-time profile for humans also showed good agreement with the observed ones. These results suggest the importance of measuring unbound fractions of drugs for more accurate prediction of human pharmacokinetic parameters by extrapolation of animal data to the human situation.

Introduction

Nimustine hydrochloride (ACNU), a water-soluble nitrosourea, is an alkylating anticancer agent currently used for treatment of chronic myelocytic leukemia, malignant lymphoma, brain tumors, and small-cell lung carcinoma [4, 25]. Pharmacokinetic and metabolic studies of ACNU in the mouse and rat have been carried out by a method using [^{14}C] ACNU with thin-layer chromatographic (TLC) separation [14, 15, 22].

We have done additional studies on the pharmacokinetics of ACNU in the mouse, rat, rabbit, and dog by the same HPLC procedure used in clinical studies [6]. Using the data obtained from these four species, we set out to predict the pharmacokinetics of ACNU in humans by means of animal scale-up.

The study of interspecies anatomic and physiological variation as a function of body or organ weight is known as allometry [1]. Animal scale-up is an allometric interspecies scaling technique using pharmacokinetic data [5]. This technique has been extended and reviewed by Boxenbaum and others [1, 3, 12, 18]. For example, the application of this method to an anesthetic drug, phencyclidine [16], and an anticancer agent, acivicin [11], has recently been reported.

In some interspecies scaling studies, data on the total plasma concentration of drugs were used for allometric relationships because of either negligible binding of drugs to plasma (or serum) proteins or little difference in the protein binding among species. However, Sawada et al. [19–21] stressed the importance of using the pharmacokinetic parameters that correspond to the unbound drug concentration. Consistent with the results of their studies, the

* *Present address:* Department of Pharmacology School of Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160, Japan

Offprint requests to: Yoshihiro Mitsuhashi

present study of ACNU revealed that data analysis based on total plasma concentration was inadequate for accurate prediction of human pharmacokinetics, even if the level of protein binding was not high, and that the prediction of human pharmacokinetics was remarkably improved by use of the unbound plasma concentration of ACNU instead of the total plasma concentration.

The prediction of the drug-plasma concentration profile in humans by animal scale-up is of particular importance for anticancer agents, because plasma pharmacokinetics is closely related to clinical efficacy and anticancer agents virtually always have narrow therapeutic ratios due to their low selective toxicities. The successful prediction of pharmacokinetics in humans based on animal data could reduce the number of patients in phase I clinical trials and would enable evaluation of the efficacy of anticancer agents in man by treatment of human-tumor-bearing nude mice at doses pharmacokinetically equivalent to clinical doses.

Materials and methods

Chemicals

ACNU was synthesized in a laboratory of the Sankyo Company just before use, and its purity was >99% as determined by HPLC analysis.

Pharmacokinetic studies

Plasma concentration-time profile. Although studies in the mouse and the rat have previously been published [22], additional investigations were conducted in male CDF1 mice and male Donryu rats to unify the experimental design and yield appropriate data for calculation of pharmacokinetic parameters. To extend the ACNU data base to other mammalian species, we conducted further studies in male Japanese white rabbits and male beagle dogs.

ACNU was dissolved in physiological saline just before administration to the animals. Groups of mice were injected with ACNU (40 mg/kg) given as an i.v. bolus (in 0.1 ml sterile saline/10 g body weight) in the tail vein and were bled by decapitation at appropriate times so as to assess the pharmacokinetics. A blood sample from each mouse was collected in a heparinized tube (three mice/time point). A similar study in rats was carried out using i.v. doses of 40 mg/kg and sampling (six rats/time point), and yet another study was done in rabbits and dogs that received i.v. doses of 2.5 mg/kg, followed by serial blood sampling.

The heparinized blood sample from each species was centrifuged at 10,000 *g* for 3 min, and plasma was recovered and rapidly frozen by immersion in liquid nitrogen. The plasma was stored at −80°C until analysis; in these studies all specimens were analyzed individually. Serum and heparinized blood samples for other experiments performed in this study were obtained as described above.

Hepatic extraction ratio in rats. Male Wistar rats weighing 313 ± 0.032 g were lightly anesthetized by ether, and ACNU was given by infusion pump (KN-201, Natsume-Seisakushyo) into a femoral vein cannulated with polyethylene tubing (PE50, Clay Adams). After drug administration, blood samples (0.1 ml) were drawn at specified times from both the femoral artery and the hepatic vein through PE50 polyethylene tubing. Plasma samples were prepared as described above and ACNU concentrations were determined. The hepatic extraction ratio (Eh) was calculated from the plasma concentrations of ACNU measured in the hepatic vein (Chv) and femoral artery (Cp) after steady state had been achieved:

$$Eh = 1 - Chv,ss/Cp,ss,$$

where Chv,ss and Cp,ss represent the steady-state plasma concentrations of the hepatic vein and femoral artery, respectively. The hepatic blood clearance (CLh,b) was calculated from:

$$CLh,b = Eh \times Qh,$$

where Qh represents the blood-flow rate of the liver (14.7 ml/min per 0.25-kg rat). The blood-flow rate was taken from the report by Igari et al. [7]. CLh,b was converted to hepatic plasma clearance (CLh,p) by the following equation:

$$CLh,p = CLh,b \times RBP,$$

where RBP represents the whole-blood-to-plasma concentration ratio. For this calculation, the RBP value for humans was used, considering that RBP is almost constant among species. The total plasma clearance was calculated by:

$$CLtot,p = I/Cp,ss,$$

where I represents the infusion rate (0.33 mg kg^{-1} min^{-1}). The results were expressed as the mean ± SD of three rats.

Analytical methods

ACNU concentrations in the specimens were measured by the HPLC method previously described by Hori et al. [6]. ACNU was extracted with 1,2-dichloroethane on ice under shaded conditions and then measured by HPLC. ACNU was separated by a 30 cm × 4 mm (inside diameter) μ-Bondapak C_{18} reverse-phase column (Waters Associates, Inc.) and eluted with a solvent system consisting of 0.1% PIC-B7 (Waters Associates) in an aqueous solution of 50% methanol at a flow rate of 1 ml/min. The detection was done by absorption at 254 nm, and the quantity was estimated from the peak height. The lower limit of detection by this method was 40 ng/ml.

Pharmacokinetic analysis

The ACNU plasma concentration-time data were analyzed by a microcomputer (PC-9801, NEC) using the MULTI program [24], an automated pharmacokinetic-analysis system for determination of pharmacokinetic parameters, including AUC. The damping Gauss-Newton method was used as an algorithm of calculation for nonlinear curve fitting. CLtot,p and Vd,ss were calculated as follows:

$$CLtot,p = Dose/AUC \text{ and}$$

$Vd,ss = Dose(A/\alpha^2 + B/\beta^2)/AUC^2$, where A, B, α, and β are coefficients of the fitted two-exponential equation $Cp = Aexp(-\alpha t) + Bexp(-\beta t)$. Correlations between body weight and total-body plasma clearance (CLtot,p) or steady-state volume of distribution (Vd,ss) were investigated among the animal species studied. Linear regression of the logarithmic values was calculated by the least-squares method. The kinetic parameters for unbound ACNU were calculated by dividing CLtot,p and Vd,ss by the unbound fraction (fu) of ACNU in the serum of each species.

Calculation of the human unbound plasma concentration-time profile, based on allometric interspecies scaling of the animal parameters, was executed according to the following equation described by Boxenbaum and Ronfeld [3], with slight modification. By their equation, plasma concentration (Cp) is expressed as follows:

Let $CLtot,p = aW^x$; $V1 = cW^y$; $Vd,_\beta = dW^y$; $Vd,ss = eW^y$; then $Cp = [D/acW^y(g-f)]\ \{\ (cfg-af)exp[-f(t/W^{y-x})] - (cfg-ag)exp[-g(t/W^{-x})]\}$,

where D represents the dose, f = a(d-c)/c(d-e), and g = a/d.

In our calculation, $V1^u$ and $Vd,^u_\beta$ were also correlated with body weight. The power values of allometric equations were determined according to the following equation. Then three different power values (y_1, y_2, y_3) of distribution volume were used for the calculation instead of the same power value (y) used in the original equation. The unbound plasma concentration (Cp^u) is expressed as follows:

Let $CL^u_{tot,p} = aW^x$; $V1^u = cW^{y1}$; $Vd,^u_\beta = dW^{y2}$; $Vd^u_{ss} = eW^{y3}$; then

$Cp^u = [D/acW^{x+y_1}\ (gW^{x-y_2} - fW^{x-y_1})] \times$

$\{\ (cfgW^{2x-y_2} - afW^{2x-y_1})exp[-f(t/W^{y_1-x})] -$

$(cfgW^{2x-y_2} - agW^{2x-y_2})exp[-g(t/W^{y_2-x})]\}$,

where $f = a(dW^{y_2} - cW^{y_1})/c(dW^{y_2} - eW^{y_3})$ and g = a/d.

The individual patient data were normalized for dose and then converted to unbound plasma concentration data using RBP and fu values for humans.

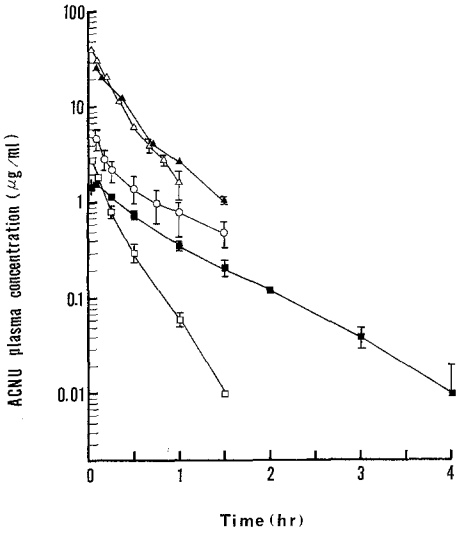

Fig. 1. Plasma concentration-time course of ACNU in the mouse (△), rat (▲), rabbit (□), dog (■), and human (○)) after an i.v. bolus dose. The delivered doses were 40 mg/kg for mice and rats, 2.5 mg/kg for rabbits and dogs, and 1.84 mg/kg for humans. Determination of ACNU plasma levels was made by the same HPLC method used in clinical studies. Each value represents the mean of 3 mice and 6 rats (plasma samples from animals killed at each time point), 3 rabbits, 3 dogs, and 7 patients (plasma samples from individual animals or patients). Clinical data (human) are quoted from the literature [6] and the original whole-blood data were converted to plasma levels by dividing by the RBP value. Error bars represent the SEM

Serum protein binding

Serum protein binding of ACNU was determined by an ultrafiltration method using an MPS-3 (Centrifree, Amicon). ACNU in physiological saline was added to fresh serum and incubated at 37° C for 5 min. An aliquot was loaded onto an MPS-3 and centrifuged at 2,000 *g* for 5 min at room temperature. Another aliquot of serum was used to determine the amount of ACNU that decomposed in serum during both incubation and centrifugation. As a control, nonspecific binding of ACNU to a YMT filter of the MPS-3 was measured in Sörensen buffer (0.113 M Na_2HPO_4, 0.017 M KH_2PO_4; pH 7.4). The unbound fraction was calculated by dividing the concentration of ACNU in the ultrafiltrate by that in the serum (or buffer) after subtracting nonspecific binding. The nonspecific binding of ACNU to the filter membrane was approximately 10% of the delivered dose. The pooled serum sample for each species was obtained from more than three individuals.

Degradation rate of ACNU in serum and its filtrate

Human serum filtrate was prepared by centrifugation using a Centrifree as described above. ACNU was added to the serum or filtrate, and its initial concentration was 10 μg/ml. Serial sampling of the spiked serum or filtrate was performed at 10-min intervals. After determination of ACNU concentrations in these samples, degradation rate constants for ACNU were calculated using the MULTI program. In this study, pooled serum obtained from three individuals was used.

Whole-blood-to-plasma concentration ratio in humans

The whole-blood-to-plasma concentration ratio (RBP) for ACNU was determined after a 5-min incubation at 37° C, with a 3-min preincubation taking place prior to the addition of ACNU. This 5-min incubation time was sufficient for the occurrence of equilibrium. The RBP was calculated by dividing the concentration of ACNU in whole blood by that in plasma.

Results

Plasma levels of ACNU as a function of time after i.v. bolus administration to mice, rats, rabbits, dogs, and humans are shown in Fig. 1. The mean value for RBP in human blood was 0.905±0.011. The RBP values determined at three different ACNU concentrations (1, 2, and 4 μg/ml) were the same. The whole-blood ACNU concentrations of seven patients receiving i.v. ACNU (mean dose, 1.84 mg/kg) reported by Hori et al. [6] were converted to plasma concentrations by dividing the whole-blood concentrations by the RBP value. Pharmacokinetic parameters calculated from the plasma concentration-time data for the four animal species and for humans are listed in Table 1, along with the predicted values for humans. Recovery of ACNU from urine was <3% of the total in the species studied (no data for rabbits). Thus, the renal clearance of ACNU was very low as compared with the total-body clearance.

Figure 2 shows the ACNU concentration-time curves for both the hepatic vein and the femoral artery in rats subjected to continuous administration of ACNU via the femoral vein. The hepatic extraction ratio of ACNU in rats was 0.223±0.068, and the calculated hepatic plasma clearance was 11.9±3.6 ml min^{-1} kg^{-1}. The total plasma clearance of these rats was 34.6±3.6 ml min^{-1} kg^{-1}.

Table 1. Pharmacokinetic parameters of ACNU and average body weight for various species

Species (*n*)	Body weight (kg)	Urinary excretion[a] (% dose)	CLtot,p (ml/min)	Vd,ss (l)	V1 (l)	Vd,β (l)
Mouse (3)	0.0281±0.0009	<0.1	1.60±0.06	0.0301±0.0011	0.0233±0.0018	0.0371±0.0013
Rat (6)	0.200±0.007	<0.1	10.7±1.5	0.296±0.045	0.210±0.036	0.408±0.042
Rabbit (3)	3.37±0.24	ND	220±22	3.25±0.27	2.26±0.19	3.88±0.24
Dog (3)	8.30±0.95	2.6	299±15	14.1±0.5	12.5±0.5	15.0±0.5
Human (7):						
Predicted	70[b]	ND	2,950	103	83.4	110
Observed	70[b]	0.81±0.21	805±36	37.3±2.2	15.4±1.8	46.8±3.1

Values represent means ±SD. ND, not determined

[a] Percentage of recovery from urine as intact ACNU. Data for mice and rats were previously published elsewhere [3]. Values for dogs and humans were obtained through personal communications from T. Nishigaki and N. Saijo, respectively

[b] Tentative body weight of humans

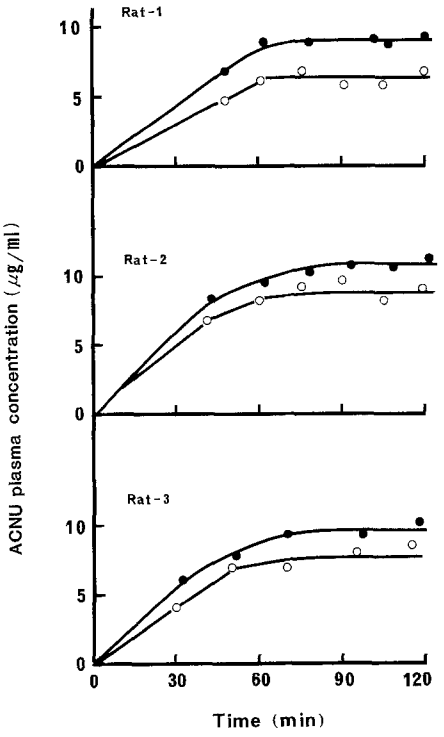

Fig. 2. Femoral artery and hepatic vein plasma concentration-time courses of ACNU in three rats that were given ACNU by i. v. infusion. *Closed and open circles,* plasma concentrations of ACNU in the femoral artery and hepatic vein, respectively

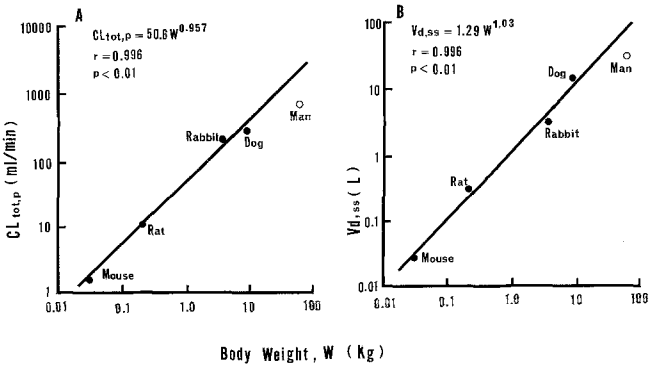

Fig. 3 A, B. A Allometric relationship between total-body plasma clearance and body weight for estimation of human data. **B** Allometric relationship between steady-state distribution volume and body weight for estimation of human data. The *open circle* denotes the value observed in a 70-kg human

Table 2. Serum protein binding of ACNU in various species[a]

Species	Serum unbound fraction
Mouse (50)[b]	0.559±0.019
Rat (3)	0.656±0.016
Rabbit (5)	0.675±0.172
Dog (4)	0.894±0.055
Human (3)	0.351±0.007

Values represent the means ± SD of 3 or 4 sample determinations

[a] Serum protein binding of ACNU at a concentration of 4 μg/ml was determined by an ultrafiltration method

[b] Figures in parentheses indicate the number of individuals from which pooled serum was obtained

The degradation rate constants of ACNU in human serum and its filtrate were 0.0816 min^{-1} ($t_{1/2}$ = 8.5 min) and 0.0824 min^{-1} ($t_{1/2}$ = 8.4 min), respectively. On the other hand, the degradation rate constant in rat plasma was found to be 0.0400 min^{-1} ($t_{1/2}$ = 17 min).

Figure 3 presents the allometric relationships of ACNU pharmacokinetic data in animal species and shows the CLtot,p and Vd,ss values for mouse, rat, rabbit, dog, and human plotted against body weight on log-log coordinates. These plots were linear, with high correlation coefficients of 0.996 for both parameters if the data for humans were excluded. These allometric equations describing data from only the four animal species were extrapolated to yield parameter estimates for a 70-kg human as indicated in Table 1. The observed values, however, were considerably lower than those predicted from the animal data.

In general, the metabolism and renal excretion of a drug depend on its unbound plasma concentration. Therefore, we examined the question as to whether the disagreement between the predicted and the observed values could be explained by a species difference in serum protein binding. Table 2 shows serum protein binding as expressed in unbound fraction (fu) values for ACNU, determined by an ultrafiltration method in the five species. At least in rats and humans, there was no difference in the fu value between plasma and serum. The fu values did not change within the range of 2–8 μg/ml ACNU. These results suggest that protein binding of ACNU is linear within the concentration range studied.

Table 3. Pharmacokinetic parameters of the unbound fraction of ACNU and average body weight for various species[a]

Species (*n*)	Body weight (kg)	$CL^u_{tot,p}$ (ml/min)	$Vd,^u_{ss}$ (l)	Vl^u (l)	$Vd,^u_\beta$ (l)
Mouse (3)	0.0281±0.0009	2.86±0.11	0.0538±0.0020	0.0417±0.0032	0.0664±0.0023
Rat (6)	0.200±0.007	16.3±2.3	0.451±0.069	0.320±0.055	0.622±0.064
Rabbit (3)	3.37±0.24	326±33	4.81±0.40	3.35±0.28	5.75±0.36
Dog (3)	8.30±0.95	334±17	15.8±0.6	14.0±0.6	16.8±0.6
Human (7):					
Predicted	70[b]	3,160	110	89.5	117
Observed	70[b]	2,290±103	106±6	43.9±5.0	133±9

[a] Data in Table 1 were converted to parameters for unbound ACNU

[b] Tentative body weight of humans

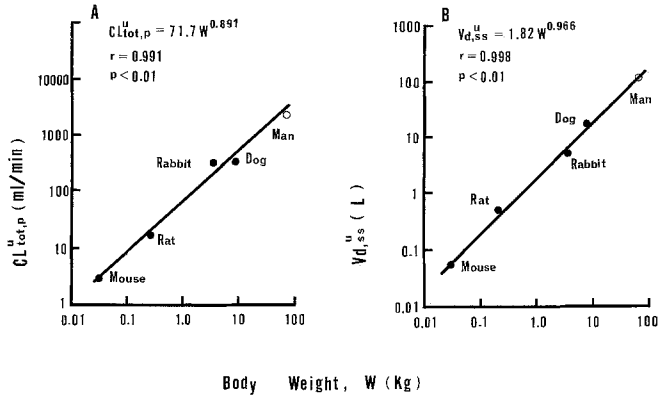

Fig. 4 A, B. **A** Allometric relationship between total-body unbound plasma clearance and body weight for estimation of human data. **B** Allometric relationship between unbound steady-state distribution volume and body weight for estimation of human data. The *open circle* denotes the value observed in a 70-kg human

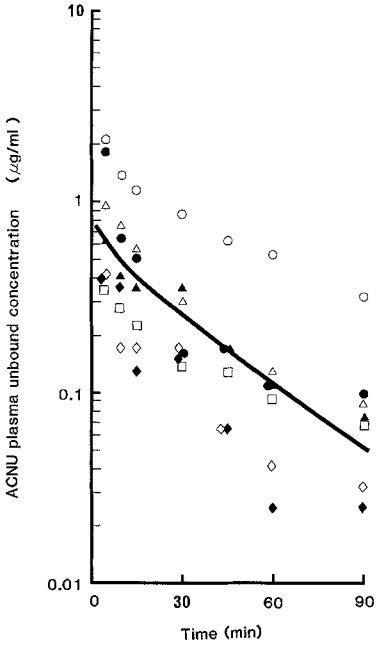

Fig. 5. Comparison of predicted unbound plasma concentration-time course for 70-kg human given 1 mg/kg ACNU as an i.v. bolus (*solid line*) with clinically observed values. For comparison, ACNU plasma levels of seven patients were normalized for the delivered dose, and these were converted to unbound plasma levels by dividing by RBP and multiplying by fu. The symbols denote data from individual patients

Table 3 shows the pharmacokinetic parameters for unbound ACNU, and Fig. 4 illustrates the allometric relationships of unbound ACNU. The values measured for human parameters compared favorably with those predicted from the animal data. The allometric equations for $CL^{u}_{tot,p}$ and $Vd,^{u}_{ss}$, which include human data, are as follows: $CL^{u}_{tot,p} = 68.2\ W^{0.859}$ ($r = 0.994$, $P < 0.01$) and $Vd,^{u}_{ss} = 1.81\ W^{0.962}$ ($r = 0.999$, $P < 0.01$). The correlation coefficient between fu and Vd,ss (per kg body weight) was 0.861 (including human data).

For the purpose of calculating the unbound plasma concentration-time course of ACNU in humans, additional

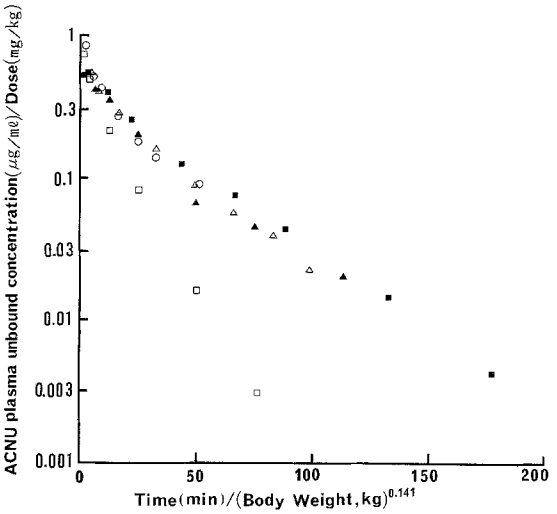

Fig. 6. Dedrick plot of ACNU unbound plasma concentration of the five species. This figure represents the data in Fig. 1, replotted after normalization of the plasma concentrations for the delivered dose and conversion to unbound concentrations by fu values for each species. The chronological times were also normalized by dividing each time value by body weight to the power 0.141. Symbols: mouse (△), rat (▲), rabbit (□), dog (■), human (○)

allometric relationships of $V1^{u}$ and $Vd,^{u}\beta$ were also determined from the animal data as follows: $V1^{u} = 1.40\ W^{0.979}$ ($P < 0.01$, $r = 0.996$) and $Vd,^{u}\beta = 2.18\ W^{0.938}$ ($P < 0.01$, $r = 0.997$). These relationships were used for the simulation of ACNU plasma profiles in humans. Figure 5 shows the time course of dose-normalized unbound ACNU plasma concentrations in individual patients. The unbound plasma concentration-time profile in a person (70 kg) given 1 mg/kg ACNU as an i.v. bolus was then predicted using the animal scale-up technique and the curve obtained from the equation $Cp^{u} = 0.230\exp(-0.136t) + 0.556\exp(-0.0269t)$. Thus, the predicted timeprofile was in good accordance with the observed values, although they showed large interindividual differences.

Figure 6 shows the Dedrick plots of the five species studied. The plasma concentration data from the mouse, rat, dog, and human were found to be dispersed on a single curve.

Discussion

In the present study, we attempted to predict the pharmacokinetics of ACNU in humans from animal pharmacokinetic data only and demonstrated that it could be accurately predicted by the animal scale-up technique, once species differences in serum protein binding had been considered.

ACNU is bound to proteins in the serum of mice, rats, rabbits, dogs, and humans, but the extent of binding is not very high. The unbound fraction of ACNU in human serum was the smallest among the species tested. This seems to be a major reason for the discrepancy between the observed and predicted parameters in humans when parameters based on the total plasma concentrations of ACNU were used. Therefore, for accurate prediction of the pharmacokinetics of drugs in humans, it might be important to

examine the species difference in serum protein binding, even if the extent of binding is not high.

The power value of the allometric exponent of total plasma clearance for unbound ACNU was 0.859 (including human data), similar to those (0.61–0.75) reported for other drugs [2, 11, 16]. This means that the intrinsic ability of the body (per kg body weight) to eliminate ACNU tends to decrease as body weight increases. On the other hand, the power value for the distribution volume of unbound ACNU was nearly 1, implying that the value per kilogram body weight is almost constant for any animal species. Furthermore, the correlation between fu and Vd,ss values suggests that the distribution volume for unbound ACNU is relatively constant and species-independent.

The renal clearance of ACNU might be underestimated because of the instability the drug in urine, as it degrades rapidly under physiological conditions [15, 17]. However, in the rat ACNU is eliminated mainly by nonrenal clearance, since the recovery of unchanged drug in urine was <0.1% of the total dose. Assuming that ACNU is excreted in the urine only via glomerular filtration, renal clearance is calculated as 3.3 ml min^{-1} kg^{-1} using the fu value (0.656) multiplied by the glomerular filtration rate (5 ml min^{-1} kg^{-1}). This corresponds to 6% of the total clearance (55 ml min^{-1} kg^{-1}) of ACNU. Although the possibility of secretion by renal tubules cannot be completely denied, the urinary excretion calculated above is a rather small value as compared with the total plasma clearance. Based on these considerations, we speculate that urinary excretion itself may be small even if spontaneous decomposition of ACNU in urine occurs.

The nonrenal clearance of ACNU can be divided into hepatic and nonhepatic clearance. The hepatic plasma clearance calculated from the hepatic extraction ratio corresponded to about 34% of the total plasma clearance, suggesting that this elimination route might play a major role in total plasma clearance of ACNU in the rat. Nonhepatic clearance would be accounted for by metabolism in organs other than the liver and by decomposition of ACNU as described above. By investigating the metabolites in rat urine, Nishigaki et al. [15] showed that ACNU was metabolized to four major metabolites, and these authors proposed three metabolic pathways for the formation of these metabolites.

Metabolite A (intramolecular cyclization product) was produced by spontaneous decomposition; the reaction giving rise to metabolite B (a denitrosated product) was catalyzed by both cytosolic and microsomal enzymes that were not inhibited by SKF-525A; and the reactions generating metabolites C and D (products of oxidative dechlorination, with metabolite D being a denitrosated product of metabolite C) were catalyzed by hepatic P-450-dependent mixed-function oxidase. The ratio of the metabolite contents in rat urine was 1.2(A):1(B):1(C):3.7(D). These results imply that the bulk of ACNU would be enzymatically metabolized, in spite of its instability under physiological conditions. The decomposition rate constant, k, in rat plasma was approximately 0.04 min^{-1}; thus, the clearance by decomposition ($V \times k$; V = volume of plasma and extracellular fluid, approximately 200 ml/kg) of ACNU in total plasma and in the extracellular fluid space corresponded to 15% of the total plasma clearance in this species. Furthermore, comparison of the decomposition rates of ACNU in human serum and its filtrate showed that the instability of the drug was not affected by protein binding in the serum.

These results suggest that enzymatic metabolism in the body is the main process for elimination of ACNU in the rat. Thus, it seems reasonable that the prediction would become more accurate after correction for the species difference in serum protein binding, because the intrinsic clearance in the liver, kidney, and other organs that have drug-eliminating ability may depend on the unbound plasma concentration of the drug.

Accurate prediction of the pharmacokinetics of anticancer agents in humans is of value for improvement of the predictability of clinical effects of anticancer agents based on animal-evaluation systems. Inaba et al. [8–10] and Tashiro et al. [23] reported a pharmacokinetic approach that could improve the predictability of the clinical effect in humans of anticancer agents tested in a human-tumor-bearing nude mouse system. In those studies, the delivered doses were pharmacokinetically equivalent to the clinical doses. Therefore, if we can determine plasma concentration-time profiles of new anticancer agents in humans, it might be possible to predict their clinical anticancer effects by treating nude mice at doses that reproduce clinical plasma levels in mice. However, to obtain the plasma concentration-time profile of a drug in humans, we must predict both its pharmacokinetic parameters and its effective delivered dose. The former prediction may be achievable by the animal scale-up technique, and the latter requires prediction of the maximum tolerated dose (MTD) in humans. The possibility of predicting the MTD in humans by the animal scale-up technique has also been discussed by Mordenti [13].

The number of anticancer agents whose pharmacokinetic similarity among species has been studied using allometric equations is rather limited. Thus, the application of the animal scale-up technique for predicting the pharmacokinetics of anticancer agents in humans by using animal data is important for evaluation of the efficacy of such agents in the preclinical stage using the above-mentioned human-tumor-bearing nude mouse system.

References

1. Boxenbaum H (1982) Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. J Pharmacokinet Biopharm 10: 201
2. Boxenbaum H (1984) Interspecies pharmacokinetic scaling and the evolutionary-comparative paradigm. Drug Metab Rev 15: 1071
3. Boxenbaum H, Ronfeld R (1983) Interspecies pharmacokinetic scaling and the Dedrick plots. Am J Physiol 245: R768
4. Cooperative study group of phase I study on ACNU (1976) Phase I study of 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU). Jpn J Clin Oncol 6: 55
5. Dedrick RL (1973) Animal scale-up. J Pharmacokinet Biopharm 1: 435
6. Hori T, Muraoka K, Saito Y, Sasahara K, Inagaki H, Inoue Y, Adachi S, Anno Y (1987) Influence of modes of ACNU administration on tissue and blood drug concentration in malignant brain tumors. J Neurosurg 66: 372

7. Igari Y, Sugiyama Y, Sawada Y, Iga T, Hanano M (1984) In vitro and in vivo assessment of hepatic and extrahepatic metabolism of diazepam in the rat. J Pharm Sci 73: 826
8. Inaba M, Kobayashi T, Tashiro T, Sakurai Y (1988) Pharmacokinetic approach to rational therapeutic doses for human tumor-bearing nude mice. Jpn J Cancer Res 79: 509
9. Inaba M, Tashiro T, Kobayashi T, Sakurai Y, Maruo K, Ohnishi Y, Ueyama Y, Nomura T (1988) Responsiveness of human gastric tumors implanted in nude mice to clinically equivalent doses of various antitumor agents. Jpn J Cancer Res 79: 517
10. Inaba M, Kobayashi T, Tashiro T, Sakurai Y, Maruo K, Ohnishi Y, Ueyama Y, Nomura T (1989) Evaluation of antitumor activity in a human breast tumor/nude mouse model with a special emphasis on treatment dose. Cancer 64: 1577
11. McGovren JP, Williams MG, Stewart JC (1988) Interspecies comparison of acivicin pharmacokinetics. Drug Metab Dispos 16: 18
12. Mordenti J (1985) Man versus beast: pharmacokinetic scaling in mammals. J Pharm Sci 75: 1028
13. Mordenti J (1986) Dosage regimen design for pharmaceutical studies conducted in animals. J Pharm Sci 75: 852
14. Nishigaki T, Nakamura K, Kinoshita T, Kuwano H, Tanaka M (1985) Identification of major urinary metabolites of ACNU, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride, in rats. J Pharmacobio-Dyn 8: 401
15. Nishigaki T, Nakamura K, Tanaka M (1985) In vitro metabolism of ACNU, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride, a water-soluble antitumor nitrosourea. J Pharmacobio-Dyn 8: 409
16. Owens SM, Hardwick WC, Blackall D (1987) Phencyclidine pharmacokinetic scaling among species. J Pharmacol Exp Ther 242: 96
17. Ozawa S, Sugiyama Y, Mitsuhashi Y, Kobayashi T, Inaba M (1988) Cell killing action of cell cycle phase-non-specific antitumor agents is dependent on concentration-time product. Cancer Chemother Pharmacol 21: 185
18. Rowland M (1985) Physiological pharmacokinetic models and inter-animal species scaling. Pharmacol Ther 29: 49
19. Sawada Y, Hanano M, Sugiyama Y, Iga T (1984) Prediction of the disposition of β-lactam antibiotics in humans from pharmacokinetic parameters in animals. J Pharmacokinet Biopharm 12: 241
20. Sawada Y, Hanano M, Sugiyama Y, Harashima H, Iga T (1984) Prediction of the volumes of distribution of basic drugs in humans based on data from animals. J Pharmacokinet Biopharm 12: 587
21. Sawada Y, Hanano M, Sugiyama Y, Iga T (1985) Prediction of the disposition of nine weak acidic and six weak basic drugs in humans from pharmacokinetic parameters in rat. J Pharmacokinet Biopharm 13: 477
22. Tanaka M, Nishigaki T, Nakajima S, Totsuka S, Nakamura K (1980) Distribution, excretion, and metabolism of 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (ACNU) in rats and mice after i.v. administration. Cancer Treat Rep 64: 575
23. Tashiro T, Inaba M, Kobayashi T, Sakurai Y, Maruo K, Ohnishi Y, Ueyama Y, Nomura T (1989) Responsiveness of human lung cancer/nude mouse to antitumor agents in a model using clinically equivalent doses. Cancer Chemother Pharmacol 24: 187
24. Yamaoka K, Tanigawara Y, Nakagawa T, Uno T (1981) A pharmacokinetic analysis program (MULTI) for microcomputer. J Pharmacobio-Dyn 4: 879
25. Yokoyama M (1980) A review on 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride (ACNU). Jpn J Cancer Chemother 7: 339 (in Japanese, with English summary)