Pharmacokinetic and Toxicity Scaling of the Antitumor Agents Amsacrine and CI-921, a New Analogue, in Mice, Rats, Rabbits, Dogs, and Humans¹

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ABSTRACT

The aim was to investigate interspecies relationships between body weight (W) (kg) and various pharmacokinetic parameters for the antitumor agents amsacrine and its 4-methyl-5-(N-methylcarboxamide) analogue, CI-921, and examine which pharmacokinetic parameter, if any, might be used to predict the toxicity of these agents. Pharmacokinetic, plasma protein binding, and toxicity data were available for CI-921 in mice, rats, rabbits, dogs, and humans. For amsacrine, similar interspecies pharmacokinetic data were available but toxicity and protein-binding data were available for only 3 species. Significant linear relationships were obtained for CI-921 between log W and log Vₘₐₓ (liters) (r = 0.971, P = 0.006), and log W and log Cl (liters/h) (r = 0.911, P = 0.031) resulting in the allometric equations Vₘₐₓ = 2.22W⁻⁰·₅ and Cl = 0.91W⁻⁰·⁵. For amsacrine these corresponding equations were Vₘₐₓ = 3.37W⁻¹·⁵ (r = 0.996, P < 0.001), and Cl = 2.28W⁻¹·⁵ (r = 0.952, P = 0.012). When interspecies differences in plasma protein binding were taken into account, the allometric relationships improved and the exponents of the power equations increased. For CI-921 the allometric equations for the kinetic parameters calculated from plasma “free” concentrations were: Vₘₐₓ (liters) = 247W⁻¹·⁵ (r = 0.984, P = 0.002) and Cl (liters/h) = 186W⁻¹·⁵ (r = 0.961, P = 0.009). The dog was a noticeable outlier in the relationship between the log maximum tolerated dose (MTD) (mg/kg) of CI-921 and log W. Omission of the latter resulted in a highly significant allometric relationship, MTD = 5.37W⁻¹·⁵ (r = 0.998, P = 0.012). For amsacrine there was no significant allometric relationship between MTD and W. CI-921s prolonged t₉₀ in the dog and the dog’s increased susceptibility to CI-921 toxicity suggested a relationship between MTD and t₉₀ (h). A significant linear relationship was observed between MTD and t₉₀ (r = -0.994, P < 0.001), from which the following equation was developed

MTD = 47.5e³·⁵tn₀

Combining the amsacrine toxicity data in the latter relationship yielded a similar equation

MTD = 44.7e³·⁵tn₀

(r = -0.933, P < 0.0001). It was concluded that allometric equations may be developed for CI-921 and amsacrine from animal pharmacokinetic data which allow a reasonable prediction of CI and Vₘₐₓ in patients, despite these agents being eliminated mainly by biotransformation. However, similar relationships between toxicity and body weight were susceptible to variation between individual species. Species differences in the toxicity of these agents were predictable from the t₉₀. This study emphasized the importance of pharmacokinetic data in preclinical toxicity and efficacy testing of antitumor agents.

INTRODUCTION

N-[4-(9-Acridinylamino)-3-methoxyphenyl]methanesulfonamide (amsacrine) and 9-[2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino-N,5-dimethyl-4-acridinocarboxamide (CI-921) are novel antitumor agents with similar structures (Fig. 1). Amsacrine has an established role in the treatment of leukemia (1). An analogue of amsacrine, CI-921, was synthesized in the Auckland Cancer Research Laboratory in an attempt to develop an agent with greater solid tumor activity. CI-921 showed significantly greater activity in both in vitro and in vivo solid tumor test systems (2) and has recently completed Phase I clinical trials with encouraging results (3). Phase II trials in patients with specific tumors are now under way. Both agents appear to act by inducing the formation of covalent links between DNA and the enzyme topoisomerase II (4).

It is important in cancer chemotherapy to be able to determine from preclinical pharmacological studies an appropriate safe starting dose for humans which also maximizes the chance that the dose an individual receives has therapeutic potential. This is of particular importance with anticancer drugs which usually exhibit an extremely narrow therapeutic index. Typically with antineoplastic agents, a biologically inactive dose may be within 1 log of a lethal dose. The aim of this retrospective study was to investigate interspecies relationships between the various pharmacokinetic parameters and body weight, and examine which pharmacokinetic parameter, if any, might be used to predict the toxicity of these agents.

MATERIALS AND METHODS

Toxicity Data. Toxicity data were obtained in animals after a single i.v. dose of amsacrine (5) or CI-921.⁴ The biological end point was the lethal dose in 10% of mice and the highest nonlethal dose (i.e., the MTD) in rats, rabbits, and dogs. The MTD in humans was obtained from Phase I trials using a single i.v. dose treatment schedule (6, 7).

Pharmacokinetic Data. Pharmacokinetic data were available for amsacrine in patients (8), dogs,⁴ rabbits (9), rats (10), and mice (11); and for CI-921 in patients (12), dogs,⁴ rabbits (13), rats,⁴ and mice (11). Plasma concentrations of amsacrine and CI-921 were measured by high-performance liquid chromatography methods using UV absorbance or electrochemical detection (11, 14–16). Determination of amsacrine in the rat was by extraction of unchanged [¹⁴C]amsacrine (10). The pharmacokinetic parameters, plasma clearance (Cl), and volume of distribution at steady state (Vₘₐₓ) were estimated by noncompartmental methods based on statistical moment theory (17). The elimination half-life (t₉₀) was calculated from In 2 divided by the slope of the terminal linear portion of the log concentration-time profile, estimated by unweighted least squares regression. The plasma unbound fraction (fu) was determined by equilibrium dialysis using [¹⁴C]CI-921 (18) or [¹⁴C]amsacrine (19, 20). Plasma protein binding data for amsacrine were not available for rat or dog. Clearance (Clu) and the volume of distribution of the unbound CI-921 (Vₘₐₓ) were calculated from Cl, Vₘₐₓ, and fu.

The power equation (or equation of simple allometry)

\[ Y = aW^b \]

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¹ The abbreviations used are: CI-921, 9-[2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino-N,5-dimethyl-4-acridinocarboxamide; MTD, maximum tolerated dose (mg/kg).

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was applied to the relationships between MTD (mg/kg) and mean body weight ($W$) and between various pharmacokinetic parameters and $W$. This equation may also be written in the form

$$\log Y = \log a + b \log W$$

where $Y$ is the MTD (or pharmacokinetic variable), $\log a$ is the $Y$-intercept, and $b$ is the slope of the log $Y$ versus log $W$ relationship. The power equation was fitted to data by least squares linear regression, and Student's $t$ test was used to determine if the slope ($b$) of the log $Y$ versus log $W$ linear relationship was significantly different from zero (i.e., $P < 0.05$). The same methods were used to fit the exponential equations and test their significance.

RESULTS

Kinetic Data. Table 1 summarizes the mean CI-921 pharmacokinetic parameters normalized to body weight and mean body weight of the five species studied. When normalized to weight, humans, and rabbits had similar small values for $V_{ss}$ of CI-921. Overall there was a 14-fold range in $V_{ss}$ from 0.3 liter/kg (rabbit and human) to 4.3 liters/kg in the mouse. When the $Cl$ of CI-921 was normalized to body weight, there was a 28-fold range across species. Humans, rabbit, and dog all had similar low values, i.e., 0.16, 0.19, and 0.22 liter/h/kg, respectively. The greatest $Cl$ value was observed in the mouse, 4.46 liters/h/kg. An 11-fold range (0.6–6.7 h) was observed for $t_{\alpha}$ of CI-921 with the dog having the longest $t_{\alpha}$. There were significant linear relationships between the log $W$ (kg) and log $K_{ss}$ (liters), and log $Cl$ (liters/h) (Fig. 2), but not between log $W$ and log $t_{\alpha}$ (h).

The allometric equations for these relationships were

$$V_{ss} = 1.22 W^{0.68} \quad (A)$$

($r = 0.971, P = 0.006$), and

$$Cl = 0.91 W^{0.51} \quad (B)$$

($r = 0.911, P = 0.031$). CI-921 is highly protein bound in plasma. The 10-fold range (0.001–0.011) in the plasma free fraction of CI-921 across species may affect the relationships between body weight and $Cl$, and $V_{ss}$, which are based on total plasma concentrations (i.e., bound plus free). Improved allometric relationships were obtained between body weight and kinetic parameters based on the plasma free concentration (Fig. 3). The allometric equations for the latter were

$$V_{ss} = 247 W^{0.93} \quad (C)$$

($r = 0.984, P = 0.002$), and

$$Clu = 186 W^{0.76} \quad (D)$$

($r = 0.961, P = 0.009$).

The mean amscarine pharmokinetic parameters normalized to body weight and mean body weights are reported in Table 2. Amsacrine $V_{ss}$ values normalized to body weight were greater than those for CI-921. However, the rank order of $V_{ss}$ values across species was the same, with the largest $V_{ss}$ value observed in the mouse (7.4 liters/kg) and the smallest in the rabbit (1.7 liters/kg) and human (1.6 liters/kg). The overall range of $V_{ss}$ values across species was less for amscarine (5-fold) than for CI-921. In contrast, the overall range of $Cl$ values normalized to body weight was considerably larger (83-fold) for amscarine, with humans having the lowest (0.26 liter/h/kg) and mouse (21.6 liters/h/kg) the highest clearance. There was a 32-fold

Table 1 Mean CI-921 pharmacokinetic parameters, free plasma fractions, and body weights for various mammalian species

<table>
<thead>
<tr>
<th>Species</th>
<th>Body wt (kg)</th>
<th>Dose* (mg/kg)</th>
<th>$V_{ss}$ (liters/kg)</th>
<th>$Cl$ (liters/h/kg)</th>
<th>$t_{\alpha}$ (h)</th>
<th>$fu$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.021</td>
<td>6.7</td>
<td>4.31</td>
<td>4.46</td>
<td>0.6</td>
<td>0.0090</td>
</tr>
<tr>
<td>Rat</td>
<td>0.43</td>
<td>0.1–3.0</td>
<td>1.98</td>
<td>3.95</td>
<td>0.6</td>
<td>0.0107</td>
</tr>
<tr>
<td>Rabbit</td>
<td>4.6</td>
<td>3.0</td>
<td>0.32</td>
<td>0.19</td>
<td>1.7</td>
<td>0.0032</td>
</tr>
<tr>
<td>Dog</td>
<td>10.5</td>
<td>0.1–3.0</td>
<td>1.00</td>
<td>0.22</td>
<td>6.7</td>
<td>0.0038</td>
</tr>
<tr>
<td>Human</td>
<td>70.5</td>
<td>0.3–6.5</td>
<td>0.32</td>
<td>0.16</td>
<td>2.6</td>
<td>0.0011</td>
</tr>
</tbody>
</table>

* Free base.
ALLOMETRIC RELATIONSHIPS FOR CI-921 AND AMSACRINE

Fig. 3. Log-log plot for CI-921 of (A) \( V_{ss} \) versus species body weight, and (B) \( Clu \) versus species body weight.

Table 2 Mean amsacrine pharmacokinetic parameters, free plasma fractions, and body weights for various mammalian species

<table>
<thead>
<tr>
<th>Species</th>
<th>Body wt (kg)</th>
<th>Dose (mg/kg)</th>
<th>( V_{ss} ) (liters/kg)</th>
<th>( Cl ) (liters/h/kg)</th>
<th>( t_\alpha ) (h)</th>
<th>( f_u )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.020</td>
<td>4.5</td>
<td>7.42</td>
<td>21.59</td>
<td>0.2</td>
<td>0.0672</td>
</tr>
<tr>
<td>Rat*</td>
<td>0.28</td>
<td>10.0</td>
<td>4.5</td>
<td>5.00</td>
<td>0.5</td>
<td>NA*</td>
</tr>
<tr>
<td>Rabbit</td>
<td>4.2</td>
<td>2.5</td>
<td>1.70</td>
<td>0.46</td>
<td>2.6</td>
<td>0.0278</td>
</tr>
<tr>
<td>Dog</td>
<td>10.5</td>
<td>3.0</td>
<td>2.80</td>
<td>0.97</td>
<td>6.5</td>
<td>NA</td>
</tr>
<tr>
<td>Human</td>
<td>81.3</td>
<td>3.8-5.6</td>
<td>1.56</td>
<td>0.26</td>
<td>4.7</td>
<td>0.0298</td>
</tr>
</tbody>
</table>

* Free base.

The plasma free fraction for amsacrine was significantly greater than that for CI-921 (7.5-fold for the mouse, 8.7-fold for the rabbit, and 27-fold for humans). Because these data were available for only three species (Table 2), allometric relationships for kinetic parameters based on plasma free concentrations of amsacrine were not investigated.

Toxicity Data. The MTD for CI-921 and amsacrine and mean body weight for various species are reported in Table 3. The relationship between log MTD for CI-921 and log \( W \) is shown in Fig. 6. The dog datum was a noticeable outlier in this relationship. Omission of the dog datum resulted in an excellent linear relationship (\( r = -0.998, P = 0.012 \)) between log MTD (mg/kg) and log \( W \) (kg) for CI-921. The allometric equation for this relationship was

\[
MTD = 23.6W^{-0.14}
\]

(H)

Toxicity data for amsacrine after a single i.v. dose were available only for mice, dogs, and humans (Fig. 6). A significant allometric relationship for the MTD of amsacrine was not observed.

The prolonged \( t_\alpha \) for CI-921 in the dog and the dog's apparently increased susceptibility to CI-921 toxicity suggested that there may be a relationship between MTD (mg/kg) and \( t_\alpha \) (h). An arithmetic plot of MTD versus \( t_\alpha \) suggested that an exponential relationship may be appropriate. Transforming MTD to In values gave a significant linear relationship with \( t_\alpha \) values for CI-921 (\( r = -0.994, P = 0.0006 \)). From this log-linear relationship the CI-921 MTD could be expressed as

\[
MTD = 47.5e^{-0.51t_\alpha}
\]

(I)

The plasma free fraction for amsacrine was significantly greater than that for CI-921 (7.5-fold for the mouse, 8.7-fold for the rabbit, and 27-fold for humans). Because these data were available for only three species (Table 2), allometric relationships for kinetic parameters based on plasma free concentrations of amsacrine were not investigated.

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\[
MTD = 47.5e^{-0.51t_\alpha}
\]

(I)
There were no significant linear relationships between MTD and $V_m$ or $Cl$ on either rectilinear or log scales. Similar results were observed for amsacrine with a significant linear relationship ($r = -0.998$, $P = 0.041$) only between In MTD and $t_m$, represented by

$$MTD = 37.0e^{-0.50t_m}$$  \( \text{(J)} \)

The similarity of the latter equations suggested combining the data for CI-921 and amsacrine (Fig. 7). The resulting equation from the combined data was

$$MTD = 44.7e^{-0.51t_m}$$  \( \text{(K)} \)

($r = -0.933$, $P < 0.0001$).

**DISCUSSION**

There are many similarities in the anatomy and physiology of mammalian species, and many physiological processes (such as basal oxygen consumption, liver weight, and creatinine clearance) can be scaled as an exponential function of body weight (21). Pharmacokinetic parameters are a reflection of the complex relationships between anatomical, biochemical, and physiological variables that govern drug disposition. Similarly, pharmacokinetic parameters may also be scaled from species to species. One example of a particularly successful scale-up of animal data to humans has been methotrexate (22).

Of the pharmacokinetic parameters studied, the most statistically significant allometric relationships reported in the literature have been for the volume of distribution. The exponents of the allometric equations for most drugs (e.g., methotrexate, cyclophosphamide, phenylcyclohexene, and antipyrine) were $\approx 0.9$, indicating that volume of distribution was almost directly proportional to body weight (i.e., exponents approaching unity) (23–25). For amsacrine and CI-921, the exponents were lower (0.81 and 0.68, respectively) indicating that the volume of distribution did not increase across species in direct proportion to weight. Thus, the larger animal, such as humans, had a smaller than expected volume of distribution. This is, at least in part, due to species differences in plasma protein binding as judged by the relationship between body weight and volume of distribution calculated from the free CI-921 concentration in plasma, which gave an allometric equation with an exponent of 0.93. Thus the volume of distribution calculated from unbound drug (i.e., independent of plasma protein binding) is almost directly proportional to body weight. With clearance, the scaling from animals to humans appears to be relatively successful for renally excreted compounds (26–29). Interspecies scaling is less successful when biotransformation is the primary mechanism for drug elimination. Different metabolites and different rates and extent of metabolism may occur in different species (30). Evidence indicates that both CI-921 and amsacrine are eliminated primarily by metabolism in the liver and excretion in the bile in most species (8, 10–12, 31). Interspecies scaling of drugs that are eliminated by biliary excretion may not be successful because biliary excretion of xenobiotics may be variable across species (32, 33). The allometric relationship for clearance may also have been complicated by the dose-dependent kinetics exhibited by CI-921 (11, 13) and amsacrine (9–11). To minimize this, CI-921 and amsacrine kinetic data were utilized from the lowest dose. Another potential complicating factor in the scale-up of antitumor agent data from animals to humans is that the kinetics and toxicology studies were undertaken in normal healthy animals, whereas the human studies were in patients with cancer. There are a number of reports of impaired hepatic drug metabolism in tumor-bearing animals (34–37) and altered pharmacokinetics in cancer patients (38–41); however, we observed no significant difference in the plasma kinetics of either CI-921 or amsacrine in normal mice compared to mice with a s.c. Lewis lung tumor (11).

Despite these complicating factors, there were significant linear relationships between log $Cl$ and log $W$, resulting in power equations with exponents of 0.51 and 0.46 for CI-921 and amsacrine, respectively. These exponents are lower than reported for other drugs, which ranged from 0.57 to 1.37 with most clustered between 0.6 and 0.8 (21–24). Low allometric exponents (0.54 and 0.41) have been reported for the clearance of $\beta$-lactam antibiotics, cefotetan and cefepiramide, and it was suggested that a low exponent might be characteristic of biliary excretion (42). However, as with volume of distribution, the linear relationship improved and the exponent of the power equation rose to 0.76 when plasma clearance of unbound drug was substituted for total plasma clearance of CI-921.

### Table 3 Toxic dose (mg/kg) and body weight (kg) for CI-921 and amsacrine across species

<table>
<thead>
<tr>
<th>Drug</th>
<th>Parameter</th>
<th>Species</th>
<th>Mouse</th>
<th>Rat</th>
<th>Rabbit</th>
<th>Dog</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-921</td>
<td>Wt (kg)</td>
<td>0.020</td>
<td>0.22</td>
<td>4.25</td>
<td>9.5</td>
<td>62.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toxic dose (mg/kg)</td>
<td>42</td>
<td>30</td>
<td>17.7</td>
<td>1.6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Amsacrine</td>
<td>Wt (kg)</td>
<td>0.022</td>
<td>NA</td>
<td>NA</td>
<td>10.5</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toxic dose (mg/kg)</td>
<td>34.6</td>
<td>NA</td>
<td>NA</td>
<td>1.6</td>
<td>3.1</td>
<td></td>
</tr>
</tbody>
</table>

* NA, not available.

* Lowest dose which produces drug-induced pathological alterations in hematological, chemical, clinical, or morphological parameters (5).

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![Fig. 6. Log-log plot of MTD versus species body weight for CI-921 (●) and amsacrine (○). ——, least squares linear regression line for the CI-921 data (excluding the dog datum point).](image)

**Fig. 6.** Log-log plot of MTD versus species body weight for CI-921 (●) and amsacrine (○). ——, least squares linear regression line for the CI-921 data (excluding the dog datum point).

![Fig. 7. Log-linear plot of MTD versus $t_m$ for CI-921 (●) and amsacrine (○). ——, least squares linear regression line.](image)

**Fig. 7.** Log-linear plot of MTD versus $t_m$ for CI-921 (●) and amsacrine (○). ——, least squares linear regression line.
With regard to the hybrid kinetic parameter, $t_{0.5}$, a significant allometric relationship was observed for amsacrine and not for CI-921. Particularly noticeable for CI-921 was the long $t_{0.5}$ in the dog which, along with the dog's intolerance to CI-921, suggested a relationship between toxicity and $t_{0.5}$. An excellent allometric relationship was observed for the MTD for CI-921 between mouse, rat, rabbit, and humans; however, the dog was strikingly different, emphasizing the hazards of attempting direct allometric extrapolation of toxicity across species without regard to other factors such as pharmacokinetics. A more predictive, yet empirical relationship (incorporating data from all species investigated) was obtained between the In MTD (mg/kg) and $t_{0.5}$ (h), allowing the development of a simple exponential equation in which the MTD for CI-921 was expressed as a function of $t_{0.5}$ (the base of natural logarithms) raised to a constant

$\text{MTD} = 47.5e^{-0.5t_{0.5}}$

Despite the limited toxicity data for amsacrine, a similar equation was obtained for MTD, i.e.,

$\text{MTD} = 37.0e^{-0.5t_{0.5}}$


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