Comparative Pharmacology of the Novel Cyclopropylpyrroloindole-Prodrug Carzelesin in Mice, Rats, and Humans

Olaf van Tellingen, Willem J. Nooijen, Larry J. Schaaf, Martin van der Valk, Judith van Asperen, Roland E. C. Henrar, and Jos H. Beijnen

Department of Clinical Chemistry, The Netherlands Cancer Institute, Amsterdam, The Netherlands [M. v.d.V.]; Department of Experimental Animal Pathology, Division of Molecular Genetics, The Netherlands Cancer Institute, Amsterdam, The Netherlands [W. J. N.]; Department of Pharmacy, Sloelheraut Hospital, Louweweg 6, 1066 EC Amsterdam, The Netherlands [J. H. B.]; European Organization for Research and Treatment of Cancer, New Drug Development Office, Free University Hospital, Amsterdam, The Netherlands [R. E. C. H.]; and Pharmacia and Upjohn Company, Kalamazoo, Michigan 49001-0199 [L. J. S.]

ABSTRACT

Carzelesin is a novel cyclopropylpyrroloindole prodrug analogue that has recently been tested in Phase I clinical trials. To increase our understanding in the pharmacology of this new class of cytotoxic drugs, we have compared the pharmacology of this drug in mice, rats, and humans. The mouse was the most tolerant (10% lethal dose $LD_{10}$, 500 ng/kg), the rat was intermediate ($LD_{50}$, 40 μg/kg), and humans were the least tolerant species in this series (maximum tolerated dose, 300 μg/kg corresponding to 7.5 μg/kg). In both mice and humans, bone marrow toxicity was the primary toxic side effect. Pharmacokinetic studies, using a validated high-performance liquid chromatographic procedure, revealed that differences in drug clearance and conversion to the active drug (U-76,074) could not explain the substantial interspecies differences. The area under the plasma concentration time curve (AUCs) of carzelesin in mice and rats at their LD₅₀ were about 80- and 20-fold higher, respectively, than in humans receiving the maximum tolerated dose, whereas the respective AUCs of U-76,074 in mice and rats were 50- and 10-fold higher. By using a colony-forming assay with bone marrow stem cells from mice and humans, we observed only a 3-fold higher toxicity in the latter. Although some of this discrepancy may be explained by the fact that the in vitro and the in vivo assays probably reflect the toxicity on different populations of colony-forming units, the tolerance of the mouse bone marrow in vivo against the very high drug levels in plasma suggest the presence of a protective mechanism, which is less active in humans. An important consequence of the much higher susceptibility of the human bone marrow against the very high drug levels in plasma suggest the presence of a protective mechanism, which is less active in humans. An important consequence of the much higher susceptibility of the human bone marrow in vivo was that plasma drug levels were much below active concentrations achieved in mice, and it is clear that this may compromise the successful use of this agent in the clinic. Ultimately, however, the efficacy of this drug will be established in Phase II clinical trials.

INTRODUCTION

Carzelesin is a synthetic prodrug analogue of the naturally occurring CPI³ compound, CC-1065 (Fig. 1A), which is an extremely potent cytotoxic agent produced by the soil organism Streptomyces zelesnis. The powerful antitumor properties of CC-1065 were already recognized in the mid-1970s; however, the delayed fatal toxicity (hepatotoxicity) that was observed in mice and rabbits treated at subtherapeutic dose levels precluded further clinical development (1). Investigations to unravel the mechanism of action by which this CPI drug exerted its cytotoxicity revealed that the agent enters into the minor groove of AT-rich regions in double-stranded DNA and consequently binds covalently to the N³ position of adenine (2, 3), thus forming DNA adducts in a sequence-selective fashion. This unique DNA interactive property made CC-1065 a model compound for the design and synthesis of a series of CPI analogues, and thus far, these efforts have lead to the generation of three potentially useful compounds, i.e., adozelesin, bizelesin, and carzelesin (Fig. 1A). All three compounds share the extreme potency of CC-1065, as demonstrated by in vitro cytotoxicity studies with tumor cell lines, and have displayed a broad spectrum of activity against transplanted tumors in mice without exerting the ominous toxic side effects (4–7). Adozelesin, which still possesses the active CPI moiety, is the most closely related analogue of CC-1065 and was found to be the most reactive compound in vivo (8, 9). Bizelesin is a CPI dimer, thus providing the potential to form DNA interstrand cross-links, a characteristic that distinguishes this compound from the classical CPI drugs (6, 10). Clinical studies with this drug have started recently.

Carzelesin is a prodrug CPI analogue that requires activation through hydrolysis of the phenylurethane substituent yielding U-76,073, followed by ring closure to form the CPI-containing DNA-reactive product U-76,074 (Fig. 1B). Although carzelesin appeared to be less potent against tumor cells grown in vitro than adozelesin (4), it compared favorably in its activity against experimental tumors grown in mice (7). It has been hypothesized that differences in the pharmacokinetic behavior and DNA alkylation of this prodrug relative to adozelesin might be responsible for this enhanced in vivo antitumor activity (11). Based on these promising preclinical results, the drug was selected for Phase I clinical evaluation, using a daily times 5 schedule (12) and as single bolus administration.⁴

The CPI drugs are a relatively new class of compounds being tested in clinical trials and, consequently, the overall knowledge about the pharmacology of these drugs is still very limited. Due to the absence of an analytical method that was sufficiently sensitive, the clinical trials with adozelesin have been completed without the establishment of a full understanding of the pharmacokinetic characteristics of this agent (8, 9). To support the Phase I clinical trials with carzelesin, we have developed a method for the simultaneous determination of carzelesin and the metabolites U-76,073 and U-76,074 in human plasma that was sufficiently sensitive to enable drug monitoring from the first dose step (13). Mice and rats are the primary species used in the European Organization for Research and Treatment of Cancer preclinical toxicology studies. We here report about the validation of our analytical method for plasma from mice and rats and the comprehensive pharmacokinetic analysis in these animals. The results have been used to compare the pharmacokinetics-toxicity relationships in mice, rats, and humans and to increase our general knowledge about the pharmacology of the CPI drug carzelesin.

Received 1/14/98; accepted 4/1/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported by a grant from the Pharmacia and Upjohn Company.

2 To whom requests for reprints should be addressed, at Department of Clinical Chemistry, The Netherlands Cancer Institute/Antoni van Leeuwenhoek Huis, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Phone: 31 20 512 2792; Fax: 31 20 617 2625; E-mail: OTEL@nki.nl.

3 The abbreviations used are: CPI, cyclopropylpyrroloindole; HPLC, high-performance liquid chromatography; MTD, maximum tolerated dose; CFU, colony-forming unit; IMDM, Iscove's modified Dulbecco's medium; LD₅₀, 10% lethal dose; AUC, area under the plasma concentration time curve; IC₅₀, 50% inhibitory concentration.

4 A. Awada, C. J. A. Punt, M. J. Piccart, O. van Tellingen, L. van Manen, J. Kerger, Y. Grots, J. Wenders, J. Verwey, and D. J. T. Wagener, Phase I study of carzelesin (U-80, 244), a DNA sequence-specific alkylating agent administered on a 4-weekly intravenous bolus schedule: an EORTC-ECSG study, manuscript in preparation.
COMPARATIVE PHARMACOLOGY OF CARZELESIN

A

**Fig. 1.** A. Molecular structures of the CPI compounds CC-1065, adozolesin, bizelesin, and carzelesin. B. Molecular structures of carzelesin, the intermediate conversion product U-76,073, and the active CPI compound U-76,074.

MATERIALS AND METHODS

**Drugs and Chemicals.** Carzelesin, U-76,073, and U-76,074 were obtained as pure powders from Pharmacia-Upjohn Company. The in vivo pharmacokinetics studies were carried out with 250 μg/ml carzelesin formulated in a solution consisting of polyethylene glycol 400: absolute ethanol:Tween 80 (6:3:1, v/v/v; Ref. 14). Sterile glucose solution 5% (w/w) and CPDA solution (3.27 g/L citric acid monohydrate, 26.3 g/L sodium citrate dihydrate, 31.9 g/L glucose monohydrate, 2.51 g/L sodium dihydrogen phosphate dihydrate, 0.275 g/L adenine in water for injection) were from NPBI (Emmer Compascuum, The Netherlands). All other reagents were purchased from E. Merck (Darmstadt, Germany) and were of analytical grade, except for acetonitrile, which was of HPLC gradient quality. Blank human plasma was obtained from the Central Laboratory of the Blood Transfusion Service (Amsterdam, The Netherlands). All other reagents were purchased from J. T. Baker (Phillipsburg, NJ).

**Animal Studies.** Animal studies were carried out in male FVB mice, 6–12 weeks of age, and male WAG/Rij rats of 16–20 weeks. All animals were housed according to institutional guidelines in a protected environment in conventional plastic cages and maintained on an automatic 12-h day/night cycle at an ambient temperature of 22–24°C. The animals were given chow (Hope Farms BV, Woerden, The Netherlands) and acidified water ad libitum.

The drug formulation was diluted with 5% (w/w) sterile glucose solution to achieve final drug concentrations in the range of 20–100 μg/ml. These solutions were kept at 4°C and used on the day of their preparation. The drug solution was administered as an i.v. bolus injection in the tail vein. Each animal was weighed individually and was lightly anesthetized prior to drug administration to facilitate the injection.

The acute toxicity of the drug in mice was determined using 60 animals, divided in six groups of 10 mice each. The tested dose levels were 350, 400, 450, 500, 550, and 600 μg/kg. The acute toxicity in rats was tested at dose levels of 10, 30, 40, 50, 60, and 200 μg/kg, with three to five animals per group. The animals were monitored each day for 3 weeks.

In a separate experiment, three groups of three mice each received 400, 500, and 600 μg/kg of carzelesin. Four days later, the animals were sacrificed, and femur, sternum, spleen, liver, kidney, stomach, small gut, cecum, and colon were dissected and placed in Harrison’s fixative (15) for histological examination after H&E staining.

Pharmacokinetic studies in mice were performed at dose levels of 100, 250, and 500 μg/kg. Animals were sacrificed at approximately 1, 2, 5, 15, 30, and
45 min and 1, 2, 3, 4, 6, and 8 h after drug administration. About three to four animals were used per time point. Blood was collected by cardiac puncture under diethyl ether anesthesia. The samples were immediately transferred into heparin containing Eppendorf vials and cooled in ice water. Next, the samples were centrifuged (5 min, 2500 × g, 4°C), and the plasma fraction was stored at −20°C until analysis.

The pharmacokinetics of carzelesin in rats was studied using six animals receiving 40 μg/kg of drug. Serial blood samples were obtained by a canulae, which was placed into the carotid artery. The surgical procedure for placement of the canulae was performed 3–4 h before drug administration. Blood was collected in Eppendorf heparin-containing vials and centrifuged immediately (1 min, 11,000 × g) at ambient temperature. Aliquots of 50 or 300 μl of plasma sample (depending on the anticipated concentration) were frozen at −70°C using ethanol-solid carbon dioxide. The samples were then kept at −20°C and analyzed the same day.

**Human Pharmacokinetic Studies.** A detailed report about the clinical pharmacokinetics of carzelesin has been published recently (16). In brief, carzelesin was administered as a brief 10 min i.v. infusion during the first course of chemotherapy. Blood samples were collected in heparin or EDTA-containing tubes and immediately cooled in ice water. Next, plasma was separated by centrifugation (5 min, 2500 × g, 4°C) within 40 min and stored at −20°C until analyses.

**Analytical Procedures.** The plasma levels of carzelesin and the metabolites U-76,073 and U-76,074 were determined by using a semiautomated method based on HPLC, as described in detail previously (13). In brief, 1000 μl of human plasma was mixed with 3000 μl of 20% (v/v) acetic acid in water and subjected to solid-phase extraction on SPE C18 columns. The column was consecutively washed with 2000 μl of water and 2000 μl of acetonitrile, and the compounds were eluted with 600 μl of dimethylacetamide. An aliquot of 500 μl was subjected to HPLC. The three compounds were first separated from endogenous interferences on a Spherisorb CN column using a linear gradient from 24 to 60% (v/v) of acetonitrile in 20 mM phosphate buffer (pH 6.5). Next, final separation was achieved on a Spherisorb ODS2 column, and UV detection at 360 nm resulted in a lower limit of quantitation of 1 ng/ml of each compound.

Plasma samples from mice (5–300 μl) and rats (50–300 μl) were supplemented to 1000 μl with blank human plasma and processed further as described above. The concentrations of carzelesin, U-76,073, and U-76,074 were determined from a calibration curve constructed in human plasma. Thawing of plasma samples from all species was always performed at 0–4°C in ice water. The samples were vortex-mixed, centrifuged (3000 × g, 5 min, 4°C) to remove precipitates that could block the SPE columns, and maintained at 4°C until further analysis within 4 h.

Validity. The suitability of the analytical procedure for the determination of carzelesin, U-76,073, and U-76,074 in plasma from mice and rats has been checked by adding blank plasma of these species with various amounts of each test compound.

**Mouse.** A set of four control samples was prepared in ice-cold, drug-free plasma, and aliquots were stored at −20°C until analysis. Control 1 contained 5000 ng/ml of carzelesin, control 2 contained 1000 ng/ml of U-76,073, and controls 3 and 4 contained 200 and 10 ng/ml, respectively, of all three compounds. Aliquots of 10, 50, 100, and 300 μl supplemented to 1000 μl with blank human plasma were used for the analysis of controls 1, 2, 3, and 4, respectively. Drug determinations in these control samples were performed in duplicate at the day of preparation (day 0) and with repeated freezing and thawing of the specimen on days 1 and 2. Furthermore, an aliquot of each control was assayed after 54 days to judge long-term stability.

**Rat.** Controls 1 and 2 contained 1000 ng/ml of carzelesin and U-76,073, respectively, whereas controls 3 and 4 contained 200 and 40 ng/ml, respectively, of all three compounds. Aliquots of 50, 50, 100, and 300 μl of controls 1–4, respectively, supplemented to 1000 μl with blank human plasma were used for the analysis.

**Recovery of U-76,073 and U-76,074 in Rat Plasma.** The recovery of U-76,073 was tested by mixing 5 μl of 10 μg/ml of U-76,073 in dimethyldicetamide with 50 or 300 μl of ice-cold blank rat plasma. Next, 950 or 700 μl of ice-cold blank human plasma were added, and the sample was subjected to analysis without delay. The same procedure was used for U-76,074.

**Stability in Plasma.** The in vitro stability of carzelesin and U-76,073 in plasma of mice, rats, and humans was tested at 37°C. To 50 μl of prewarmed (37°C) blank plasma, 5 μl of 10 μg/ml of carzelesin or U-76,073 in dimethyldicetamide were added, and the samples were incubated for 5 and 15 min. Next, the samples were cooled in ice-water, and 950 μl of ice-cold blank human plasma were added. The samples were analyzed within 30 min. Ice-cold plasma supplemented with the same amount of drug was used as reference (t = 0) sample.

**Pharmacokinetic Data Analysis.** In mice and rats, the plasma concentration-time curves of carzelesin were fitted using a two-compartment open model with a weighing of 1/ν (where ν represents the concentration). The computations were performed with the MW Pharm software package version 3 (MEDIWARE BV, Groningen, The Netherlands; Ref. 17).

Because of the absence of a suitable model for the description of the concentration-time profiles of the metabolites U-76,073 and U-76,074, the plasma AUC of these compounds was calculated by using the linear trapezoidal rule up to the last time point where the concentration was above the lower limit of quantitation of the assay. The terminal half-lives [1(1/2)] of both compounds were calculated by linear regression analysis of the log concentrations (abscissa) versus time (ordinate) data, using the time points in the final log-linear (elimination) part of the concentration-time curve.

The pharmacokinetics data in humans at the MTD (300 μg/m²) has been derived from our previous report (16).

**Bone Marrow Stem Cell Assay.** The toxicity of carzelesin and U-76,074 against murine and human hematopoietic stem cells was tested by in vitro CFU assays.

Under sterile conditions, 2.5 ml of human bone marrow were added to 10 ml of ice-cold ammonium chloride solution (StemCell Technology, Inc., Vancouver, British Columbia, Canada) and incubated in ice for 10 min. After centrifugation (10 min, 300 × g, ambient temperature), the supernatant was discarded, and the cell pellet was resuspended in 10 ml of 2% (v/v) FCS in IMDM (StemCell Technology). After centrifugation (10 min, 300 × g), the supernatant was discarded, and the cell pellet was resuspended in 2 ml of 2% FCS in IMDM. The yield was 16.4 × 10⁶ cells/ml. This cell suspension was diluted 8-fold in 2% FCS in IMDM, and aliquots of 250 μl were incubated with 250 μl of carzelesin or U-76,074 diluted in 2% FCS in IMDM. The final drug concentrations used during the incubation was 0 (control), 0.1, 0.3, 1, 3, 10, and 100 ng/ml. The tubes were incubated at 37°C for 90 min and then centrifuged (5 min, 300 × g, ambient temperature). The cell pellet was washed once with 500 μl of 2% FCS in IMDM and then resuspended in 250 μl of carzelesin or U-76,074 diluted in 2% FCS in IMDM. Next, 2.5 ml of Methocult GF H4534 (StemCell Technology) were added, and the tubes were mixed vigorously. Aliquots of 1 ml were plated in duplicate in six-well culture plates (Greiner, Alphen a/d Rijn, The Netherlands) and incubated for 8 days at 37°C in 5% CO₂ in humidified air. CFUs were scored by phase-contrast light microscopy.

Mouse bone marrow stem cells were obtained from the femurs of FVB mice flushed with −250 μl of a CPDA solution. After centrifugation (5 min, 300 × g), the supernatant was discarded, and the cell pellet was washed once with 500 μl of 2% FCS in IMDM and, next, resuspended in 250 μl of carzelesin or U-76,074 diluted in 2% FCS in IMDM. Next, 2.5 ml of Methocult GF H4534 (StemCell Technology) were added, and the tubes were mixed vigorously. Aliquots of 1 ml were plated in duplicate in six-well culture plates (Greiner, Alphen a/d Rijn, The Netherlands) and incubated for 8 days at 37°C in 5% CO₂ in humidified air. CFUs were scored by phase-contrast light microscopy.

**RESULTS**

**Validation.** The assay appeared to be suited for the determination of carzelesin, U-76,073, and U-76,074 in plasma of mice. The observed concentrations of these compounds in controls 1–4 measured on the day of their preparation were within the acceptable ±15% deviation of the nominal values (Table 1). The lower limit of quantitation for each of the three compounds is 1 ng/ml when 1000 μl of human plasma is used (13). However, because the maximum volume of mouse plasma sample used for the determination was limited to 300 μl (supplemented to 1000 μl with blank human plasma), the lower limit of quantitation was 3.3 ng/ml. The observed concentration of U-76,074 in control 4 was biased from its nominal value, but this was due to the presence of a minor chromatographic impurity in the blank plasma specimen that was used to prepare this control sample. The
peak area of this impurity corresponded to a concentration of ~2.7 ng/ml. This impurity was not found in blank samples of several other mice. Although repeated thawing and freezing resulted in some conversion of carzelesin to U-76,073 and U-76,074, this effect was small and did not notably diminish the accuracy of the assay (Table 1). The conversion of carzelesin to U-76,073 and U-76,074 occurred at a much faster rate. The concentration of U-76,074 observed after storage of the sample at -20°C for 54 days was ~80% of the nominal value. Because the difference was not recovered as U-76,074, this deviation was probably due to variation in the assay and not caused by conversion.

Acceptable results for the determination of carzelesin were also obtained in the rat plasma specimens. However, the accurate determination of the metabolites in these samples was troubled by a major analytical problem. The concentrations of U-76,073 observed in controls 2, 3, and 4 were about 87, 75, and 40% of their nominal value, respectively. Additional experiments using rat plasma samples supplemented with U-76,073 alone revealed that the recovery depended on the amount of rat plasma that was used. The recovery decreased from 89% when 50 μl of rat plasma sample was analyzed to 54% with 300 μl of sample. At the same time, the amount of U-76,074 observed in these samples increased from 11.2 to 20.4% of the concentration of U-76,073 added to these samples. The recovery of U-76,074 added to rat plasma was 83% when using 300 μl of sample.

Stability in Plasma. The in vitro stability of carzelesin and U-76,073 in plasma incubated at 37°C was tested in plasma of mice, rats, and humans (Table 2). To examine the nature of the toxicity in more detail, we performed histology of some of the most relevant tissues. At all three dose levels tested (400, 500, and 600 μg/kg), there was a serious depletion of bone marrow and the red pulp of the spleen. Only minor changes were found in the other tissues. Kidney showed more or less dose-dependent protein loss (“casts”) and increasing cell density of glomeruli (glomerulitis). The intestinal crypts revealed high numbers of apoptotic cells, atypical mitoses, and dysplasia; however, there was no necrosis or appreciable inflammatory reactions detected at 4 days after administration. The rapid death of the animals might relate to insufficient water uptake due to general distress, renal protein loss, or nondetected physiological changes or pathology of other organs.
COMPARATIVE PHARMACOLOGY OF CARZELESIN

4 h after drug administration. The metabolites U-76,073 and U-76,074 were found in considerable amounts, and in contrast to the parent drug, these metabolites remained detectable for more than 7 h after administration of 500 µg/kg.

Within the range of dose levels tested in mice, carzelesin displayed linear pharmacokinetics because the clearance was independent on the dose.

A similar biphasic decline of carzelesin plasma levels with comparable α and β half-lives was observed in rats (Fig. 3 and Table 4). However, at their respective LD10, the plasma levels of carzelesin in rats were only about one-twentieth of those achieved in mice. The plasma level of U-76,073 in rats was also much lower than in mice, whereas the plasma level of U-76,074 was relatively high.

A detailed report about the clinical pharmacokinetics of carzelesin has been presented elsewhere (16). In humans, carzelesin was administered by a 10-min i.v. infusion. The MTD of carzelesin was established at 300 µg/m², corresponding to ~7.5 µg/kg. The plasma concentration decayed with α and β half-lives comparable with those in mice and rats. At this MTD, the plasma AUC of the parent compound was almost 80-fold lower than observed in mice at the LD10 (Fig. 4 and Table 4). The plasma levels of both U-76,073 and U-76,074 in humans were about 50-fold lower. Interestingly, the clearance calculated on the dose level normalized to body weight was fairly similar in all three species (Table 4).

Bone Marrow Stem Cell Toxicity Assay. Because the clinical studies with the CPI drugs have revealed that bone marrow toxicity is the dose-limiting toxicity, we have compared the relative toxicities of carzelesin and the active compound U-76,074 on human and murine hematopoietic stem cells. Based on our pharmacokinetic data, a drug incubation time of 90 min was chosen because this was a close approximation of the actual in vivo situation. From these in vitro tests, it appeared that the human CPUs were about 3-fold more sensitive to both compounds than murine CPUs (Table 5).

DISCUSSION

This pharmacological study shows that the substantial differences in tolerance to carzelesin that are found among mice, rats, and humans are not related to proportional higher weight-normalized clearance of this drug in the more tolerant species. The mouse is the most tolerant species, allowing the administration of up to 500 µg/kg (body weight) of carzelesin, resulting in a plasma AUC of 2440 ng/ml-h, whereas the plasma AUCs achieved in rats and humans at their respective LD10/MTD are 20- and 80-fold lower.

The mouse is the primary species used for testing the safety of new anticancer drugs and selecting an appropriate starting dose for Phase I clinical trials. Dose transformations from mice to humans are performed by using body surface, as Freireich et al. (18) demonstrated that toxicity of anticancer drugs across species correlates relatively well with dose levels expressed on a mg/m² basis. Usually, one-tenth of the LD10 is taken as a safe starting point, and dose escalation is performed according to a modified Fibonacci scheme. The preclinical toxicology and safety studies for carzelesin have been conducted in MF1 mice and showed an LD10 of 280 µg/kg. This is substantially lower than the LD10 in FVB mice observed in this study, indicating an intraspecies, interstrain difference in tolerance. Based on the LD10 in MF1 mice (280 µg/kg), the safe starting dose of a Phase I clinical trial with carzelesin given as a single dose schedule repeated every 4 weeks (European Organization for Research and Treatment of Cancer protocol ND-05912) would have been 84 µg/m². However, because preclinical toxicology and safety studies of carzelesin in rats demonstrated a species difference in toxicity, with the rat being three
COMPARATIVE PHARMACOLOGY OF CARZELESIN

### Table 4 Pharmacokinetic parameters of carzelesin and metabolites in mice, rats, and humans

<table>
<thead>
<tr>
<th>Dose *μg/kg</th>
<th>C_{max} ng/ml</th>
<th>t_{1/2} (α) min</th>
<th>t_{1/2} (β) min</th>
<th>AUC ng·h/ml</th>
<th>C_{max} ng/ml</th>
<th>t_{1/2} (β) min</th>
<th>AUC ng·h/ml</th>
<th>C_{max} ng/ml</th>
<th>t_{1/2} (β) min</th>
<th>AUC ng·h/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>~300</td>
<td>4.4</td>
<td>21</td>
<td>480</td>
<td>0.63</td>
<td>0.21</td>
<td>26</td>
<td>108</td>
<td>490</td>
<td>200</td>
</tr>
<tr>
<td>250</td>
<td>~750</td>
<td>5.9</td>
<td>22</td>
<td>990</td>
<td>0.76</td>
<td>0.25</td>
<td>41</td>
<td>135</td>
<td>560</td>
<td>99</td>
</tr>
<tr>
<td>500</td>
<td>~1500</td>
<td>7.6</td>
<td>36</td>
<td>2440</td>
<td>0.62</td>
<td>0.21</td>
<td>40</td>
<td>179</td>
<td>990</td>
<td>106</td>
</tr>
<tr>
<td>Rats</td>
<td>40</td>
<td>670</td>
<td>4.3</td>
<td>120</td>
<td>2.00</td>
<td>0.33</td>
<td>27</td>
<td>171</td>
<td>62</td>
<td>31</td>
</tr>
<tr>
<td>Man*</td>
<td>~7.5</td>
<td>300</td>
<td>6.3</td>
<td>26</td>
<td>9.4</td>
<td>0.23</td>
<td>38</td>
<td>135</td>
<td>18</td>
<td>46</td>
</tr>
</tbody>
</table>

- Dose transformation from μg/kg to the approximate (≈) μg/m² dose (and vice versa for humans) are based on the formula: dose (mg/kg) = km × dose (mg/m²) with km being 3, 6, and 40 for mice, rats, and humans, respectively (18).
- t_{1/2} (β) could not be calculated due to scatter in the data.
- Mean values from three patients [data compiled from van Tellingen et al. (16)].

---

**Fig. 3.** Plasma concentration versus time curves of carzelesin (•), U-76.073 (○), and U-76.074 (△) in rats after administration of 40 μg/kg of carzelesin by i.v. bolus injection. Bars, SE.

**Fig. 4.** Typical plasma concentration versus time curve for carzelesin in humans (16). The dose level of carzelesin was 300 μg/m² given as a brief 10-min i.v. infusion. •, carzelesin; ○, U-76.073; △, U-76.074.

---

Times more sensitive, a more conservative starting dose of 24 μg/m² (i.e., approximately one-thirtieth of the mouse LD_{10}) was selected.

Although dose transformations from mice to humans based on body surface have been useful, Collins et al. (19) proposed, based on a retrospective analysis of preclinical pharmacological data, that differences between species in their sensitivity toward anticancer drugs may be better correlated with their respective drug plasma concentration × time (AUC) value. It was hypothesized that using pharmacokinetic-guided dose escalation may permit accelerated dose escalations during clinical trials. Since then, pharmacokinetic-guided dose escalations have been successfully implemented in a number of cases (20). However, exceptions like carzelesin, where the target AUC established in mice is so much higher than the AUC achieved in humans receiving the MTD, make this strategy risky.

The reason why the plasma AUC of carzelesin in mice, rats, and humans at approximate equitoxic dosages is so different is not clear. Carzelesin is a prodrug CPI analogue that requires conversion, via the intermediate metabolite U-76,073, to the active CPI product U-76,074. It was thought that interspecies differences in the formation rate of the active metabolite might attribute to the differences in tolerance noted between the several species. Although the relative faster conversion rate of U-76,073 into the active compound U-76,074 in rat plasma relative to mice plasma does correspond to the differences in tolerance between these two species, it cannot account for the even much larger difference in tolerance observed between mice and humans. Under in vitro conditions, the conversion rates in mouse and human plasma were comparable, whereas in vivo the ratios between the plasma AUCs of carzelesin and U-76,073 or U-76,074 were very similar for mice and humans.

An alternative explanation might be an interspecies difference in tissue penetration, which may result from differences in the plasma protein binding between species. A lower protein binding in human plasma could result in a higher tissue penetration and thus higher toxicity. The issue of the plasma protein binding of carzelesin, however, is difficult to assess because classical methods to determine protein binding at physiological conditions (e.g., equilibrium analyses and ultrafiltration) are hindered by the rapid conversion rate of carzelesin in plasma. An alternative mathematical approach to estimate the relative tissue penetration by calculation of the apparent volumes of distribution (V_{p}) using the classical equation: V_{p} = CL/β [where CL is clearance and β is the 0.693/t_{1/2} (β)] is not appropriate. Usually a high V_{p} is associated with extensive tissue penetration. However, carzelesin is also eliminated from the peripheral compartment (i.e., carzelesin molecules that have penetrated into the tissue will be converted to U-76,074 and may then bind to intracellular constituents), and the contribution of this peripheral elimination in the various times is not accounted for by classical volume of distribution calculations.

---

**Table 5 Toxicity of carzelesin and U-76,074 on bone marrow stem cells**

<table>
<thead>
<tr>
<th>Concentration (ng/ml)</th>
<th>Carzelesin</th>
<th>U-76,074</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human</td>
<td>Mouse</td>
</tr>
<tr>
<td>0.10</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>0.30</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>1.0</td>
<td>92%</td>
<td>95%</td>
</tr>
<tr>
<td>3.0</td>
<td>55%</td>
<td>60%</td>
</tr>
<tr>
<td>10</td>
<td>0%</td>
<td>20%</td>
</tr>
<tr>
<td>30</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Mean values from three patients [data compiled from van Tellingen et al. (16)].

Downloaded from cancerres.aacrjournals.org on July 24, 2017. © 1998 American Association for Cancer Research.
species cannot be established from our data. Consequently, the value obtained for $V_e$ underestimates the extent of tissue penetration, and the use of this parameter may not result in an accurate reflection of the relative differences in tissue distribution between species. However, although $V_e$ cannot be used, the findings that in all species the $V_c$ (volume of the central compartment) approximates the plasma volume (blood volume/hematocrit) and that the distribution ($\alpha$) half-life is similar suggest that substantial differences in tissue distribution are not very likely.

In both mice and humans, the most obvious toxic events occur in the bone marrow. The higher susceptibility of the human bone marrow may result from: (a) a more efficient uptake and DNA binding of carzelesin and or metabolites by the human hematopoietic stem cells; (b) a higher susceptibility of the human stem cells to the effects caused by DNA binding in the AT-rich sequences (2, 3); or (c) both. The high susceptibility of the bone marrow to carzelesin is not unique caused by DNA binding in the AT-rich sequences (2, 3); or (c) both. The high susceptibility of the bone marrow is also affected. The in vitro culture assay, however, may only measure the effects against the more differentiated CPUs. How to identify the nature of this toxic side effect may be helpful to design more effective CPI analogues.

**REFERENCES**


Comparative Pharmacology of the Novel Cyclopropylpyrroloindole-Prodrug Carzelesin in Mice, Rats, and Humans

Olaf van Tellingen, Willem J. Nooijen, Larry J. Schaaf, et al.


Updated version  Access the most recent version of this article at:  
http://cancerres.aacrjournals.org/content/58/11/2410

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.