Cancer Chemother Pharmacol (1998) 41: 377–384

ORIGINAL ARTICLE

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A clinical pharmacokinetics study of carzelesin given by short-term intravenous infusion in a phase I study

Received: 6 May 1997 / Accepted: 5 September 1997

Abstract We investigated the pharmacokinetic behavior of carzelesin in 31 patients receiving this drug by 10-min intravenous infusion in a Phase I clinical trial, which was conducted at institutions in Nijmegen (institution 1) and Brussels (institution 2). The dose steps were 24, 48, 96, 130, 150, 170, 210, 250, and 300 μg/m^2. Carzelesin is a cyclopropylpyrroloindole prodrug that requires metabolic activation via U-76,073 to U-76,074. The lower limit of quantitation (LLQ) of the high-performance liquid chromatography (HPLC) method used in this study was 1 ng/ml for the parent drug and its metabolic products. Carzelesin was rapidly eliminated from plasma (elimination half-life 23 ± 9 min; mean value ± SD). At all dose levels, U-76,073 was found as early as in the first samples taken after the start of the infusion. However, the concentration of U-76,074 exceeded the LLQ for only short periods and only at the higher dose levels. Although the plasma levels of all three compounds were well above the respective IC_{50} values obtained by in vitro clonogenic assays, they were much lower than those observed in a preclinical study in mice. There was a substantial discrepancy in the mean plasma clearance observed between patients from institution 1 (7.9 ± 2.1 l h^{-1} m^{-2}) and those from institution 2 (18.4 ± 13.6 l h^{-1} m^{-2}; $P = 0.038$), probably reflecting problems with drug administration in the latter institution. The results recorded for patients in institution 1 indicated that the AUC increased proportionately with increasing doses. There was a good correlation between the maximal plasma concentration and the AUC, enabling future monitoring of drug exposure from one timed blood sample. Urinary excretion of carzelesin was below 1% of the delivered dose.

Key words Cyclopropylpyrroloindole prodrug · High-performance liquid chromatography · Pharmacokinetics · Plasma clearance

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Introduction

Carzelesin (U-80,244, Fig. 1) is a cyclopropylpyrroloindole (CPI) prodrug analog designed after CC-1065 and is the result of synthetic efforts to discover clinically useful CPI drugs. CC-1065 is a natural product isolated from the soil organism *Streptomyces zelenis*, which proved to be an extremely potent cytotoxic compound. However, its clinical development as an antitumor agent was halted because preclinical toxicology studies revealed that this agent caused delayed fatal toxicity in mice and rabbits at subtherapeutic doses [8]. CPI drugs such as CC-1065 are a class of compounds with unique DNA-interactive properties. These agents fit into the minor groove region of DNA, and the CPI function in the molecule mediates a covalent bonding to the N^3-position of adenine in A-T rich regions, thus forming DNA adducts in a sequence-selective fashion [3, 10].

Thus far, three new leads have emerged from this drug development program, each with unique properties. Adozelesin is most closely related to CC-1065 as it possesses the active CPI moiety (Fig. 1). The late toxic effects seen with CC-1065 were abolished by changing of

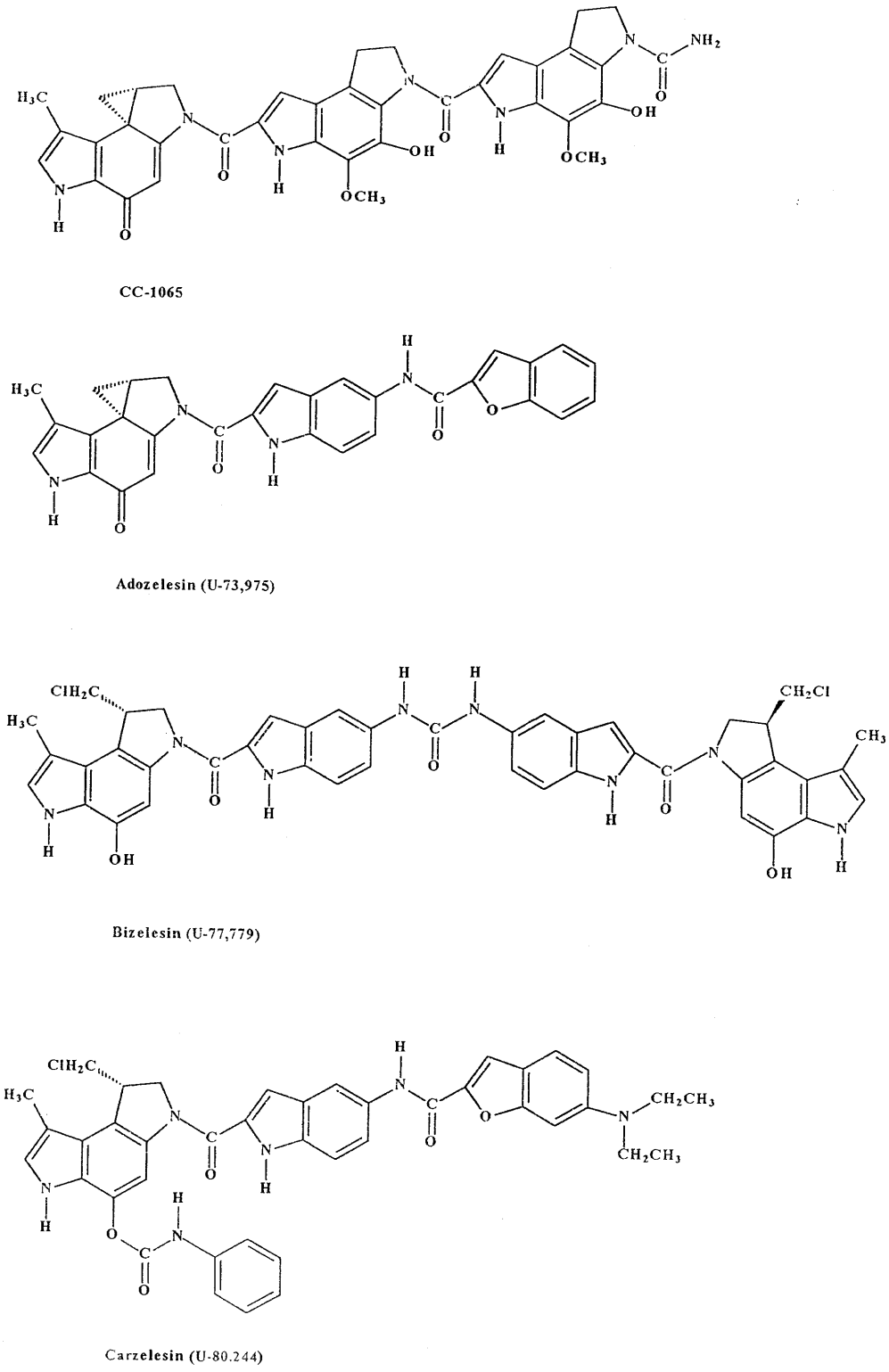

Fig. 1 Molecular structures of CC-1065, adozelesin (U-73,975), bizelesin (U77,779), and carzelesin (U-80,244)

the right-hand side of the molecule [6]. Bizelesin differs from the classic CPI drugs in that it contains two chloromethyl functions, both of which are converted (via intramolecular rearrangements) to the CPI alkylating species that interact with DNA. Consequently, this agent is capable of forming DNA interstrand cross-links [5]. Carzelesin was designed to be an inactive prodrug, as earlier studies with CC-1065 had revealed that DNA alkylation by CPI drugs occurred in a rapid fashion [10, 14]. Therefore, efforts were directed at modulating the alkylation rate by the preparation of CPI prodrugs that would require chemical or enzymatic conversion. The

activation of carzelesin is a two-step reaction involving hydrolysis of the phenylurethane substituent to form the intermediate compound U-76,073, followed by ring closure to form the active product U-76,074 (Fig. 2). Although carzelesin was found to be less potent than adozelesin against tumor cells cultured in vitro, it proved to be more efficacious in a broad panel of murine and human tumor xenografts [7]. This better efficacy may be related to the pharmacokinetic behavior of this compound. Due to the high-level reactivity of the CPI moiety, the inactive prodrug may be capable of reaching deeper into the (target) tissues before it is converted to the active species.

All three compounds had displayed promising results in preclinical testing and were selected for phase I testing. Two phase I clinical trials of adozelesin have been completed [1, 11]. The maximum tolerated dose (MTD) of this drug given by brief i.v. infusion was established at

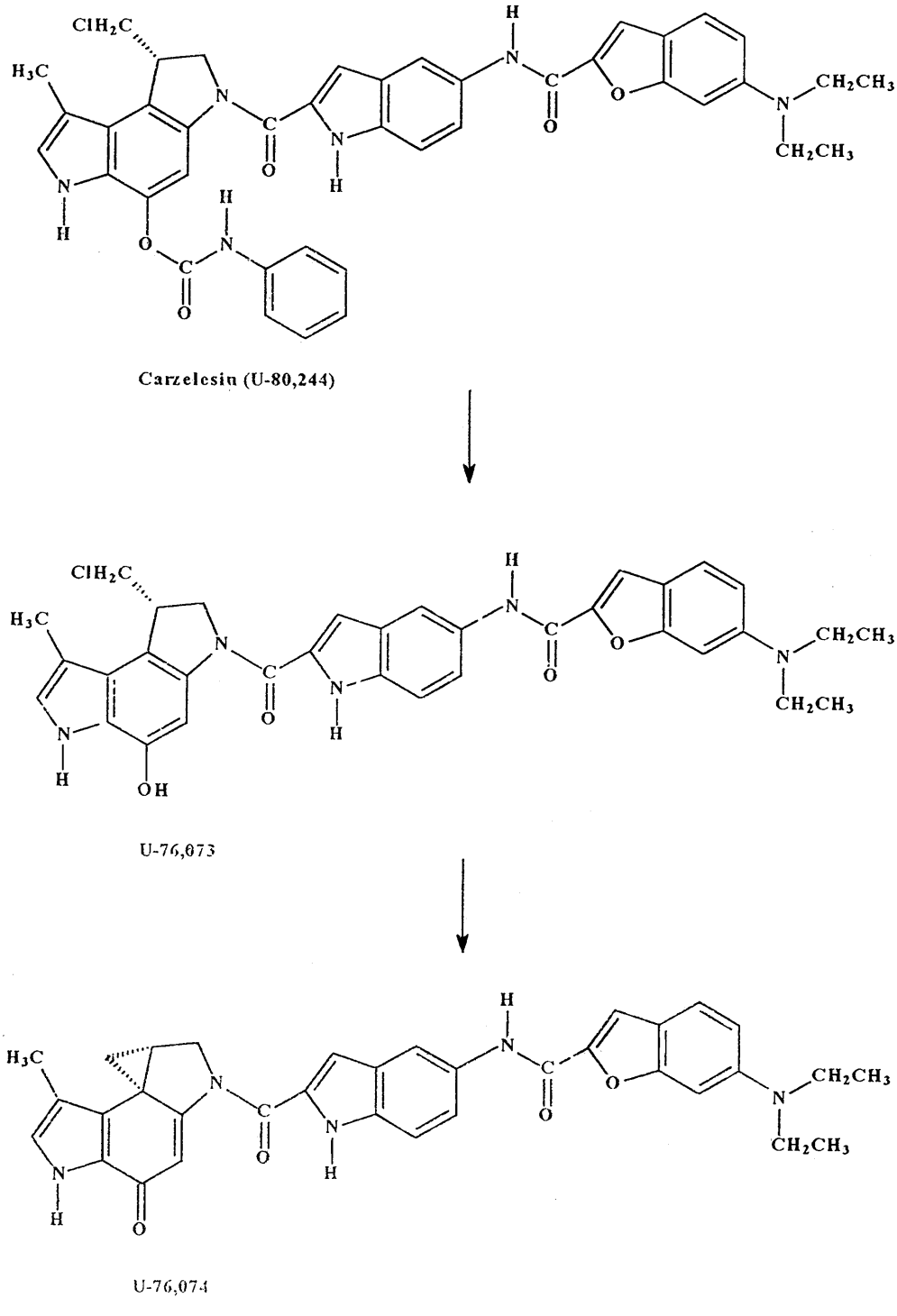

Fig. 2 Molecular structures of carzelesin (U-80,244), the intermediate metabolite U-76,073, and the active metabolite U-76,074

188 μg/m^2 with dose-limiting myelotoxicity. Due to the absence of a sufficiently sensitive analytical method, information about the pharmacokinetic behavior of this drug is lacking. The phase I trial of bizelesin remains in the planning stage.

Coordinated by the EORTC-NDDO, two multicenter phase I studies of carzelesin were started in Europe. The patients received the drug by a brief (10-min) i.v. infusion given once every 4 weeks or in a daily 5 schedule repeated every 4 weeks [15]. On the basis of preclinical toxicology studies in mice and rats, the starting dose levels in these trials were 24 μg/m^2 and 12 μg m^{-2} day^{-1} × 5, respectively. A sensitive bioanalytical method based on high-performance liquid chromatography (HPLC) had been developed to study the pharmacokinetic behavior of carzelesin and its metabolites in plasma [12]. In this report we describe the pharmacokinetics of carzelesin in patients receiving carzelesin by a brief i.v. infusion once every 4 weeks, whereas the clinical aspects of this phase I trial will be presented in detail elsewhere. This is the first pharmacokinetic analysis of a CPI drug in humans.

Patients and methods

Patients

Patients with a histologically confirmed solid tumor or lymphoma that was refractory to conventional therapy or for which no conventional therapy existed were eligible for this study. All patients gave written informed consent in accordance with federal and institutional guidelines.

Drug administration

Carzelesin was provided in 2-ml ampules containing 0.50 mg drug/ml PET vehicle (polyethylene glycol 400-absolute ethanol-Tween 80; 6/3/1, by vol. [4]. For use the carzelesin concentrate was diluted 10-fold with PET vehicle to a concentration of 25 μg/ml. Next, the appropriate volume containing the prescribed dose was diluted with 5% (v/v) dextrose in water (D5W) to a final volume of 20 ml, and contained in a sterile plastic syringe. An extra amount of drug solution of the same concentration was used to fill the infusion lines. The drug solution was infused at a dose rate of 2 ml/min (10-min infusion time) into a line in which D5W was flowing at a rate not exceeding 2 ml/min via a constant-rate infusion pump. The starting dose for this phase I trial was 24 μg/m^2 and was escalated further to 48, 96, 130, 150, 170, 210, 250, and 300 μg/m^2.

Pharmacokinetics studies

Pharmacokinetics studies were performed in at least two patients per dose level during the first course of chemotherapy. Blood samples (5 ml) were drawn before the infusion (blank), halfway during the infusion, at the end of the infusion, and at 5, 10, 15, 30, and 45 min as well as 1, 1.5, 2, 3, and 4 h postinfusion and were collected in tubes containing heparin or ethylenediaminetetraacetic acid (EDTA). The samples were immediately cooled in ice water. Within 5–40 min the plasma was separated by centrifugation (5 min, 2500 *g*, 4 °C) and stored at −20 °C. Aliquots of urine samples were taken immediately after each voiding and stored at −20 °C. The plasma and urine samples were shipped in frozen condition to the analytical laboratory in a container supplied with dry ice (solid carbon dioxide).

Analytical procedures

The plasma levels of carzelesin and the metabolites U-76,073 and U-76,074 were determined using a semiautomated method based on HPLC as described in detail elsewhere [12].

In brief, 1 ml human plasma was mixed with 3 ml 20% (v/v) acetic acid in water and then subjected to solid-phase extraction on SPE C_{18} columns. The column was consecutively washed with 2 ml water and 2 ml acetonitrile and the compounds were eluted with 600 μl 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 m*M* 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 for each compound. The method could also be used for urinalysis. In this case, urine was diluted 10-fold with blank human plasma and further treated as described above.

Pharmacokinetic analyses

The pharmacokinetic parameters were calculated using the MW\Pharm software package version 3 (MEDI\WARE BV, Groningen, The Netherlands) [9]. The plasma concentration-time curves of carzelesin were fitted using a one- or two-compartment infusion model with a weighting of $1/Y$, where Y represents the observed concentration. This curve fit was used only to calculate the distribution ($t_{1/2\alpha}$) and elimination half-time ($t_{1/2\beta}$) of carzelesin. The plasma AUC of carzelesin was calculated by the linear trapezoidal rule from time point $t = 0$ h up to the last time point at which the concentration was above the lower limit of quantitation of the assay (1 ng/ml). The clearance (Cl) of carzelesin was calculated using the classic equation $Cl = Dose/AUC$. The linear trapezoidal rule was also used to calculate the plasma AUC of the metabolites U-76,073 and U-76,074. Only concentrations above the lower limit of quantitation of the assay (1 ng/ml) were used. The elimination half-life of U-76,073 was calculated by linear regression analysis of the log concentration (abscissa) versus time (ordinate) of the data points in the final log-linear part of the concentration-time curve. Student's unpaired *t*-test was used for statistical analyses. Reported *P* values are based on a two-sided test of significance.

Results

A total of 31 patients were enrolled in this pharmacokinetics study. One patient (NDDO number 5, Table 1) scheduled to receive 48 μg/m^2 apparently did not receive any drug, as the plasma concentration of carzelesin remained below the detection limit. The data of all other patients were used in this analysis.

The analytical method was sufficiently sensitive to establish a plasma concentration-time profile for carzelesin starting from the first dose level of 24 μg/m^2 [12]. The peak plasma concentration at this dose was about 10 ng/ml and the plasma level dropped to below 1 ng/ml within 1 h of drug administration. At this dose level, very low quantities of the intermediate metabolite U-76,073 were detected, but the concentration of the active metabolite U-76,074 remained below the detection limit (1 ng/ml).

At the lower dose levels the plasma concentration of carzelesin declined monoexponentially, whereas at higher dose levels, two-compartment kinetics usually

Table 1 Pharmacokinetic parameters of carzelesin, the intermediate metabolite U-76,073, and the active metabolite U-76,074 (C_{max} Peak plasma level, T_{max} time of peak plasma level, $t_{1/2\alpha}$ distribution half-life, $t_{1/2\beta}$ elimination half-life)

Dose ($\mu g/m^2$)	Patient (NDDO number)	Institution	Carzelesin (U-80,244)						U-76,073			U-76,074	
			C_{max} (ng/ml)	T_{max} (min)	($t_{1/2\alpha}$) (min)	($t_{1/2\beta}$) (min)	AUC (ng ml^{-1} h)	Cl (l h^{-1} m^{-2})	C_{max} (ng/ml)	($t_{1/2}$) (min)	AUC (ng ml^{-1}h)	C_{max} (ng/ml)	AUC (ng ml^{-1} h)
24	2	1	9.7	10	–[a]	13	3.4	7.0	1.0	–[b]	–[c]	< 1.0	–[c]
	3	2	7.8	17	–[a]	20	3.6	6.7	2.2	–[b]	1.1	< 1.0	–[c]
	4	1	6.8	5	–[a]	12	1.7	14.0	< 1.0	–[b]	–[c]	< 1.0	–[c]
48	5	1	< 1.0										–[c]
	6	2	7.7	22	–[a]	15	3.0	16.2	1.5	–[b]	–[c]	< 1.0	–[c]
96	8	1	34.5	15	–[a]	16	17.5	5.5	5.2	–[b]	2.8	3.4	4.9
	9	2	18.9	22	–[a]	18	9.0	10.6	3.9	–[b]	2.3	1.2	–[c]
130	13	2	17.3	15	–[a]	17	10.0	12.9	3.2	–[b]	3.9	1.1	–[c]
	15	1	48.9	10	–[a]	16	20.0	6.5	8.3	–[b]	6.2	4.8	3.7
	18	1	47.3	10	–[a]	19	20.1	6.5	5.3	–[b]	2.8	1.1	–[c]
150	19	2	26.8	10	–[a]	20	11.9	12.6	7.1	–[b]	4.8	1.1	–[c]
	22	1	48.6	22	–[a]	17	20.0	7.5	6.4	–[b]	5.1	1.0	–[c]
	23	2	15.5	10	–[a]	12	3.3	45.5	8.3	–[b]	3.5	1.5	–[c]
170	25	2	23.6	10	–[a]	22	12.4	13.8	9.6	–[b]	4.9	5.7	2.8
	26	2	28.0	10	–[a]	17	13.9	12.2	4.0	–[b]	2.4	1.8	1.2
	27	2	37.1	5	7	28	15.0	11.3	3.4	57	3.6	1.1	–[c]
	29	1	43.2	10	7	26	16.8	10.1	4.8	49	4.5	< 1.0	–[c]
	42	1	44.6	10	10	33	22.4	7.6	7.0	69	8.7	1.1	–[c]
	44	1	44.3	15	–[a]	16	23.8	7.2	10.8	33	8.3	2.2	0.8
210	32	1	47.3	15	9	32	21.2	9.9	9.1	70	11.0	1.5	1.2
	33	1	56.6	14	8	27	31.1	6.8	7.4	74	9.9	< 1.0	–[c]
	34	1	61.2	10	12	27	29.5	7.1	9.2	54	9.6	1.4	0.7
	35	2	21.9	11	–[a]	14	5.0	41.7	7.6	–[b]	2.1	4.5	2.5
	47	1	52.6	10	13	48	26.9	7.8	10.6	50	14.4	1.7	0.8
	48	1	62.1	10	9	30	32.7	6.4	16.7	74	25.2	3.3	4.7
250	36	1	61.8	27	–[a]	22	43.2	5.8	10.9	51	16.5	1.6	1.7
	37	1	50.5	39	10	43	35.4	7.1	10.7	76	13.6	3.4	3.6
	39	1	43.4	28	–[a]	19	31.0	8.1	9.4	–[b]	6.7	1.6	1.7
300	49	1	56.9	15	5	20	24.7	12.2	17.8	41	11.9	2.5	1.4
	50	1	70.7	25	8	29	37.0	8.1	22.9	46	23.3	2.6	4.0
	51	1	62.3	13	7	28	38.1	7.9	11.8	50	12.7	2.8	2.1

[a] Distribution half-life not calculated due to monophasic decay kinetics
[b] Half-life could not be calculated accurately
[c] AUC not calculated because of too few data points above the LLQ

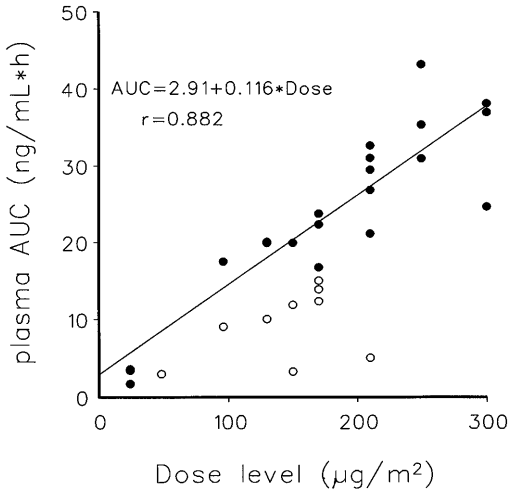

Fig. 3 Graphic presentation of the area under the plasma concentration-time curve (AUC) of carzelesin as a function of the dose (● Data from institution 1, ○ data from institution 2). The *solid line* was calculated by unweighted linear regression analysis of data points from patients treated in institution 1 only

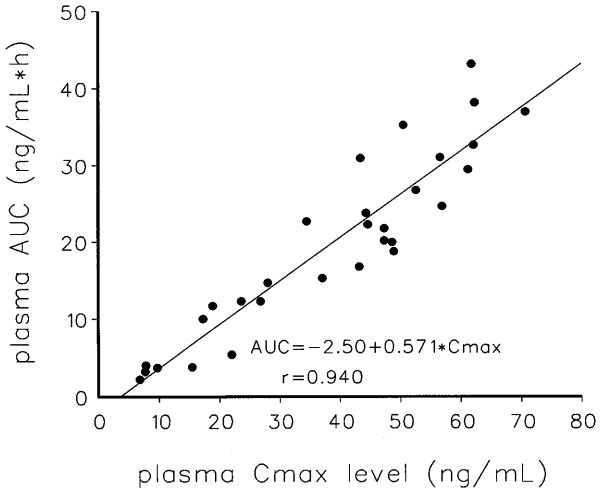

Fig. 4 Graphic presentation of the relationship between the maximal carzelesin plasma level and the plasma AUC. The *solid line* was calculated by unweighted linear regression analysis

gave an improved curve fit. However, the differences observed between ($t_{1/2\alpha}$) and ($t_{1/2\beta}$) values were small (Table 1). The ($t_{1/2\beta}$) value over the whole dose range was 23 ± 9 min (mean ± SD).

There appeared to be substantial interpatient variation in the pharmacokinetic parameters (Table 1). However, for the greater part this variation was caused by differences between patients from the two participating institutions. Overall, the peak plasma concentrations (C_{max}) and plasma AUC recorded for carzelesin in patients treated in institution 1 were higher than those noted for patients treated in institution 2. The derived Cl values were lower and more consistent for patients treated in institution 1 than for those treated in institution 2, *viz.*, 7.9 ± 2.1 and 18.4 ± 13.6 l h^{-1} m^{-2} (mean ± SD; $P = 0.038$), respectively. As based on the data obtained from patients treated in institution 1, the AUC of carzelesin increased proportionately with increasing dose (Fig. 3).

Although practical problems made the duration of the infusion longer than the appointed 10 min in a number of patients, most patients received the dose within 15 min. In a few patients (e.g., NDDO number 37, Table 1) the infusion lasted much longer. The finding that the C_{max} was not much lower in those patients might be explained by the observation that such problems usually occurred during the start of the infusion. Next, when the problem had been resolved, the infusion was continued normally. Overall, there was a good correlation between the plasma AUC and the C_{max} plasma level of carzelesin (Fig. 4).

A substantial concentration of the intermediate metabolite U-76,073 was detected in plasma from all patients receiving carzelesin at dose levels of 96 μg/m^2 and higher (see Fig. 5). Overall, the plasma AUC of U-76,073 was about 45% ± 5% (mean ± SD; $r = 0.84$) of the plasma AUC of carzelesin. In all patients the peak plasma level of U-76,073 was observed within 10 min of the cessation of the infusion. The elimination half-life of U-76,073 could be calculated only at higher dose levels and was always longer than the elimination half-life of carzelesin. In most patients the concentration of the active metabolite U-76,074 ranged between 1 and 2 ng/ml, although higher levels were observed in a few patients. Due to these low plasma levels of U-76,074 and the scatter in the results it was not possible to calculate the elimination half-life of this metabolite.

We also analyzed carzelesin in the first urine specimen that was voided after drug administration from patients

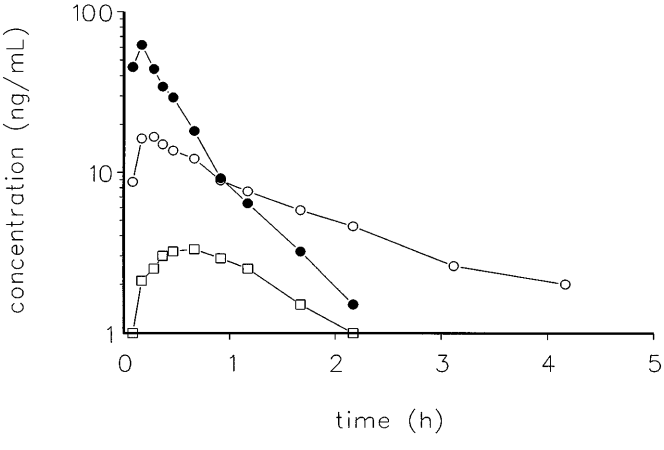

Fig. 5 Plasma concentration versus time curve of a patient (NDDO number 48) receiving 210 μg/m^2 carzelesin. (● Carzelesin, ○ intermediate metabolite U-76,073, □ active metabolite U-76,074)

who received carzelesin at a dose level ranging from 170 to 300 μg/m^2. The concentrations of all three compounds were below the limit of detection (10 ng/ml in urine), and since the volume of these portions was about 400 ml (range 160–500 ml) the urinary excretion was less than 1% of the dose.

Discussion

We studied the pharmacokinetic behavior of carzelesin and its major metabolites in cancer patients, and our results show that the required conversion of this prodrug to its active species does occur in patients. While carzelesin disappeared from plasma at a mean half-life of about 23 min, the intermediate metabolite U-76,073 was readily formed. The plasma level of the active metabolite U-76,074 exceeded 1 ng/ml for short periods in most patients who had received a dose of 130 μg/m^2 or higher.

However, it is not clear whether these levels are sufficiently high to induce an antitumor response in patients. Although these concentrations are well above the concentration required to produce more than 50% tumor cell kill (IC_{50}) in in vitro cell culture [7], these levels are much lower than those achieved in mice receiving this drug at the maximum tolerated dose (MTD) [13]. Furthermore, recent results obtained in tumor-bearing mice suggested that the dose-response curve was very steep [2]. When the dose was lowered to 50% of the MTD, the antitumor efficacy was significantly reduced.

It was hypothesized that the superior in vivo antitumor efficacy of carzelesin relative to other CPI analogs might be related in part to the unique pharmacokinetic properties of this agent [7]. Although the alkylation of DNA by CPI drugs is a rapid process, administration of an inactive prodrug is thought to enable a more optimal distribution into the tumor tissue. Next, activation to the active CPI-containing species and subsequent bonding to the target sites in DNA should take place. As DNA binding is rapid, the fraction of U-76,074 found in plasma very likely does not come from these tissues but is probably formed in the central plasma compartment itself. The conversion of carzelesin, which is probably an enzymatic process, occurs readily in vitro (and, likely, in vivo also) in plasma and whole blood. The temperature is a critical factor. Whereas this conversion is rapid in plasma incubated at 37 °C, carzelesin is stable at temperatures below 4 °C for at least 60 min. According to the instructions in this study, the patients' samples had to be cooled immediately after collection in ice water and had to be stored at −20 °C within 45 min. Problems with the stability of the compounds in these samples upon handling, storage, and transport might have contributed to the finding of relatively high levels of U-76,074 in some of the patients (e.g., NDDO numbers 8 and 35). However, this probably cannot explain the apparent differences in plasma AUC of carzelesin observed between patients from the two institutions, because the conversion to U-76,074 is quantitative and this end product is relatively stable even at 37 °C.

The relatively complex procedures that were required to deliver the drug to the patients might have contributed to the observed variation. Due to the drug's very low aqueous solubility, administration of carzelesin required the use of a complex double-pump infusion system to ensure that the concentration of PET vehicle in the infusion lines remained above the critical micellar concentration so as to prevent the precipitation of carzelesin [4]. Furthermore, the infusion lines needed to be filled properly with drug solution of the same concentration to ensure that when the drug-containing infusion lines were discarded the patient had received the correct dose. Because the low plasma concentrations observed in a number of patients may reflect improper drug delivery, monitoring of plasma levels during subsequent phase II clinical investigations may help to reduce the potential risk of erroneously missing a relevant antitumor effect in these studies.

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