Cancer Chemother Pharmacol (1991) 28: 475–479
034457049100137F

Cancer Chemotherapy and Pharmacology

Clinical pharmacokinetics of high-dose DTIC*

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Summary. The pharmacokinetics of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, dacarbazine) given at a dose of 850–1,980 mg/m^2 as a 10- to 30-min infusion was studied in cancer patients, and the plasma concentration-time curves were adjusted to a two-compartment model, with a mean $t_{1/2\alpha}$ value of 0.17 h (range, 0.1–0.26 h) and a mean $t_{1/2\beta}$ value of 2 h (range, 1.5–2.7 h) being found. The mean volume of the central compartment (V_C) and the apparent volume of distribution (V_B) were 0.42 l kg^{-1} (range, 0.24–0.54 l kg^{-1}) and 1.49 l kg^{-1} (range, 0.88–1.74 l kg^{-1}), respectively. The mean total body clearance of DTIC was 0.58 l kg^{-1} h^{-1} (range, 0.26–0.82 l kg^{-1} h^{-1}), and the mean renal clearance was 0.28 l kg^{-1} h^{-1} (range, 0.17–0.49 l kg^{-1} h^{-1}). Unchanged DTIC recovered from urine within 24 h varied from 11% to 63% of the delivered dose, with an inverse correlation being found between the DTIC dose and the amount excreted. The metabolite aminoimidazole carboxamide (AICA) was detectable in plasma from the start of DTIC infusion, and its concentration-time curve showed a monophasic decay, exhibiting a mean $t_{1/2}$ value of 3.25 h (range, 1.77–5.82 h). Mean AICA renal clearance was 0.15 l kg^{-1} h^{-1} (range, 0.05–0.32 l kg^{-1} h^{-1}). The amount of AICA excreted in urine increased with increasing DTIC dose and varied from 1.2% to 13.6% of the delivered DTIC dose. Both DTIC distribution and disposition and AICA production and renal excretion seemed to be limited after high DTIC doses as compared with the pharmacokinetics of low-dose DTIC. Nonlinear pharmacokinetics for high-dose DTIC could not be clearly excluded.

* This work was supported in part by CAICYT grant 1182

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Introduction

5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (DTIC, dacarbazine) is an antineoplastic drug that is considered to belong to the alkylating agents, although its mechanism of action has not been fully elucidated [1, 4, 11, 15, 23, 29]. In vivo, the drug is *N*-demethylated by liver, resulting in the production of methyltriazenoimidazole carboxamide (MIC), which eventually liberates a carbonium ion that is responsible for the alkylating property of DTIC. This metabolic pathway produces aminoimidazole carboxamide (AICA), which is eliminated in the urine [21, 24, 27, 28].

When DTIC is exposed to light, however, the triazene bond dissociates, yielding dimethylamine and diazoimidazole carboxamide (DZC), which cycles to 2-aza-hypoxanthine [24, 25]. DZC has shown antitumor activity in vitro, but its contribution to the cytotoxicity of DTIC in vivo is not well known [1, 11, 20]. This photodecomposition of DTIC is considered to be undesirable, and the drug must be protected from light exposure during its use.

DTIC has shown definite therapeutic activity in the treatment of malignant melanoma, soft-tissue sarcomas and Hodgkin's lymphoma [8, 14, 30]. Initial phase I–II studies were conducted with repeated low doses of DTIC, and the schedule of 250 mg/m^2 daily for 5 consecutive days became standard [19]. Subsequently, to decrease the duration of and the side effects produced during each cycle, a schedule of high intermittent doses was developed [9]. We conducted a formal phase I study using this schedule and found a maximal tolerated dose of 1,980 mg/m^2 DTIC; the dose-limiting toxicity was hypotension, and a dose of 1,200 mg/m^2 seemed to be recommendable for further trials [6]. DTIC given at high intermittent doses has shown an antitumor activity similar to that previously reported for low-dose DTIC against soft-tissue sarcomas and malignant melanoma [7, 22].

To obtain additional data, we decided to study the pharmacokinetics of DTIC given at high intermittent doses to patients who had been entered in different phase I–II studies at our institution.

Patients and methods

Patients. Patients who had been entered in different phase I and II trials of high-dose DTIC participated in the present study. All subjects exhibited normal bone marrow (leukocytes, >3,500/mm^3; platelets, >100,000/mm^3), hepatic (bilirubin, <1.2 mg/dl) and renal (serum creatinine, ≤1.2 mg/dl; creatinine clearance, ≥60 ml/min) functions. Informed consent was obtained in all cases.

Therapy. The dose of DTIC was ≥850 mg/m^2 given as a 10- to 20-min infusion; in the patient treated at 1,980 mg/m^2, the infusion was given for 30 min so as to decrease local pain caused by the drug. The dose to be given was quickly reconstituted and then infused within 10 min of preparation. The total dose was diluted to a final volume of 200 ml 0.9% saline, and both flask and tubing were protected from light exposure. To prevent hypotensive episodes secondary to the presence of citric acid in the pharmaceutical preparation of DTIC, we concomitantly infused 5 ml of a 10% solution of calcium gluconate at 0, 10 and 20 min after the beginning of DTIC treatment [6].

Blood samples were collected at 0, 5, 15, 30 and 45 min as well as at 1, 2, 4, 6, 8, 12, 16, 20 and 24 h after the start of the DTIC infusion. Plasma was separated and frozen until analysis. After each voiding episode and for 24 h, urine samples were also collected; no attempt was made to obtain urine specimens at regular intervals. Special care was taken to protect all samples from light exposure.

Quantitation methods. DTIC and AICA were quantitatively analyzed using high-pressure liquid chromatography. The equipment consisted of an M-6000 pump, a Universal Injector U6K and a UV detector Lambda Max 481 (Waters Associates). DTIC was a gift from Miles-Martin Laboratories (USA) and AICA was purchased from Sigma (Poole, UK). All reagents were of analytical grade.

DTIC was extracted from plasma with 100% methanol, whereas AICA was extracted with a 20% (v/v) solution of trichloroacetic acid (TCA); in both instances recovery was >90%. Urine samples were injected directly and were diluted when necessary. DTIC was separated in a μBondapak C_{18} column using 0.05 M acetic acid buffer (pH 4.5) as the mobile phase, and UV detection was performed at 326 nm. For AICA, a μBondapak C_{18} column was also used, the mobile phase was a buffer of 0.005 M heptane-sulphonic acid (pH 3.5), and UV detection was carried out at 270 nm [5]. Retention times for DTIC and AICA were 8 and 14 min, respectively. The lower limit of sensitivity for DTIC was 23 ng/ml and that for AICA was 34 ng/ml.

No internal standards were used, and concentrations of both DTIC and AICA were determined by comparison of the chromatographic peak heights with a calibration plot of external standards. The calibration graph was linear from 10^{-7} to 10^{-3} M for both DTIC and AICA. The administration of calcium gluconate or metoclopramide to some patients did not interfere with the analytical technique.

The method of residuals was followed to identify the different components of the concentration-time curves for DTIC and AICA [31]. Values obtained for DTIC were corrected for the duration of infusion [12, 16]. To calculate the various pharmacokinetic parameters, we followed the guidelines established by Gibaldi and Perrier [13]. Renal clearance values for DTIC and AICA were calculated from a plot of the excretion rate vs the plasma concentration at the midpoint of each urine-collection period.

Results

Plasma concentration-time curves for high-dose DTIC were best described by a two-compartment model (Fig. 1). After the administration of doses varying from 850 to 1,980 mg/m^2, DTIC reached a maximal concentration in plasma that ranged from 54 to 150.5 μg ml^{-1}. Concentrations corrected for the duration of infusion at time zero increased with increasing DTIC dose. Table 1 shows the

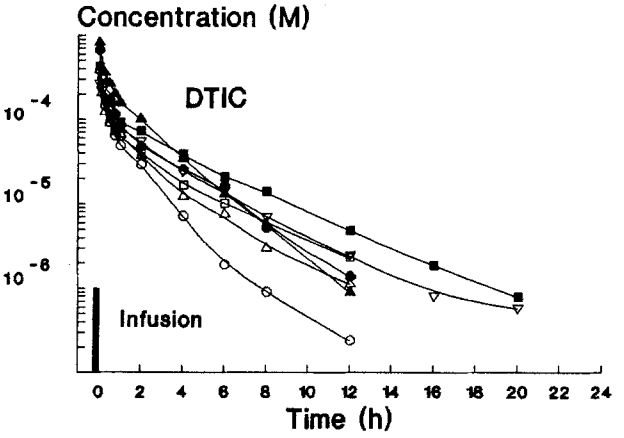

Fig. 1. Mean plasma concentration-time curves for DTIC after doses of 850 (□, 1 patient), 1,000 (■, 1 patient), 1,200 (▽, 3 patients), 1,380 (○, 3 patients), 1,500 (●, 3 patients), 1,650 (△, 3 patients), and 1,980 (▲, 1 patient) mg/m^2

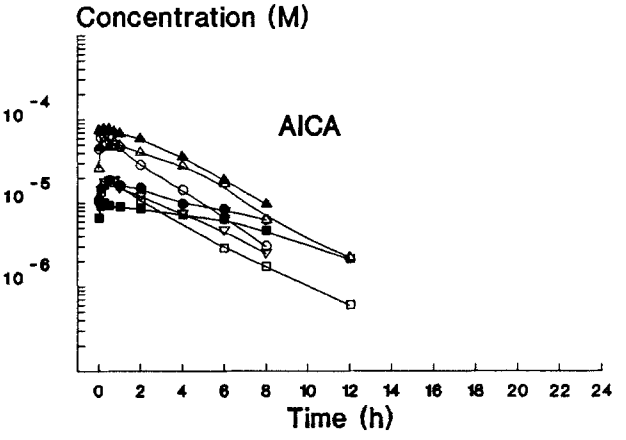

Fig. 2. Plasma concentration-time curves for AICA after doses of 850 (□, 1 patient), 1,000 (■, 1 patient), 1,200 (▽, 5 patients), 1,380 (○, 1 patient), 1,500 (●, 1 patient), 1,650 (△, 1 patient), and 1,980 (▲, 1 patient) mg/m^2

different pharmacokinetic parameters of DTIC according to the dose given. The mean volume of the central compartment (V_c) was 0.42 l kg^{-1} (range, 0.24–0.54 l kg^{-1}), and the apparent volume of distribution (V_B) for DTIC was 1.49 l kg^{-1} (range, 0.88–1.74 l kg^{-1}).

The disappearance of DTIC from plasma exhibited a mean $t_{1/2\alpha}$ value of 0.17 h (range, 0.1–0.26 h) and a mean $t_{1/2\beta}$ value of 2 h (range, 1.5–2.7 h). In one patient who was treated with a dose of 1,200 mg/m^2 and in another who received 1,650 mg/m^2, we detected a third phase showing a $t_{1/2\gamma}$ value of 5.33 and 10.19 h, respectively. The mean total body clearance of DTIC was 0.58 l kg^{-1} h^{-1} (range, 0.26–0.82 l kg^{-1} h^{-1}), and the mean renal clearance was 0.28 l kg^{-1} h^{-1} (range, 0.17–0.49 l kg^{-1} h^{-1}). Unchanged DTIC excreted in the urine within 24 h after therapy varied from 11% to 63% of the dose, with 90% of these amounts being excreted in the first 9 h. There was an inverse correlation between the DTIC dose and the amount excreted ($r = 0.712$, $P = 0.0005$; Table 1).

The data were checked for nonlinear pharmacokinetics [31]. The area under the plasma concentration-time curve

Table 1. Pharmacokinetic parameters of DTIC

DTIC dose (mg/m²)	Patients (*n*)	c_{max} (mM)	V_c (l kg^{-1})	V_B (l kg^{-1})	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	DTIC excreted in urine (%)	Total body clearance (l kg^{-1} h^{-1})	Renal DTIC clearance (l kg^{-1} h^{-1})	Creatinine clearance (l kg^{-1} h^{-1})
850	1	0.3	0.36	1.39	0.26	2.56	54.87	0.37	0.4	0.14
1,000	1	0.42	0.24	0.99	0.11	2.7	63.52	0.26	0.23	0.08
1,200	5	0.27±0.04	0.54±0.12	1.58±0.55	0.24±0.03	2.3 ±0.8	42.64	0.53±0.27	0.28	0.07±0.02
1,380	3	0.38±0.09	0.42±0.05	1.74±0.01	0.15±0.04	1.61±0.5	11.77	0.82±0.21	0.17±0.01	0.1 ±0.01
1,500	3	0.66±0.15	0.45±0.01	1.61±0.53	0.16±0.01	1.73±0.25	11.84	0.66±0.22	0.18±0.08	0.08±0.01
1,650	3	0.46±0.13	0.3 ±0.11	1.37±0.41	0.1 ±0.01	1.6 ±0.1	24.91	0.59±0.15	0.49±0.15	0.08±0.02
1,980	1	0.83	0.27	0.88	0.18	1.5	–	0.41	–	0.06
Mean			0.42	1.49	0.17	2		0.58	0.28	0.08

Data represent mean values ± SD. c_{max}, Maximal plasma concentration; V_c, volume of the central compartment; V_B, apparent volume of distribution

Table 2. Pharmacokinetic parameters of the metabolite AICA

DTIC dose (mg/m²)	Patients (*n*)	c_{max} (μg ml^{-1})	$t_{1/2}$ (h)	DTIC excreted as AICA (%)	Renal AICA clearance (l kg^{-1} h^{-1})	Creatinine clearance (l kg^{-1} h^{-1})
850	1	3.04	2.27	1.2	0.07	0.14
1,000	1	1.62	5.82	2.5	0.08	0.08
1,200	5	3.68±1.54	3.07±1.31	4.75±3.25	0.21±0.15	0.08±0.02
1,380	1	10.23	1.77	11.2	0.12	0.1
1,500	1	3.04	4.81	13.6	0.32	0.08
1,650	1	7.93	2.41	–	0.05	0.07
1,980	1	12.35	2.56	–	–	–
Mean			3.25		0.15	0.09

did not increase proportionally with the DTIC dose, and a plot of those curves normalized for a dose of 1,000 mg/m² did not show unequivocal dose-related changes. On the other hand, the first-order intercompartmental distribution rate constants k_{12} and k_{21} did not vary with the DTIC dose when all patients were considered, although we found a positive correlation for k_{12} and a positive trend for k_{21} in relation to the dose when patients who had been treated at doses of 1,000 and 1,980 mg/m² were excluded from the analysis (data not shown).

The rate of appearance and disposition of AICA followed apparent first-order kinetics (Fig. 2). AICA was detectable in plasma from the beginning of the DTIC infusion, exhibiting a peak at 15 min that ranged from 1.53 to 12.35 μg ml^{-1}. No positive correlation was found between the DTIC dose and the maximal AICA peak. The mean plasma half-life of the metabolite was 3.25 h (range, 1.77–5.8 h), and the mean renal clearance was 0.15 l kg^{-1} h^{-1} (range, 0.05–0.23 l kg^{-1} h^{-1}). The different pharmacokinetic parameters for AICA are presented in Table 2.

If it is assumed that all of the AICA quantified in the urine samples came from DTIC, then the amount of DTIC excreted as this metabolite varied from 1.2% to 13.6% of the dose, with no positive correlation existing between the DTIC dose and the amount of AICA excreted. The total amount of DTIC accounted for in urine (i.e. intact drug plus that excreted as AICA) ranged from 36%–55% for doses of ≤1,200 mg/m² to 19%–38% for doses of ≥1,380 mg/m².

Discussion

The pharmacokinetics of high-dose DTIC best fits a two-compartment model in most patients, as has been described for that of low, conventional doses of the drug [5]. However, experimental data in the dog have revealed a third gamma phase, which we encountered in two patients in the present study [17]. The pharmacokinetic data obtained suggested that high-dose DTIC might follow nonlinear pharmacokinetics, with saturation occurring in the renal disposition of unchanged drug and in its metabolism to AICA, but the different tests performed for nonlinearity yielded negative results [31]. It cannot be excluded that the photosensitivity of this drug altered some of the experimental data obtained.

High-dose values for V_c and V_B found in the present study were higher than the values of 0.202 l kg^{-1} (range, 0.132–0.281 l kg^{-1}) and 0.622 l kg^{-1} (range, 0.406–0.931 l kg^{-1}), respectively, that have previously been reported for low-dose DTIC; these data indicate that the drug accumulates in peripheral tissues, confirming previous clinical and experimental work [5, 17, 18]. For DTIC given at low doses, $t_{1/2\alpha}$ values of 2.9 min (range, 2.2–3.6 min) and $t_{1/2\beta}$ values ranging from 38 to 190 min have been reported by different authors [3, 5, 18, 26]. The $t_{1/2\alpha}$ value found for high-dose DTIC was 3.5-fold that previously obtained for low doses of the drug, and the present $t_{1/2\beta}$ value lay within the upper limit of the previously reported

low-dose values, which probably indicates the slower distribution and disposition of high DTIC doses.

Renal clearance of DTIC exceeded that of creatinine, as previously described in preclinical and clinical research, supporting the presence of a mechanism of tubular secretion for the drug [5, 18]. This mechanism most likely becomes saturated at doses of >1,200 mg/m^2 as shown by the inverse correlation encountered between the dose and the amount of intact drug excreted. At the recommended phase-II dose of 1,200 mg/m^2, about 40% of the dose was excreted unaltered in the urine, which was similar to the 20%–50% value previously found for DTIC doses ranging from 120 to 270 mg/m^2 [15, 18, 26].

After the administration of high-dose DTIC, the appearance of metabolite AICA in plasma followed apparent first-order kinetics, exhibiting a mono-exponential decay previously described for low DTIC doses (Fig. 2). Although AICA can be reutilized, the data obtained suggest that this metabolic pathway may become saturated after the administration of high DTIC doses. In the study by Breithaupt et al. [5], the AICA peak in plasma varied from 3 to 5 μg ml^{-1} following a dose of 6.5 mg kg^{-1} DTIC, whereas in our patients who had received doses of 1,200 mg/m^2 (30 mg/kg^{-1}), we found a mean AICA plasma concentration of 3.7 μg ml^{-1} (range, 1.53–6.22 μg ml^{-1}).

The elimination half-life of AICA following low doses of DTIC ranged from 43 to 120 min in two different studies, which was considerably lower than the mean 3.25 h (range, 1.77–5.82 h) we found in the present study [3, 5]. The values for the renal clearance of AICA in our patients were approximately 10 times lower that those previously reported for low DTIC doses, with recovery of the metabolite in urine also being comparatively lower following high doses of DTIC [5]. These data seem to indicate that renal disposition of AICA is also limited after the administration of high-dose DTIC.

The DTIC analog 1-(4-carboxyphenyl)-3,3-dimethyltriazene, which has recently been studied, is *N*-demethylated in vivo and undergoes conjugation with glucuronic acid and glycine [2]. During a phase I clinical study, the plasma levels of the metabolites formed were determined, and the *N*-desmethyl derivative reached a plasma concentration of 18–32 μ*M* at the maximal tolerated dose, whereas the glucuronic and glycine conjugates accounted for 31% and 19% of the dose, respectively [10]. As we did not investigate the presence of other possible metabolites of DTIC in the present study, their relevance in relation to the DTIC dose and to DTIC disposition cannot be evaluated.

In summary, the pharmacokinetics of high-dose DTIC is best described by a two-compartment model associated with possible saturation of the distribution and disposition phases, including renal clearance, as compared with the pharmacokinetics of low-dose DTIC. Both the metabolism of the drug to AICA and the renal disposition of this metabolite appeared to be limited following the administration of high DTIC doses. We could not confirm nonlinear pharmacokinetics for this DTIC schedule.

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