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Pharmacokinetics of Dacarbazine (DTIC) and Its Metabolite 5-Aminoimidazole-4-Carboxamide (AIC) Following Different Dose Schedules

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Summary. *The pharmacokinetics of dacarbazine (DTIC) and its main metabolite 5-aminoimidazole-4-carboxamide (AIC) have been studied in eight patients with malignant melanoma or sarcoma receiving 2.65–6.85 mg DTIC/kg body weight by intravenous bolus injection or by continuous 0.5–6-h infusions on 5 consecutive days. The plasma disappearance of DTIC was biphasic, with a terminal half-life of 41.4 min (range 30.3–51.6 min). The mean distribution volume of DTIC was 0.632 liters/kg and the total clearance was 15.4 ml/kg · min (range 8.7–23.3 ml/kg · min). The renal clearance of DTIC was 5.2–10.9 ml/kg · min, indicating that about 50% of DTIC was eliminated by extrarenal mechanisms. The plasma decay of AIC was mono-exponential with a half-life of 43.0–116 min. A renal clearance of 2.6–5.3 ml/kg · min was calculated for AIC. The urinary recovery was 46%–52% for DTIC and 9%–18% for AIC. The plasma concentrations of DTIC observed during 0.5–6-h infusions of DTIC (5.45–6.85 mg/kg) were 0.66–6.2 μg/ml. Comparison of various dosage schedules within the same patient did not reveal relevant differences of the areas under the concentration-time curves. Immunotherapy with Bacillus Calmette-Guérin (BCG) did not significantly influence the pharmacokinetics of DTIC.*

During isolated extremity perfusion with DTIC (75–130 mg/kg extremity) for treatment of malignant tumors of the extremities concentrations of DTIC ranged from 150–500 μg/ml perfusate. There was no evidence of AIC formation. In isolated liver perfusion experiments in anesthetized dogs metabolic degradation of DTIC to AIC was demonstrated.

Introduction

Dacarbazine [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide; DTIC] has been shown to be useful in the therapy of malignant melanoma [6, 21], soft tissue sarcoma [12, 38], and Hodgkin's disease [5]. Since gastrointestinal absorption of DTIC is incomplete and variable [21], various dosage schedules for IV administration have been proposed. Only a small number of reports on the distribution of DTIC is available, in particular with regard to different treatment schedules and repeated administrations [21, 22, 36].

DTIC itself is assumed to be inactive. Its degradation involves photodecomposition to 2-azahypoxanthine, which is considered to be a toxic and inactive derivative [11, 31]. Microsomal *N*-demethylation of DTIC in the liver appears to be the major metabolic pathway of DTIC, giving rise to AIC and a methyl carbonium ion, the agent presumably alkylating DNA [11, 24, 34]. *N*-Demethylation of DTIC has been reported to be inhibited by previous immunotherapy with *Bacillus Calmette-Guérin* (BCG) or *Corynebacterium parvum* [10, 13, 29].

It was the aim of this study to investigate the pharmacokinetics of DTIC and its major metabolite AIC following different dosage schedules and to examine the influence of BCG on the kinetics of DTIC. Furthermore, the kinetics of DTIC in isolated perfusion experiments (extremity, liver) are presented.

Patients and Methods

Patients. Eight patients with various forms of malignant disease (Table 1), for whom DTIC therapy was considered appropriate, gave informed consent for participation in this study. Routine hematologic and chemical tests including a complete blood count, serum electrolytes, serum urea nitrogen, creatinine, serum aspartate aminotransferase (SGOT), serum alanine aminotransferase (GPT), alkaline phosphatase (AP), γ-glutamyltransferase (γ-GT), lactate dehydrogenase (LDH), and bilirubin were normal in all patients before each cycle of DTIC and were monitored for at least 5 days thereafter.

Clinical Modalities. Doses of 2.65–6.85 mg DTIC/kg (Dome, Miles) were administered on 5 consecutive days by IV bolus injection or by continuous IV infusion over 0.5–6 h (Table 1). Drug courses were repeated six times or more, at 4-week intervals. The four patients with malignant melanoma received DTIC monotherapy combined with a BCG immunotherapy (Institute Pasteur) applied between the DTIC drug cycles. BCG (about 10^8 organism/m^2) was given weekly by ID injections. The four patients with sarcomas received DTIC on 5 consecutive days. Vincristine (1.5 mg/m^2), adriamycin (40 mg/m^2), and cyclophosphamide (1 g/m^2) were given on day 1. During DTIC drug cycles all patients (except pt 3) received antiemetic treatment in the form of daily doses of 0.1–0.3 mg levomepromazine/kg body weight. No other drugs were administered. DTIC was administered without exposure to UV light.

Drug monitoring was performed during each day of the first and second drug cycles, except in pt 2 who was monitored during the twelfth drug cycle of DTIC. Blood samples of 1.5 ml were collected into heparinized tubes at the following time intervals after bolus injection of DTIC: 0, 1, 2, 4, 6, 8, 10, 15,

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Table 1. Characteristics of patients prior to therapy with DTIC

Patient	Age/sex (years)	Weight (kg)	Clinical diagnosis	Treatment before DTIC	Dose of DTIC		Mode of administration
					(mg)	(mg · kg^{-1})	
1	47/M	85	Malignant melanoma stage IV (neck)	Excision. Neck dissection	225	2.65	IV bolus
2	49/F	62	Malignant melanoma stage IV (extremity)	Excision. Radiation	425	6.85	IV bolus
3	42/M	71	Malignant melanoma stage IV (axilla)	Excision. Bloc dissection	450	6.34	IV bolus
4	49/M	92	Malignant melanoma stage IV (back)	Excision. Bloc dissection	600	6.52	IV bolus
5	43/M	70	Fibrosarcoma (distal femur)	cis-Platinum. Vincristine. Bleomycin	400	5.71	6-h infusion
6	22/F	55	Osteosarcoma (distal femur)	Amputation. High-dose methotrexate	300	5.45	6-h infusion
7	48/M	78	Synovialsarcoma (distal femur)	Amputation	500	6.41	IV bolus 0.5- to 4-h infusions
8	57/M	73	Fibrosarcoma (proximal femur)	Bloc dissection	500	6.85	IV bolus 1- to 4-h infusions

20, 30, 45, 60, and 90 min; 2, 3, 4, 5, and 6 h. During and following continuous infusion of DTIC blood samples were collected at comparable time intervals. Urine samples were collected at 4-h intervals during and following several drug cycles and were stored frozen at −16° C until use. Exposure to daylight was avoided.

The technique of isolated extremity (man) and liver (dog) perfusion will be described elsewhere [2]. In five patients a total of 75−130 mg DTIC/kg perfused extremity was administered by three bolus injections at 0, 5, and 10 min after the start of isolated extremity perfusion. Isolated perfusions of the liver were performed in six anesthetized dogs. DTIC doses of 50−250 mg were administered by bolus injection. Samples from the extracorporeal circuit were collected at 5-min intervals. Exposure to UV light was avoided.

Laboratory Procedures. Plasma samples were directly analyzed without protein precipitation or other pre-clean-up procedures. Urine samples were diluted 1 : 20 in distilled water before analysis. The quantitative determination of DTIC and AIC in plasma and urine was performed using a paired-ion high-performance liquid chromatographic method developed in our laboratory. The chromatographic system for analysis of DTIC consisted of a Spherisorb 5 μ ODS column (5 × 0.4 cm i.d.) as the stationary phase and a 0.005 *M* tetrabutylammonium phosphate solution (PIC A, Waters) as the mobile phase. Samples (20 μl) of AIC and 2-azahypoxanthine were analyzed on a 30 × 0.4 cm Spherisorb 5 μ ODS column, using 0.005 *M* 1-heptane sulfonic acid (PIC B-7, Waters) as the eluent. DTIC was eluted at 2 min (45 bar, 2.5 ml/min, 326 nm), whereas 2-azahypoxanthine was eluted at 8 min and AIC at 13 min (200 bar, 2 ml/min, 270 nm). The lower limit of sensitivity for DTIC, 2-azahypoxanthine, and AIC was 60 ng/ml plasma with an intra-assay precision better than 7%. The calibration graph was linear in the range of 0.06−600 μg/ml plasma. The recovery from plasma was 96%−103% when compared with the peak areas obtained by injection of the pure compounds. DTIC, AIC, and 2-azahypoxanthine were found to be stable in human blood, plasma, and urine, respectively, when stored for a period of 4 h at room temperature without exposure to UV light. Reference standards for DTIC, 2-azahypoxantine, and AIC were obtained from Miles (Frankfurt, FRG). All other chemicals were of analytical reagent grade.

2-Azahypoxanthine was not detected in any of the plasma or urine samples tested in this study.

Pharmacokinetics. Pharmacokinetic computations were done by rotative iterative procedures on a Hewlett-Packard 9825 calculator (Prof. Dr. med. von Hattingberg, Zentrum für Kinderheilkunde, Feulgenstrasse 12, 6300 Giessen, FRG). Half-lives ($t_{50\%}$) were calculated from plasma data by nonlinear curve fitting, which is based on the Gauss-Newton method with dynamic Hartley's modification. The total clearance (Cl) was obtained by dividing the total dose by the area under the curve (AUC) between the start of IV infusion and infinity. AIC was computed from the experimental data by parabolic interpolation and was extrapolated to infinity using the terminal slope. Extrapolation contributed less than 4% to the area of DTIC and AIC. The total volume of distribution (V_{ss}) was obtained as the product of Cl and the mean transit time (T). Transit time is computed as the area between the curve of cumulative disposition and its asymptote divided by this asymptote [14]. The mean time of constant infusion, e.g., 0.5 × infusion time is subtracted from the mean system time to obtain the mean transit (or residence) time T for the drug in the body. The fraction of DTIC converted to AIC has not been determined in this study. Volume terms are therefore not representative for the metabolite. The mean time T for AIC contains formation as well as distribution and elimination of AIC in the body. For AIC a fictive clearance ('Cl') has been calculated as total dose of DTIC divided by AUC of DTIC. From urinary data the renal clearances of DTIC and AIC (Cl_{renal}) were evaluated. The values of $t_{50\%}$, V_{ss}, Cl, 'Cl', and Cl_{renal} are presented in Tables 2−4.

Differences of pharmacokinetic data were calculated using the paired *t*-test at a level of significance of 5%.

Results

Following IV bolus injection of DTIC (2.65–6.85 mg/kg) maximal plasma concentrations of DTIC were between 10 and 30 μg/ml (Fig. 1). In all patients plasma disappearance of DTIC was biphasic, with rapid half-life values between 2.4 and 3.6 min (mean 2.9 min; Table 2) and slow half-life values between 30.0 and 51.6 min (mean 41.4 min; Table 2). The rapid half-life ($t_{50\%}$) accounted for about 28 ± 7% (mean ± SE) of the areas under the curves. The plasma values of DTIC obtained for an individual patient on 5 consecutive days were reproducible within a standard deviation range of less than 12%. Interindividually the kinetic data of DTIC were also found to be in a narrow range (Tables 2 and 3). The following mean values for DTIC were calculated from patients' data: $t_{50\%\beta}$ 41.4 min, T 42.9 min, V_{ss} 0.632 l/kg, and Cl 15.4 ml/kg · min.

Immunotherapy with BCG did not result in significant changes in the pharmacokinetics of DTIC (Table 2).

The major metabolite AIC was demonstrable in plasma immediately after bolus injection of DTIC. The plasma concentration of AIC increased up to 5 μg/ml at 15 min (Fig. 2), followed by a mono-exponential decay with half-life values between 43 and 116 min (Table 3). Intraindividually plasma kinetics of AIC varied within a range of ± 13% when followed after DTIC bolus injections on 5 consecutive days.

The cumulative excretion of DTIC and AIC in urine was measured in three patients (Table 4). The recovery of DTIC was 46%–52% and that of AIC 9%–18%, giving a total recovery of 55%–70%. The renal clearance of DTIC ranged from 5.2 to 10.9 ml/kg · min and that of AIC from 2.6 to 5.3 ml/kg · min.

Following continuous IV infusion of DTIC, steady-state concentrations were observed 4 h after starting infusions (Figs. 3 and 4). Depending on the infused dose-flow, maximum plasma concentrations of DTIC ranged from 0.66 to 6.2 μg/ml (Table 5). The plasma levels of AIC increased up to 1.0 μg/ml. Following different IV dosage schedules the areas under the curves of DTIC were of comparable magnitude (Table 5).

During isolated liver perfusion (50–250 mg DTIC) performed in six anesthetized dogs DTIC concentrations of 38.3–295 μg/ml perfusate were obtained (Table 6). DTIC decreased mono-exponentially with a half-life of 66.4 ± 47.5 min (mean ± SE). The clearance of DTIC was 18.4 ± 10.2 ml/min. AIC was demonstrable in the perfusate 2–5 min after bolus injection of DTIC into the extracorporeal circuit (Fig. 5). AIC levels continuously increased. The concentration of AIC in the perfusate doubled within 18.5 ± 6.6 min. At the end of the perfusion experiments (at 30 min) AIC concentra-

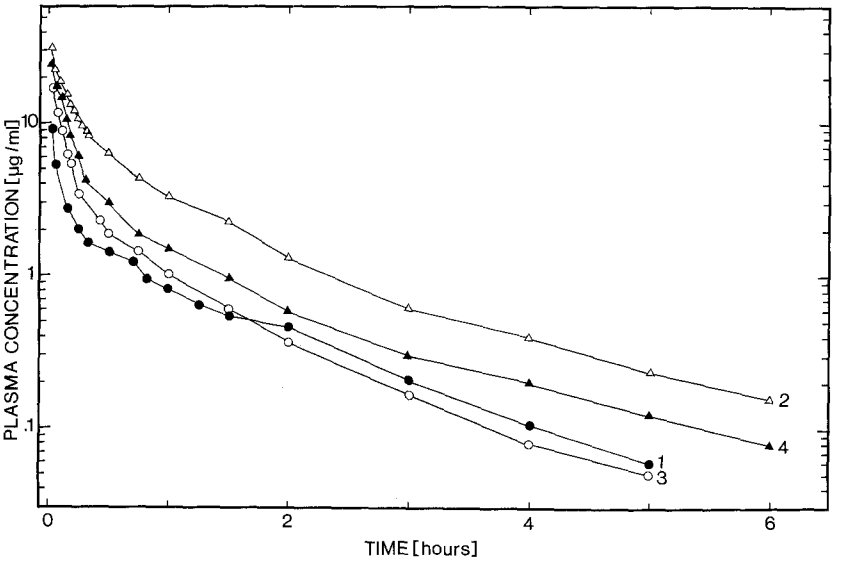

Fig. 1. Time-concentration curves of DTIC following IV bolus injection of 2.65–6.85 mg DTIC/kg bodyweight. Each *point* represents the mean value after five IV bolus injections performed on 5 consecutive days. Standard deviation (SE) was less than 12%. Patient 1 (●) received 2.65 mg/kg; pt 2 (△) 6.85 mg/kg; pt 3 (○) 6.34 mg/kg; and pt 4 (▲) 6.52 mg/kg

Table 2. Pharmacokinetic data of DTIC. Mean values following repeated IV injections on 5 consecutive days

Patient	Dose of DTIC ($mg \cdot kg^{-1}$)	$t_{50\%\alpha}$ ± SE (min)	$t_{50\%\beta}$ ± SE (min)	T ± SE (min)	V_{ss} ± SE ($l \cdot kg^{-1}$)	V_c ± SE ($l \cdot kg^{-1}$)	Cl ± SE ($ml \cdot kg^{-1} \cdot min^{-1}$)
1	2.65	2.5 ± 0.4	45.8 ± 8.0	51.7 ± 8.8	0.700 ± 0.097	0.182 ± 0.023	13.6 ± 1.0
2	6.85	3.6 ± 1.1	40.0 ± 7.2	46.9 ± 5.7	0.406 ± 0.055	0.165 ± 0.021	8.7 ± 0.3
3	6.34	2.6 ± 1.3	30.9 ± 13.3	30.7 ± 12.7	0.719 ± 0.331	0.225 ± 0.107	23.3 ± 2.2
	6.34[a]	3.6 ± 0.6	39.3 ± 7.7	39.8 ± 6.1	0.731 ± 0.064	0.241 ± 0.021	19.0 ± 1.5
4	6.52	3.5 ± 0.8	36.0 ± 13.7	34.9 ± 10.5	0.549 ± 0.153	0.193 ± 0.022	15.8 ± 5.0
	6.52[a]	2.2 ± 1.2	30.3 ± 17.3	32.3 ± 14.7	0.438 ± 0.212	0.132 ± 0.046	13.5 ± 5.3
7	6.41	2.4 ± 0.4	51.6 ± 12.4	44.3 ± 5.2	0.931 ± 0.180	0.281	21.9 ± 3.6
8	6.85	2.9 ± 0.9	44.0 ± 19.0	49.0 ± 19.0	0.487 ± 0.148	0.166 ± 0.018	9.3 ± 1.3
Mean[b]		2.9	41.4	42.9	0.632	0.202	15.4

[a] The drug cycle was preceded by immunotherapy with BCG
[b] The drug cycles preceded by BCG were excluded

Table 3. Pharmacokinetic data of AIC following IV injection of DTIC

Patient	Dose of DTIC ($mg \cdot kg^{-1}$)	$t_{50\%} \pm$ SE (min)	T ± SE (min)	'Cl' ± SE ($ml \cdot kg^{-1} \cdot min^{-1}$)
3	6.34[a]	43.0 ± 2.7	31.2 ± 11.9	27.6 ± 2.7
4	6.52[a]	67.6 ± 9.0	60.3 ± 18.8	25.5 ± 2.3
8	6.85	116.4	120.2	26.0

[a] Repeated IV bolus injections on 5 consecutive days

tions of 5.2–31.0 μg/ml were measured. The entrance of AIC ($= k_{invasion} \times V_{ss}$) was calculated to be 56.6 ± 27.5 ml/min. In plasma samples collected from the systemic circulation of each dog DTIC and AIC were not detectable. All animals survived without severe harm [2].

Isolated extremity perfusions with DTIC (75–130 mg/kg extremity) performed in four patients with advanced malignant tumors of extremity resulted in DTIC concentrations of 150–500 μg/ml without evidence of AIC formation (Fig. 6). Eliminatioin of DTIC from the perfusate occurred slowly, with a half-life of about 16 h. DTIC and AIC levels in the systemic circulation were below the limit of the assay system (60 ng/ml).

Table 4. Renal clearances and urinary recoveries of DTIC and AIC following IV bolus injection of DTIC

Patient	Dose of DTIC ($mg \cdot kg^{-1}$)	Cl_{renal} of DTIC ($ml \cdot kg^{-1} \cdot min^{-1}$)	Cl_{renal} of AIC ($ml \cdot kg^{-1} \cdot min^{-1}$)	Recovery of DTIC (%)	Recovery of AIC (%)
3	6.34[a]	10.9 ± 4.8	3.8 ± 2.0	52 ± 10	18 ± 5
4	6.52[a]	5.2 ± 2.0	2.6 ± 0.7	46 ± 11	9 ± 2
8	6.85	8.2	5.3	51	11

[a] Repeated IV bolus injections on 5 consecutive days

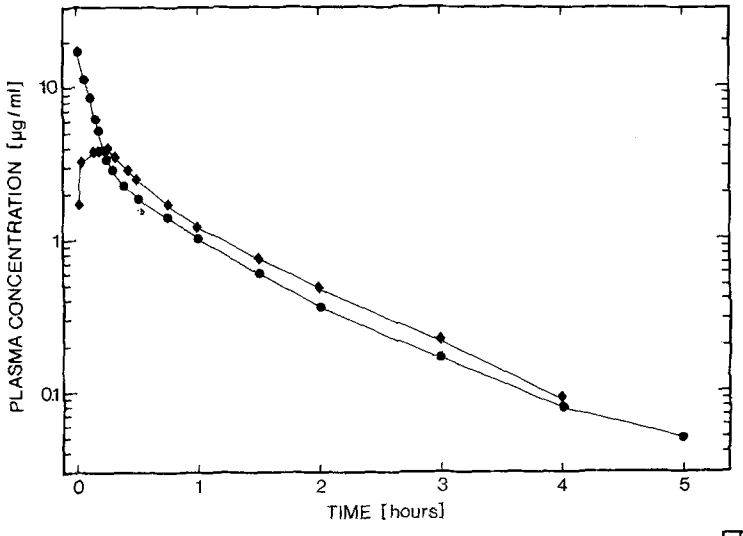

Fig. 2. Time-concentration curves of DTIC (●) and AIC (◆) following IV bolus injection of DTIC (6.34 mg/kg) to pt 3. Each *point* represents the mean value of five injections performed on 5 consecutive days. Standard deviation (SE) was less than 13%

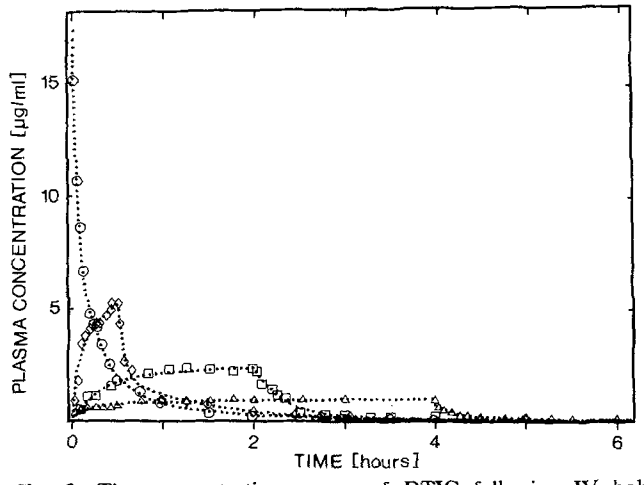

Fig. 3. Time-concentration curves of DTIC following IV bolus injection (○) or during and following continuous infusions of DTIC (6.41 mg/kg; pt 7) for 0.5 h (◇), 2 h (□), and 4 h (△). The *small points* (•) represent the time courses obtained by computerized curve fitting

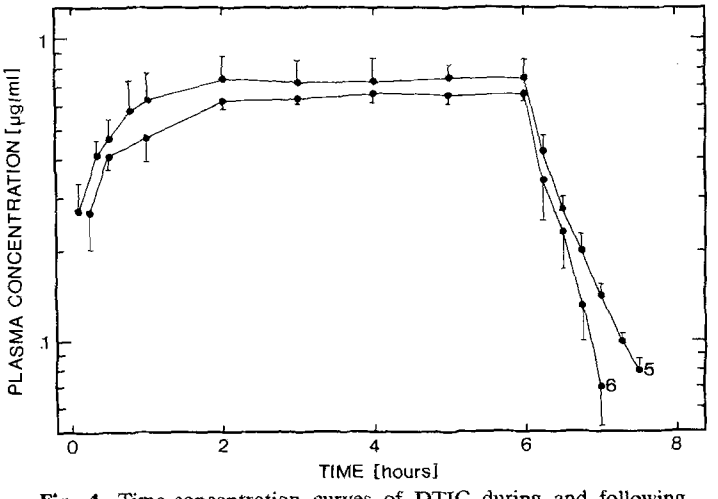

Fig. 4. Time-concentration curves of DTIC during and following continuous 6-h infusions of DTIC (5.45 mg/kg to pt 6 and 5.71 mg/kg to pt 5). Each *point* represents the mean value ± SE of five infusions given on consecutive days. Standard deviation was less than 16%

Table 5. Peak plasma concentrations of DTIC and AIC and areas under the curves following different dosage schedules

Patient	Dose of DTIC	Duration of infusion	C_{max}[a]		AUC $(0-\infty)$[b]
			DTIC	AIC	DTIC
	$(mg \cdot kg^{-1} \cdot h^{-1})$	(h)	(μg/ml)	(μg/ml)	(%)
5	0.95	6	0.74		
6	0.91	6	0.66		
7	1.6	4	0.94		
	3.2	2	2.3		127
	6.4	1	3.7		118
	12.8	0.5	5.3		107
8	1.7	4	2.9	0.7	101
	3.4	2	5.0	0.8	118
	6.8	1	6.2	1.0	96

[a] Maximal plasma concentration observed
[b] Related to AUC $(0-\infty)$ following IV bolus injection

Table 6. Kinetic data from isolated liver perfusion of the dog

Dose of DTIC (mg)	DTIC			AIC		
	C_{max} (μg/ml)	$t_{50\%}$ (min)	Cl $(ml \cdot min^{-1})$	C_{max} (μg/ml)	Rate of formation (min)	Entrance [8] $(ml \cdot min^{-1})$
50	38.3	29.8	30.3	5.2	11.4	79.4
100	55.1	147	8.5	5.2	18.1	69.1
100	53.2	43.1	30.5	7.4	15.2	86.9
150	132	44.3	17.2	18.4	13.4	56.7
150	151	102	6.8	9.7	25.5	27.2
250	295	32.2	17.2	31.0	27.5	20.2
Mean		66.4	18.4		18.5	56.6
SE		47.5	10.2		6.6	27.5

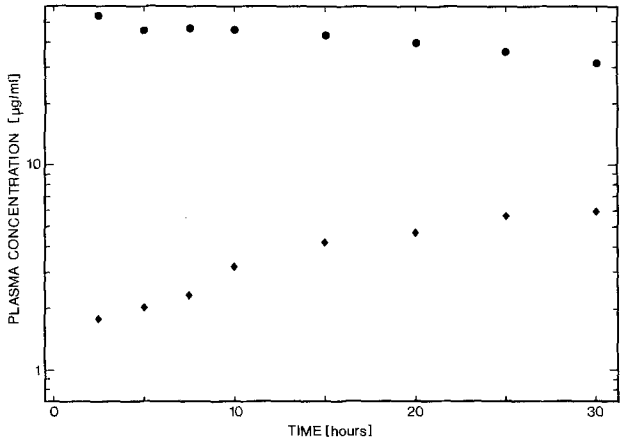

Fig. 5. Time-concentration curves of DTIC (●) and AIC (◆) during isolated liver perfusion with DTIC in a dog. A bolus of 50 mg DTIC was given into the extracorporeal circuit (1.3 l). The liver was perfused for 30 min

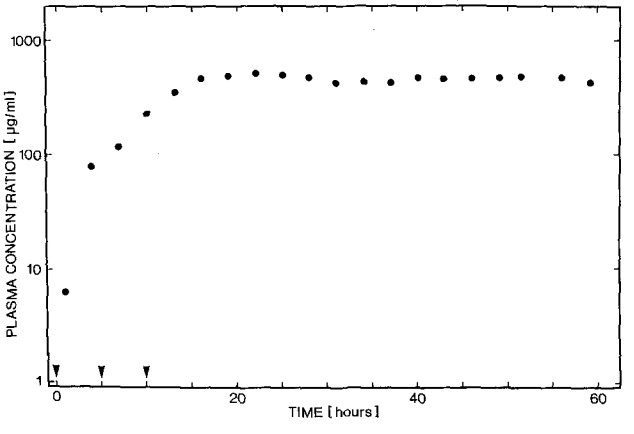

Fig. 6. Time-concentration curve of DTIC during isolated arm perfusion in a woman suffering from angiosarcoma. DTIC (400 mg) was administered into the extracorporeal circuit by bolus injections (▼) at 0, 5, and 10 min. AIC was not detectable up to 60 ng/ml

Discussion

Conventional DTIC therapy consists of single IV injections on 5 consecutive days with subsequent courses given at 4-week intervals. Patients treated with this regimen have suffered from severe gastrointestinal side-effects. Since efficacy of DTIC has been shown to be unrelated to dose schedules in animal experiments [25] daily IV infusion of DTIC over several hours may be used instead to avoid nausea and vomiting. On the other hand, a single-day schedule with high-dose DTIC $(600-1500\ mg/m^2)$ at 5-week intervals was equally effective [7, 27, 30]. In these studies nausea and vomiting were less

prolonged and other toxic side-effects occurred less frequently.

Our knowledge of the pharmacokinetics of DTIC following different dosage schedules is limited. It was one aim of this study to investigate the kinetics of DTIC and AIC in plasma and urine of patients given DTIC repeatedly by IV bolus injection and/or by continuous infusion over several hours. For analysis of DTIC, AIC, and 2-azahypoxanthine (produced by photodecomposition of DTIC [22, 33]) a rapid and selective high-performance liquid chromatographic assay was developed which was sufficiently sensitive for clinical monitoring of DTIC and its metabolites in plasma and urine. Previously described methods are restricted to colorimetry [20] and polarography [19] for determination of DTIC, or are based on ion exchange chromatography for analysis of AIC [35] or 2-azahypoxanthine [3]. Recently, a high-performance liquid chromatographic assay for determination of DTIC and AIC by separate runs has been proposed [4]. However, in our hands this method did not result in sufficient separation of DTIC and AIC from endogenous constituents of plasma or urine.

The pharmacokinetic data recorded in this study are compatible with the assumption of a two-compartment model for distribution and elimination of DTIC. A similiar model has been proposed for disposition of DTIC in dogs [23]. In humans, bolus injection of DTIC was followed by a biphasic disappearance of DTIC from plasma, with mean half-lives of 2.9 and 41.4 min. The rapid half-life represents 21%–35% of the total area under the curve. The second half-life is close to previously reported half-life values of 35 and 75 min [21, 36], but in contrast to the half-lives of 3.5 h and 5 h [13, 22] found in DTIC plasma levels, which are comparable to those of this study. The volume of distribution of DTIC was found to exceed total-body water content, thus suggesting some localization in body tissue. Consistent with this finding, a low protein binding was reported for DTIC [21]. Our data with regard to the renal clearance of DTIC confirm a previous study reporting a clearance rate of 221 ml/min [36]. These findings support the assumption that a tubular secretory mechanism is involved in the renal excretion of DTIC. Following bolus injection DTIC was measurable in urine over 12 h, with cumulative recoveries somewhat higher than those reported by others [16, 21, 36].

AIC is the only major metabolite of DTIC that has been identified in rats, dogs, and man [16, 17, 36, 37]. Previous pharmacokinetic data on AIC were restricted to its urinary recovery [16, 21, 36], which is confirmed by our results. In plasma, AIC was demonstrable immediately after bolus application of DTIC. Peak levels of AIC at 15 min were followed by a mono-exponential disappearance from plasma, with half-life values similar to those for DTIC. Since plasma half-life, mean transit time, and renal clearance of AIC and DTIC are comparable, we assume similar mechanisms for renal and nonrenal excretion of the two compounds.

Steady-state concentrations of DTIC were observed upon continuous infusions over 4- and 6-h periods. It has been reported previously that steady-state conditions were not obtained following 8-h infusions of 4.5 mg/kg [36]. The areas under the curves obtained with different dose schedules were equivalent, which indicates that saturation of the mechanisms of metabolism and excretion of DTIC did not occur.

When the infusion period for identical doses of DTIC is more and more prolonged, plasma levels of DTIC may fall below therapeutically efficient levels. Since the minimal therapeutic plasma concentration of DTIC is not known, one is forced to give the highest tolerable dose likely to produce the maximal therapeutic effect. The minimal clinically effective dosage of DTIC has been reported to be a daily dose of 2 mg/kg given by bolus injection for 10 days [26].

The possible effect of daily applications on DTIC pharmacokinetics was investigated. Following plasma levels and cumulative excretion of DTIC during 5-day courses there were no trends or significant differences in kinetic parameters.

BCG has been reported to cause reduction in hepatic *N*-demethylation of DTIC in rats [10, 22]. In humans, prolonged half-lives of DTIC were observed following immunotherapy with IV *Corynebacterium parvum* [13]. However, after ID BCG we did not find any significant alteration in the kinetics of DTIC.

DTIC per se is believed to be inactive and to exert its cytotoxic activity by metabolic activation in liver microsomes with liberation of AIC and a methyl carbonium ion with alkylating potential [11, 24, 34]. Among various tissues tested for DTIC-degrading activity, only liver [15] and sarcoma 180 cells [24] convert DTIC to AIC. It has been suggested, however, that DTIC itself exerts antineoplastic activity. In isolated perfused rabbit ears DTIC induced an impressive regression of VX2-carcinoma [1]. Intra-arterial infusion therapy with DTIC in patients with tumor metastases in skin, node, and soft tissue were reported to induce favorable clinical results [9, 18,32]. Intrathecal application of DTIC (8 × 5–25 mg) in a patient with leptomeningeal metastases of malignant melanoma was followed by clinical improvement [28]. Encouraged by these findings, four patients with advanced extremity sarcomas were treated by isolated extremity perfusion with high-dose DTIC [2]. As with isolated extremity perfusion in dogs [2], DTIC concentrations of 150–500 μg/ml perfusate were well tolerated during and following 1-h perfusions of the extremity. During isolated extremity perfusion DTIC levels decreased very slowly with no evidence of conversion to AIC. In contrast to extremity perfusion, in isolated dog liver perfusion experiments AIC formation was demonstrable after bolus administration of DTIC. About 30% of DTIC that had left the extracorporeal circulation reappeared as AIC.

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