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Alteration of dacarbazine pharmacokinetics after interleukin-2 administration in melanoma patients*

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Summary. In an effort to improve the treatment of metastatic malignant melanoma, we evaluated the sequential administration of the chemotherapeutic agent dacarbazine (DTIC) and the biological response modifier interleukin-2 (rIL-2) in a phase I–II study. Since the combination of biological response modifiers and chemotherapeutic agents could alter drug disposition, we evaluated the pharmacokinetics of DTIC and its major metabolite, 5-amino-imidazole 4-carboxamide (AIC), before and after rIL-2 administration. DTIC (1 g/m^2, 24-h i.v. infusion) was given on day 1 and rIL-2 (2–4 million Cetus units/m^2, 30-min i.v. injection), on days 15–19 and 22–26 of each course of therapy. The second DTIC dose was given on day 29, i.e., 3 days after the last rIL-2 administration. DTIC and AIC were assayed by reversed-phase HPLC. DTIC plasma levels showed a significant decrease after rIL-2 administration as compared with DTIC values obtained in the same patients before rIL-2 administration. DTIC area under the curve (AUC) values obtained after rIL-2 were lower than those obtained on day 1 before rIL-2 administration ($P = 0.02$). After rIL-2, the total body clearance (Cl_T) was increased ($P = 0.04$), as was the volume of distribution at steady state (V_{ss}; $P = 0.02$). The decrease in AUC after rIL-2 administration became more pronounced as the rIL-2 dose was increased ($P = 0.03$). No significant difference was detected in the elimination phase of DTIC when half-lives obtained before and after rIL-2 administration were compared; the mean half-lives were 0.7 and 2.8 h for the α- and β-phases, respectively. The model-independent mean residence time was 3.4 h. The plasma AUC for the metabolite AIC did not charge after rIL-2 administration. AIC biphasic plasma elimination was also similar after rIL-2 administration, with α- and β-half-lives of 0.7 and 11.4 h, respectively. Urinary excretion of DTIC and AIC did not differ after rIL-2 administration; the overall DTIC excretion was 39% of the dose over 48 h, and AIC urinary excretion was 25% of the DTIC dose. The observed decrease in the DTIC plasma AUC after rIL-2 administration appears to be due to an increase in the volume of distribution, since other factors such as half-lives, urinary excretion, and metabolism were not significantly altered. The clinical consequences of the rIL-2-DTIC interaction remain difficult to assess based on presently available data, but this drug interaction should be taken into consideration in the development of future chemo-immunotherapy regimens that include high-dose rIL-2.

Introduction

In an effort to improve the treatment of metastatic malignant melanoma, we evaluated the sequential administration of the chemotherapeutic agent dacarbazine (DTIC) and the biological response modifier interleukin-2 (rIL-2) in a phase I–II study [4]. The choice of these agents is based on their demonstrated activity in metastatic malignant melanoma and their non-overlapping toxicities. DTIC is considered to be the standard single chemotherapeutic agent in metastatic malignant melanoma when treatment is indicated [8], and rIL-2 was recently reported to be active in metastatic malignant melanoma [9].

Since the combination of biological response modifiers and chemotherapeutic agents can alter the pharmacokinetics of the latter [1, 3, 6, 7, 10], we evaluated the pharmacokinetics of DTIC and its major metabolite, 5-amino-imidazole 4-carboxamide (AIC), before and after rIL-2 administration. We report that the plasma pharmacokinetics of DTIC is significantly altered after rIL-2 administration.

Patients and methods

Patients. Patients with histologically confirmed, measurable and unresectable metastatic malignant melanoma were eligible for this study.

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Table 1. DTIC and AIC plasma pharmacokinetics in melanoma patients before and after rIL-2 administration[a]

Patient number	rIL-2 dose (10^6 U/m^2)	Course[b]	DTIC								AIC		
			C_{end}[c] µg/ml	Half-lives α (h)	Half-lives β (h)	MRT_{iv} (h)	AUC (µg ml^{-1} h)	V_{ss} (l/m^2)	Cl_T (l h^{-1} m^2)	% change in AUC	C_{end}[c] (µg/ml)	AUC (µg ml^{-1} h)	% change in AUC
1	0	1	11.2	0.1	1.6	3.3	197	16.8	5.1		0.6	13.3	
	2	2	10.4	0.2	1.8	2.4	194	12.2	5.2	–1.5	0.4	8.9	–50.3
2	0	1	11.2	0.2	2.3	3.3	203	16.5	4.9		0.6	14.7	
	2	2	9.6	0.2	2	3.8	157	24.2	6.4	–22.7	0.8	14.2	–3.6
3	0	1	6.1	0.3	1.3	2.6	121	21.5	8.3		0.4	10.4	
	2	2	13.4	0.4	2.5	3.7	146	25.2	6.8	20.7	0.6	10.4	0.3
4	0	1	10.5	0.5	2.5	3.2	201	15.8	5		1	11.5	
	2	2	9.8	0.7	2.4	3.5	192	18.2	5.2	–4.5	0.6	13.8	17.0
5	0	1	10.9	0.7	4.4	3.7	224	16.7	4.5		0.5	15.1	
	2	2	7.8	0.6	3.6	3.8	132	28.5	7.6	–41.1	0.5	10.4	–44.8
6	0	1	8	1.3	1.3	3.1	156	20	6.4		0.5	11.3	
	3	2	8	1.1	1.5	3.2	159	20.4	6.3	2	0.7	14	19.2
7	0	1	7.2	0.8	1.8	2.9	137	21.1	7.3		0.6	10.8	
	3	2	5.5	0.7	1.8	3.9	107	36	9.3	–21.9	0.6	11.2	3.5
8	0	1	7.4	1.5	3	3.7	144	25.8	6.9		0.5	11.9	
	2	2	7.9	1.2	3.8	3	147	20.4	6.8	2.1	0.4	11.6	–2.3
9[d]	0	1	9.1	1.1	3.1	5.9	153	38.5	6.5		0.4	10.7	
10[d]	0	1	10.1	1.2	1.3	3.4	161	21.3	6.2		0.4	11.1	
11	0	1	9	1.5	1.5	3.9	167	23.3	6		0.5	12.6	
	3	2	5.1	0.7	3.8	3.2	113	28	8.8	–32.3	0.5	8.9	–42.7
12	0	1	7.5	1	2.3	2.4	160	14.8	6.3		0.4	12.1	
	4	2	8.1	0.2	1.9	4.2	123	34	8.1	–23.1	0.5	10.5	–14.9
13	0	1	8.6	0.6	6.2	3.3	154	21.3	6.5		0.5	12	
	4	2	4.3	0.5	7.4	3.5	64	55.4	15.7	–58.4	0.5	12.1	0.4
14	0	1	3.2	0.7	5.1	3	71	42.7	14		0.6	10.2	
	4	2	2.2	0.6	3	2.7	40	67.5	25	–43.7	0.5	8.4	–17.6
Before rIL-2 (mean)			8.6	0.8	2.7	3.4	161	22.6	6.7		0.5	12	
(SE)			±0.6	±0.1	±0.4	±0.2	±10	±2.2	±0.6		±0.04	±0.4	
After rIL-2 (mean)			7.7	0.6	2.9	3.4	131	30.8	9.3		0.6	11.2	
(SE)			±0.9	±0.1	±0.5	±0.1	±13	±4.6	±1.6		±0.03	±0.6	

[a] DTIC dose was 1 g/m^2 given as a 24-h i.v. infusion
[b] Course 1, before rIL-2 administration; course 2, after rIL-2
[c] C_{end} = concentration at the end of infusion
[d] Did not receive a second course
U/m^2 = Cetus units/m^2

Subjects were allowed to have received prior radiation therapy, chemotherapy, or immunotherapy other than those involving DTIC or rIL-2. Patients had to have a Zubrod's performance score of 0–2, with a life expectancy of at least 4 months. At the beginning of the study, subjects were required to have recovered from prior treatments. Written informed consent was obtained from each patient according to institutional guidelines, and the protocol was approved by both Wayne State University and by Cetus Corporation (Emeryville, Calif.).

rIL-2 administration. Human rIL-2 was obtained from Cetus Corporation (Emeryville, Calif.) and was injected i.v. over 30 min on an outpatient basis. All rIL-2 doses indicated in this paper are expressed in Cetus units. For comparison purposes, 1 mg rIL-2 = 3 million Cetus units = 18 million IU. The rIL-2 dose was 2×10^6 Cetus units/m^2 for patients 1–5 and 8; 3×10^6 Cetus units/m^2 for patients 6, 7, and 9–11; and 4×10^6 Cetus units/m^2 for patients 12–14.

Treatment regimen. DTIC was given every 28 days, beginning on day 1 (course 1), and rIL-2 was injected on days 15–19 and 22–26. Groups of three patients each began treatment at 2, 3, and 4×10^6 Cetus units/m^2. Intrapatient dose escalations of 1×10^6 Cetus units/m^2 were allowed for two additional cycles in the absence of disease progression or grade III toxicity. The second DTIC course (course 2) was given on day 29 (or day 1 of a second cycle), i.e., 3 days after the last rIL-2 administration.

DTIC administration. DTIC (Miles Pharmaceuticals) at 1 g/m^2 in 1 l 5% dextrose in water was given as a 24-h continuous i.v. infusion via a double-lumen Hickman catheter. The drug was protected from light at all times (from drug preparation to patient administration, blood samples acquisition, storage, and analysis).

HPLC assay for DTIC. We used the HPLC procedure described by Breithaupt et al. [2]. Briefly, the drug was extracted with methanol, and 20 µl extract (or 20 µl concentrated methanolic extract) was injected onto a reversed-phase octadecylsilane column. DTIC was eluted with a mobile phase composed of 5 m*M* tetrabutyl ammonium phosphate at a flow rate of 2.5 ml/min. The retention time of DTIC under these conditions was about 4 min. The UV detection was set at 326 nm. The calibration curves were linear to 50 µg/ml, with the correlation coefficient being near unity. The sensitivity of this assay was about 50 ng/ml, with a concentration step, if required.

HPLC assay for AIC. The metabolite was extracted with methanol, and 20 µl was injected onto an octadecylsilane column [2]. AIC was eluted with a mobile phase composed of 5 m*M* heptane sulfonic acid : methanol (80 : 20, v/v) at a flow rate of 1.0 ml/min. The retention time of AIC was about 6 min under these conditions. The UV detection was set at 270 nm. The calibration curves were linear to 50 µg/ml, with the correlation coefficient being near unity. The sensitivity of this assay was about 125 ng/ml.

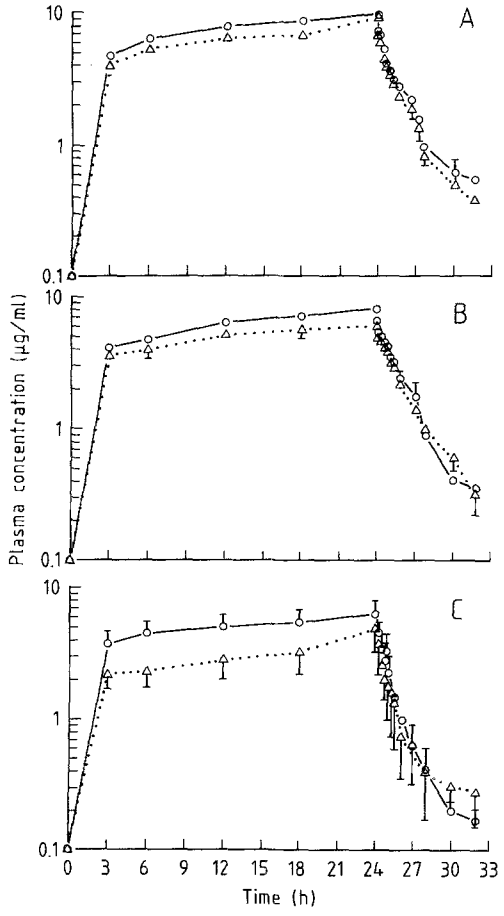

Fig. 1 A–C. DTIC plasma pharmacokinetics in patients with metastatic melanoma before and after rIL-2 administration, as a function of the rIL-2 dose. DTIC was given at a dose of 1 g/m^2 as a 24-h i.v. infusion. **A** 2×10^6 Cetus units/m^2. **B** 3×10^6 Cetus units/m^2. **C** 4×10^6 Cetus units/m^2. *Circles*, DTIC before rIL-2 administration; *triangles*, DTIC after rIL-2 administration. Each point represents the mean ±SE (error bars not shown if smaller than symbol size). DTIC was assayed by HPLC

Pharmacokinetic analysis. DTIC/AIC plasma concentrations as a function of time were fitted with the nonlinear regression program PC-NONLIN (Statistical Consultants, Lexington, Ky.) to a two-compartment open model with continuous zero-order input, using a data weight of 1/Y. The area under the curve (AUC) was determined by the trapezoidal method from zero to infinity. Model-independent analysis was used to determine the mean residence time (MRT), the total body clearance (Cl_T; dose/AUC), and the volume of distribution at steady-state (V_{ss}) [5].

Statistical analyses. All data are expressed as the mean ±SE. The statistical difference between a pharmacokinetic parameter before vs after rIL-2 administration was assessed using Student's *t*-test for paired observations, each patient being his own control. A *t*-test was done on the correlation coefficient, *r*, of the linear regression analysis of the percentage of change in Cl_T as a function of the rIL-2 dose. A *P*-value of <0.05 was considered to be statistically significant.

Results

DTIC plasma pharmacokinetic values obtained before and after rIL-2 administration are presented in Table 1. DTIC plasma concentrations obtained before rIL-2 administration (on day 1) were generally higher than those measured in the same patients on day 29, after administration of rIL-2 on days 15–19 and 22–26. As a consequence of lower DTIC levels, the AUCs after rIL-2 injection were smaller ($P = 0.02$) than those obtained on day 1 before IL-2 administration. After rIL-2 injection, the Cl_T was increased ($P = 0.04$), as was the V_{ss} ($P = 0.02$).

The decrease in the DTIC plasma AUC after rIL-2 administration became more pronounced as the rIL-2 dose increased from 2×10^6 to 4×10^6 Cetus units/m^2 (Fig. 1, Table 1). A linear regression analysis of the percentage of change in AUC as a function of the rIL-2 dose yielded a significant ($P = 0.03$) correlation coefficient of $r = -0.61$ ($y = 25.83 - 16.23x$). In the group of patients that received the highest dose of rIL-2 (4×10^6 Cetus units/m^2), the mean percentage of decrease in AUC was 42%.

No significant difference was detected in DTIC half-lives obtained before and after rIL-2 administration (Fig. 1, Table 1). The mean α- and β-half-lives were 0.7 and 2.8 h, respectively. The model-independent MRT was 3.4 h.

Since DTIC is not active per se and needs microsomal activation to be cytotoxic, we also examined the pharmacokinetics of its major metabolite AIC, which is formed along with the cytotoxic carbonium ion. The plasma AUC of AIC did not show a significant change after rIL-2 administration (Table 1). AIC plasma elimination was similar before and after rIL-2 administration: before rIL-2, the α- and β-half-lives were 0.79 ±0.12 and 10.4 ±2 h, respectively; after rIL-2 injection, these values were 0.66 ±0.08 and 12.4 ±2.7 h, respectively.

The 48-h values for urinary excretion of DTIC and AIC were obtained in 17 courses. No statistical difference in the urinary excretion of DTIC was noted between the first course of DTIC (30.2% ±8.2%) and the second course given after rIL-2 (42.3% ±6.7%). Similarly, rIL-2 administration had no significant influence on AIC urinary excretion. The AIC urinary excretion before rIL-2 was 24.6% ±5.5%, and after rIL-2 administration it accounted for 26.1% ±6.4% of the DTIC dose excreted as AIC.

Discussion

The influence of rIL-2 administration on DTIC pharmacokinetics was evaluated during a phase I–II study of this biological response modifier-drug combination in patients with metastatic malignant melanoma. It was observed that rIL-2 could significantly decrease the DTIC plasma AUC after rIL-2 injection; moreover, this decrease was correlated with the rIL-2 dose, giving a 42% decrease in the AUC at the highest rIL-2 dose given (4×10^6 Cetus units/m^2). Cl_T and V_{ss} values were increased significantly after rIL-2 administration.

The decrease in the DTIC plasma AUC after rIL-2 injection appears to be caused by an increase in the V_{ss} value, since other possible factors such as shorter half-lives, increased urinary excretion, or increased metabolism were not observed in this study. The increase in the V_{ss} could be related to the edema frequently observed in rIL-2-treated patients, since most of the side effects of rIL-2 appear to be secondary to its ability to mediate a decrease

in systemic vascular resistance and a presumed increase in capillary permeability [9].

This drug interaction is different from those previously reported for DTIC and other biological response modifiers, by which, contrary to the decrease in DTIC exposure (AUC) observed in the present study, an increase in DTIC exposure after administration of the biological response modifier was noted due to a depression of DTIC metabolism. A decrease in drug metabolism was observed with bacillus Calmette-Guérin and *Corynebacterium parvum* [1, 3, 6, 7, 10]. For example, *C. parvum*, an immune adjuvant used with DTIC in the treatment of metastatic malignant melanoma, causes a prolongation of the DTIC plasma half-life to 4.8 h as opposed to 3 h for controls; actinomycin D given with *C. parvum* can reverse this effect and return the half-life to control values [1]. *C. parvum* treatment has also been shown to depress the *N*-demethylation of other drugs [7].

The clinical consequences of this rIL-2-DTIC interaction remain difficult to assess based on presently available data. In our previous study [4], this drug regimen was well tolerated and did not produce overlapping toxicity. Also, encouraging responses were observed in visceral metastases; these responses could be partly due to the changes in DTIC distribution reported in this paper.

This drug interaction should be taken into consideration in the development of future chemo-immunotherapy regimens that include DTIC or other drugs, especially when high doses of rIL-2 are used, since changes in the pharmacokinetics of anticancer drug(s) used with rIL-2 may also change the pharmacodynamics of the latter.

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