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Phase I clinical trial and human pharmacokinetics of 2,4-diamino-5-adamantyl-6-methyl pyrimidine ethane sulfonate (DAMP-ES): a lipid-soluble antifolate*

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Summary. A phase I and pharmacokinetic study of a novel lipid-soluble antifolate, 2,4 diamino-5-adamantyl-6-methyl pyrimidine ethane sulfonate (DAMP-ES) has been carried out on two schedules: I – daily ×5; II – 24-h continuous infusion. In schedule I, doses of 10–90 mg/m^2 per day were evaluated. Dose-limiting toxicity was hematologic, but nausea and vomiting, skin rash, diarrhea, anorexia, alopecia, mucositis, and neurotoxicity were also noted. In schedule II, doses of 192 and 240 mg/m^2 were evaluated. Dose-limiting toxicity was neurotoxicity, but hematologic toxicity was also marked. Recommended starting doses for phase II studies are 75 mg/m^2 per day for 5 days or 192 mg/m^2 by continuous infusion for 24 h. Pharmacokinetic studies indicated a β-phase plasma half-life of 12.4–24 h and a large and variable volume of distribution.

Introduction

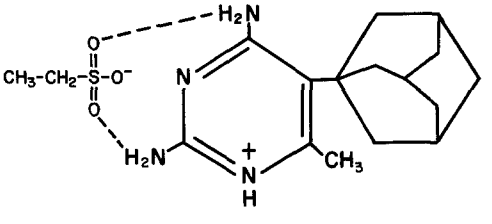
2,4-Diamino-5-adamantyl-6-methyl pyrimidine (DAMP-ES) is a lipid-soluble antifolate developed by one of us (SFZ) in the course of a study on the structure-activity relationships of substituted pyrimidines as inhibitors of dihydrofolate reductase [7]. DAMP has an ID_{50} for TA-3 mouse mammary carcinoma cells in vitro of 6 nM and an ID_{50} for sarcoma 180 cells in vitro of 400 nM [6]. It is active against the Walker 256 carcinosarcoma in the rat [15], a tumor which is essentially resistant to methotrexate. For this reason DAMP underwent preclinical toxicology and pharmacokinetic analysis in preparation for initial clinical trial.

The clinical form of the drug and the form evaluated in preclinical toxicology is the ethane sulfonate salt (Fig. 1). When given by rapid intravenous (i.v.) injection, the drug produced acute central nervous system toxicity. However, this could be obviated by giving it in divided doses on a 12-h schedule for 10 doses. On this schedule the drug produced antiproliferative toxicity consisting of toxic gastroenteritis with bloody diarrhea and some degree of myelosuppression [17]. Pharmacokinetics in the dog showed a very rapid α-phase half-life of 0.38 min, a β-phase half-life of 178 min, and a large volume of distribution [17]. These data indicate that the drug was being removed extremely rapidly from the central compartment and presumably localized in the fat tissues because of its high degree of lipid solubility. On the basis of these data, a phase I clinical trial was designed using a daily ×5 schedule, in the course of which the pharmacokinetics of [^{3}H] DAMP-ES were studied in selected patients. A second schedule of 24-h infusion was then explored. Because of acute CNS toxicity in the dog when the drug was given rapidly, the drug was given to patients by infusion over at least 1 h. The study was conducted under an IND from the FDA after approval of the clinical protocol by the Institutional Review Board. Preliminary reports of some of these data have appeared [2, 12, 13].

Materials and methods

DAMP-ES for clinical use was manufactured by Starkes Associates and formulated in 10- and 50-mg vials by the research pharmacy at this institute. The drug was administered, dissolved in 250 ml 5% dextrose in water, over a period of 1–2 h daily for 5 days.

Patients with advanced cancer not amenable to other treatments, or for whom other treatments had proven ineffective, were entered into the study, after having given written informed consent and after the experimental nature of the treatment, the possible hazards, the alternatives, and the freedom to withdraw at any time from the study had been carefully explained to them in both written and oral form. Patients had a minimum life expectancy of at



DAMP-ES

Fig. 1. Structure of DAMP-ES

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least 2 months. They were required to have a white blood cell count (WBC) of at least 4,000/mm^3 and a platelet count of at least 100,000/mm^3. A serum bilirubin of less than 1.5 mg/100 ml, serum glutamic oxalacetic transaminase (SGOT) of less than 100 IU/ml, and serum creatinine of less than 1.5 mg/100 ml were also required. The minimum creatinine clearance for entry into the study was 60 ml/min.

Before treatment and following each course of treatment, a complete blood count (CBC) and platelet count were carried out twice weekly and serum Na^+, K^+, Ca^{2+}, and PO_4^{3-}, creatinine, uric acid, total protein, albumin, bilirubin, alkaline phosphatase, LDH, SGOT, and BUN were measured weekly. Pretreatment EKGs and EEGs were also carried out.

Except as noted below, each patient received two 5-day courses of the drug. In the absence of toxicity, the second course was begun on day 22. When toxicity was observed with the first course, the second course was given when the WBC was $>4{,}000/mm^3$ and the platelet count was $>100{,}000/mm^3$. In cases where disease was stable, patients were continued on DAMP-ES beyond two doses, with adjustment of the dose to produce moderate reversible toxicity.

Nontoxic patients were retreated at higher dose levels if clinically indicated. However, to avoid the possibility of cumulative toxicity, such patients were permitted to be reentered into the study at a higher dose level only after a minimum of 8 weeks off the drug.

The planned dose escalation was by 100% increments until biological effect was seen, then by 50% increments to mildly toxic dose levels, and 33% increments to moderately reversible toxicity.

After completion of the daily $\times 5$ schedule (schedule I), an evaluation of a 24-h continuous infusion (schedule II) was carried out. The starting dose was 192 mg/m^2 as a single 24-h infusion.

Radioactive material. [^{3}H] DAMP-ES was prepared by direct tritiation of nonradioactive DAMP-ES by the Radiochemical Centre (Buckinghamshire, England). The crude material was purified by repeating twice a process of extraction of the free base with dichloroethane, conversion to the ethane sulfonate salt, and extraction with water. This was followed by chromatography on a Sephadex G-25 column (Pharmacia K16/40) as previously described elsewhere [14]. This simplified procedure produced material 96% radiopure which was the same as that obtained by an earlier described method [14]. The standardized aqueous solutions of [^{3}H] DAMP-ES were sterilized by ultrafiltration and distributed into sterile ampules, each containing 250 μCi in 100–400 μl water. Nine patients received [^{3}H] DAMP-ES (250–750 μCi) added to an infusion of the daily $\times 5$ study, of whom seven gave data that were sufficiently complete to permit pharmacokinetic analysis. One patient was studied with a 24-h infusion of [^{3}H] DAMP-ES.

Blood and urine collection. Blood samples were drawn into heparinized tubes at midinfusion, at the end of infusion, and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 8, 12, and 24 h thereafter. Urine was collected at 6-h intervals for 24 h or longer.

Analytical procedures. The blood samples were centrifuged as soon as possible after collection, and the total radioactivity of plasma was determined by direct counting of 500-μl aliquots.

The unchanged free drug was estimated by a slightly modified procedure, used earlier in the dog study [17], which was based on the extractability of DAMP with dichloroethane. The modification involved centrifugation to assure better separation of the organic from the aqueous layer rather than separation by standing. Since this extraction of DAMP from plasma is only 76%±6% efficient, all data were corrected accordingly. To determine the extent of binding of the drug by the blood cells, experiments were carried out in which [^{3}H] DAMP-ES was added to the freshly drawn, heparinized blood. Aliquots were removed for counting, and after 1 min the blood was centrifuged and radioactivity remaining in plasma was determined. Only 53%±2% of the original radioactivity was recovered in plasma. By chromatography of lysed red cells on the Sephadex G-25 [17], it was established that the drug in red cells was not tightly bound.

The total volume of urine from each collection was determined and 200-μl aliquots were counted for radioactivity. Aliquots were made alkaline and extracted with dichloroethane. After separation of the phases, 200 μl of each phase were counted for radioactivity.

Thin-layer chromatography of urine fractions was carried out on 1-in.-wide strips cut from plastic plates coated with silica gel (Merck 60F-254). The eluting solvent was: 30 parts ethylacetate, 20 parts ethoxyethanol, 10 parts 4% formic acid (EEF). After drying, the strips were cut in 0.5-cm-wide segments. Each segment was placed in a scintillation vial and covered with 100 μl water. After a brief shaking, 10 ml scintillation solution was added and radioactivity was determined. The analytical chromatography on Sephadex G25 columns was carried out as described earlier [17].

Counting of radioactivity. All counting was performed in a Searle Mark III scintillation spectrometer Model 6880 using Liquiscint 2 scintillation solution (Research Products Intern. Corp.). Whole blood was counted using soluene-250 by a procedure described in the Packard instruction manual *Soluene-250 soluene-100.*

Data analysis. For the estimation of pharmacokinetic parameters, Eq. 1 [4] was fit to the data with nonlinear regression using the PCNONLIN software package on an IBM PC/AT microcomputer. A weighting factor of the reciprocal of the square of the observed dependent variable was used.

$$C_p = \frac{A}{\alpha\tau}\left[e^{-\alpha t^*} - e^{-\alpha t}\right] + \frac{B}{\beta\tau}\left[e^{-\beta t^*} - e^{-\beta t}\right] \qquad (1)$$

Equation 1 is an empirical model for a two-compartment system with constant i.v. infusion. In Eq. 1, Cp is the plasma concentration of free DAMP; τ is the infusion time; t is the time from the beginning of the infusion; $t^* = 0$ during infusion; and $t^* = t-\tau$ after the end of infusion; A, B, α, and β are estimable empirical parameters. From A, B, α, and β, model independent parameters such as $t_{1/2}\alpha$ (the α-phase half-life), $t_{1/2}\beta$ (the β-phase half-life), V_d (the volume of distribution), AUC (the area under the C_p vs t curve

Table 1. Patient characteristics, daily × 5 administration

Pt. no.	Age	Sex	Diagnosis	Prior therapy	P.S. (ECOG)	Dose mg/m²	No. courses
1	58	M	Adenocarcinoma of the prostate	DES, DDP, 5FU, CTX, PRED, ADR, MTX	3	10	1
2	48	M	Adenocarcinoma of the colon	R, 5FU, MIT-C, DITC, BCNU	0	10	1
3	62	F	Adenocarcinoma of the colon	5FU, HO-UREA	0	10	3
4	47	F	Carcinoma of the colon	5FU, MIT-C	1	10	2
5	59	M	Mixed-cell parotid carcinoma	5FU, MTX, CTX, ADR, METHYL-GAG, ARA-C/DAUR	0	10	2
6	52	M	Adenocarcinoma of the prostate	DES, DDP, CTX, 5FU, Estracyt	2	10	2
7	70	M	Adenocarcinoma of the colon	MECCNU, METHYL-GAG, MIT-C	3	20	2
8	52	M	Adenocarcinoma of the colon	5FU	0	20	2
9	70	M	Adenocarcinoma of the colon	MTX, 5FU/TDR	0	20 40	2 4
10	52	M	Adenocarcinoma of the colon	5FU, MECCNU	0	20 60	2 2
11	64	M	Adenocarcinoma of the prostate	DDP, CTX, 5FU, Estracyt, MTX/CF	3	20 60	2 2
12	65	F	Adenocarcinoma of the colon	R, 5FU, VCR, MECCNU	0	40	1
13	64	M	Adenocarcinoma of the prostate	5FU, CTX, ADR, MTX/CF	0	40 90 75	2 2 6
14	54	M	Adenocarcinoma of the colon	5FU, HO-UREA, CTX, VCR, MTX	1	60	1
15	51	M	Adenocarcinoma of the rectum	MER	0	60 75 90	2 2 1
16	50	M	Carcinoma of the rectum	5FU	1	90 75	1 1
17	67	M	Carcinoma of the colon	5FU, HO-UREA, MECCNU, DTIC	0	90 75	1 4
18	51	F	Adenocarcinoma, rectum	MECCNU, VCR, 5FU	0	75	2
19	60	M	Squamous-cell carcinoma, tongue	R, DDP, CTX, ADR, BLEO, MTX, VCR	0	75	2
20	53	M	Melanoma	DDP, DTIC, BCNU, ACT.D, VCR, MTX	2	75	1
21	65	F	Adenocarcinoma, lung	DDP, ADR, 5FU, MTX, VCR, CTX, BLEO	2	75	1 (only rec. 3 doses)
22	69	M	Adenocarcinoma, prostate	DDP, CTX, Estracyt, MTX, 5FU/CF	2	75	4
23	52	M	Adenocarcinoma, colon	R, 5FU, MIT-C	1	75	1
24	56	M	Adenocarcinoma, rectum	R	0	75 80	2 2
25	62	F	Adenocarcinoma, colon	MIT-C	1	75	1
26		M	Adenocarcinoma, colon	5FU	1	75	1
27	66	F	Adenocarcinoma, colon	CTX, VCR, MTX, CHIP	1	75	1
28	54	F	Adenocarcinoma, colon	MTX, FUDR	0	75	2
29	45	M	Carcinoma, colon	5FU, MTX, CCNU	3	90	1
30	60	M	Adenocarcinoma, colon	5FU	1	90	4
31	63	M	Adenocarcinoma, colon	5FU, CCNU, VCR, MIT-C, BCG	0	90	1
32	57	M	Renal cell carcinoma	VBL, BCNU	3	90	2
33	44	F	Adenocarcinoma, breast	R, CTX, MTX, 5FU, Tamoxifen, ADR, VBL, MIT-C, Thiotepa	2	90	3

Table 1. (cont'd)

Pt. no.	Age	Sex	Diagnosis	Prior therapy	P.S. (ECOG)	Dose mg/m²/24 h CI
34	58	F	Small-cell carcinoma, lung	R, DDP, VCR, hyperthermia	2	192
35	38	M	Fibrosarcoma	ADR, DTIC, DDP, MTX	1	192
36	58	M	Liposarcoma	R, ADR, DTIC, MTX, DDP, CTX, VCR, ARA-C/DAUR	0	192
37	54	F	Adenocarcinoma, liver	–	3	192
38	58	M	Adenocarcinoma, rectum	R, MIT-C	2	192
39	58	M	Adenocarcinoma, colon	R, 5FU, MECCNU, VCR	3	192
40	71	M	Adenocarcinoma, colon	5FU, MIT-C	0	192
41	49	F	Bronchoalveolar carcinoma, lung	BLEO, MTX, 5FU, ADR	4	192
42	61	M	Adenocarcinoma, colon	5FU	0	192
43	66	M	Adenocarcinoma, colon	5FU	0	192
44	44	M	Malignant mesenchymoma	R, CYC, VCR, ADR, DTIC, DDP	1	192
45	64	M	Adenocarcinoma, colon	5FU, HOUREA, MIT-C	0	192
46	46	M	Adenocarcinoma, rectum	5FU, MECCNU	0	240
47	60	M	Adenocarcinoma, colon	5FU	0	240
48	62	F	Adenocarcinoma, colon	–	0	240 192
49	68	M	Squamous-cell carcinoma, lung	CTX, ADR	0	240

Abbreviations: R, Radiation therapy; 5-FU, 5-fluorouracil; MECCNU, 1-(2-chloroethyl)-3(4-methylcyclohexyl)-1-nitrosurea; BLEO, bleomycin; MTX, methotrexate; ADR, doxorubicin; DDP, cisplatin; VCR, vincristine; CTX, cyclophosphamide; VBL, vinblastine; MIT-C, mitomycin C; BCNU, 1,3-*bis*-chloro(2-chloroethyl)-1-nitrosourea; CHIP, iproplatin; DTIC, dacarbazine; ACT.D, actinomycin D; DES, diethylstilbestrol; CF, citrovorum factor; PRED. prednisone; HO-UREA, hydroxyurea; FUDR, 5-fluoro-2'deoxyuridine; MER, methanol extractable residue; BCG, bacillus calmette guerin; CCNU, 1-(2-chloroethyl)-3(cyclohexyl)-1-nitrosourea; ARA-C, cytosine arabinoside; DAUR, 3-deazauridine; TDR, thymidine; METHYL-GAG, methyl glyoxal-*bis*-guanyl hydrazone

from time 0 to infinity), and Cl_s (the systemic clearance) were calculated [3].

Results

Schedule I

In schedule I, 33 patients received 83 complete courses. One patient received an incomplete course at 75 mg/m² per day and is not included in the toxicity evaluation. There were 24 males and 9 females with a median age of 58 (range 44–72) years. The patients treated, with their pretreatment characteristics and the doses received, are listed in Table 1. The starting dose was 10 mg/m² per day for 5 days. At a dose of 40 mg/m², a platelet count of 23,000/mm³ was reported in one patient after the second course and a WBC of 3,000/mm³ after the third course, so the escalation was reduced to 50%. A dose of 90 mg/m² per day ×5 produced severe hematologic toxicity in two of the first three patients treated. An intermediate dose of 75 mg/m² per day ×5 was therefore evaluated, but when this proved to be easily tolerated, 90 mg/m² was again cautiously explored in additional patients.

The dose-limiting toxicity was hematologic; both leukopenia and thrombocytopenia were seen 10 to 17 days after administration of the drug. This was seen in 5 of 23 evaluable courses at 75 mg/m² per day and in 8 of 16 evaluable courses at 90 mg/m² per day. However, at 90 mg/m² per day ×5, all eight patients evaluable for hematologic toxicity developed myelosuppression, with WBC ≤3,000/mm³ in seven and platelet count ≤13,000/mm³ in three (Table 2). One patient developed mucosal hemorrhages related to thrombocytopenia. Recovery was prompt and there were no fatalities or drug-related sepsis, and there was no evidence of cumulative toxicity. A variety of nonhematologic toxicities were seen and are listed in Table 3. Nausea and vomiting were mild or moderate (grades 1 and 2) except in one patient, who had grade 3 vomiting lasting 36 h. The skin rash was a mild (grade 1), evanescent maculopopular eruption, usually on the trunk, disappearing within 2–3 days of termination of the treatment. A variety of symptoms related to the neurotoxicity of this agent were seen and are listed in Table 3, indicating the neurotoxic potential of the drug. These symptoms were generally mild and of short duration (usually 1–6 h). There were no long-term sequelae (follow-up 1–4 months). Diarrhea was mild (grade 1) and lasted for 1–3 days; oral toxicity was grade 1 or 2.

Schedule II

An evaluation of Schedule II was carried out in 16 patients, whose pretreatment characteristics are listed in Table 1. The starting dose was 8 mg/m² per hour for 24 h, for a total dose of 192 mg/m². Escalation to 10 mg/m² per hour (240 mg/m² total dose) was carried out and four patients were treated at this dose; however, it proved too tox-

Table 2. Hematologic toxicity of DAMP-ES

Daily dose	Schedule	Evaluable patients	WBC <4,000/mm³			Platelet count <100,000/mm³		
			N	Median[a]	Range[a]	*N*	Median[a]	Range[a]
75	I	12	4	3.4	1.6–3.6	4	80	43–99
90	I	8	7	2.5	0.4–3.0	5	13	8–99
192	II	9	6	3.1	0.9–3.9	2	67	36–97
240	II	4	3	1.7	0.1–2.1	3	12	4–16

[a] Median and range are for WBCs below 4,000/mm³ or platelet counts below 100,000/mm³
Schedule I – 1 h infusion daily for 5 days
Schedule II – 24 h CI

ic, so the study was completed by adding further patients at a dose of 192 mg/m². The hematologic toxicity is shown in Table 2, and the nonhematologic toxicity in Table 3. Hematologic toxicity was severe in three of four patients treated at the higher dose, with drug-related sepsis in one patient. In addition, severe neurotoxicity was seen in one patient (convulsions) and severe (grade 3) skin toxicity in one patient. A dose of 192 mg/m² per day by continuous infusion (CI) was well tolerated.

Patient benefit

One partial response was seen in patient no. 16, who showed a greater than 50% decrease in an abdominal mass by computerized tomography (CT) scan. Other patient benefits noted were an increase in performance status from 3 to 0–1 in patient no. 33 and disappearance of bone pain in patient no. 13. All these patients were treated on schedule I.

Pharmacokinetic studies

The estimated pharmacokinetic parameters for seven patients (eight courses) are summarized in Table 4. The first seven courses used a 1- to 1.5-h continuous infusion, whereas the eighth course used a 24-h continuous infusion. The number of data points used for the estimations ranged from 12 to 17. A two-compartment model, taking into account the administration by infusion (Eq. 1), fit all of the data well. The 95% confidence limits for the estimated parameters were wide enough to include 0 only for the eighth course.

This study used infusion for time periods (1–24 h) much longer than the anticipated half-life of the true α-phase from an i.v. bolus injection [$t_{1/2}\alpha$ was found to be 23 s in an i.v. bolus study in the dog [17]. Therefore, the parameter estimates reported in Table 4 should be interpreted as purely empirical: no inferences to hypothetical central and peripheral compartments should be made. However, several clear conclusions can be made from the empirical parameters in Table 4: 1) The volume of distribution for free DAMP ranges from 39.4 to 578 l. This greatly exceeds the weight of the patients and implies a sequestration of DAMP in the tissues. Such behavior is in good agreement with results found in the dog [17] and in cell culture [5]. 2) The apparent α phase of DAMP disposition is quite rapid (6.12–31.7 min for the patients receiving a 1-h infusion), and the apparent β-phase half-life is quite long (12.4–24.4 h). 3) There is large variation in the pharmacokinetic parameters among patients, and even between two courses given to the same patient (see patient no. 9).

In 24 h the recovery of total radioactivity in urine (13%–30%) was incomplete. Of the recovered radioactivity, less than 20% was extractable with dichloroethane.

The chromatographic profiles of the radioactivity in the urine showed at least two major components in the aqueous phase and at least one compound in the organic extract, in addition to unchanged DAMP.

Discussion

DAMP is a lipid-soluble antifolate which was entered into clinical trial because of its activity against experimental tu-

Table 3. DAMP-ES phase I study: nonhematologic toxicity

Dose mg/m²/day × 5	No. pts.[a]	Nausea and vomiting	Skin rash	Diarrhea	Anorexia	Alopecia	Mucositis	Disorientation/confusion	Face numbness	Dizziness
<60	18	4	2	0	0	0	0	1	0	0
75	14	8	2	1	0	1	2	0	2	1
90	8	5	1	1	0	0	0	1	0	0
mg/m²/24 h CI										
192	11	3	0	2	0	0	0	0	0	2
240[b]	4	0	3	2	0	0	2	2	0	0

[a] Number of patients evaluable for nonhematologic toxicity
[b] One patient had convulsions and one had hallucinations

Table 4. Summary of pharmacokinetic parameters for all cases studied[a]

Pt. no.	Length of infusion (h)	Dose mg/m^2	t α (min)	t β (h)	V_d L	AUC μ*M*/h	AUC/total dose h/ml^{-1}	Cl_s L/h^{-1}
9[b]	1.00	20	7.02	16.0	89.2	5.87	58.3	17.2
13	1.33	40	6.12	24.4	39.4	7.95	45.6	21.9
9[b]	1.50	40	19.6	17.6	271	5.06	27.3	36.6
14	1.00	60	31.7	16.7	578	4.70	15.6	64.0
24	1.00	75	6.24	12.4	217	2.86	8.09	124.0
20	1.00	75	9.18	16.4	70.5	9.81	36.0	27.8
27	1.24	75	21.5	12.7	185	8.47	22.9	43.6
34	24.0	192	55.3	22.3	550	14.1	17.9	55.8

[a] Symbols used are: $t_1\alpha$, the half-life for the alpha phase of drug disappearance from plasma; $t_1\beta$, the half-life for the beta phase; V_d, volume of distribution; AUC, area under the C_p vs time curve from 0 to infinite time; Cl_s, the systemic clearance
[b] Patient 9 was given two courses of DAMP

mors resistant to methotrexate. Since it is lipid-soluble, it should be more easily taken up into the cell and, therefore, circumvent any resistance to methotrexate that occurs due to a loss of the specific transport mechanism for methotrexate into the cell. In large-animal toxicology studies, the agent produced convulsions when given by rapid i.v. injection and, for this reason, the drug in human studies was given by a 1-h infusion, and the drug administration spread over 5 days or by 24-h continuous infusion. Its dose-limiting toxicity in man was myelosuppression, producing leukopenia and thrombocytopenia at the highest dose level studied. Other evidence of antiproliferate activity such as mucositis, alopecia, and diarrhea were seen but were not marked. Nausea and vomiting occurred but were easily controlled. The toxicity of major concern, apart from myelosuppression, was the evidence of neurotoxicity. While neurotoxicity was noted in schedule I, the neurotoxic potential of the drug became more evident with schedule II. The higher dose of 240 mg/m^2 was abandoned because of neurotoxicity after four patients had received the drug (hematologic toxicity was also severe). One patient had a grand mal seizure and two others showed extreme restlessness and confusion. The latter two patients received dilantin and valium for prophylaxis.

In terms of toxicity, DAMP-ES has features common to many of the lipid-soluble antifolates. DDMP, triazinate, trimetrexate, and piritrexim all showed myelosuppression, stomatitis, and skin rash; in addition, triazinate showed major neurotoxicity which, however, does not appear to be a feature of the toxicity of trimetrexate and piritrexim in the trials reported to date [1, 8–11].

In pharmacokinetic studies, plasma and urine from in seven patients eight courses of [^{3}H] DAMP-ES treatment were analyzed for total radioactivity and for the radioactivity extractable with dichloroethane, a quantity considered to represent the free drug. The pattern of distribution of DAMP-ES ($t_{1/2}\alpha$. Vd, Cl_s) agrees well with the lipophilic character of the drug and with the results observed in animal studies [6, 16, 17].

In 24 h the urinary recovery of radioactivity was incomplete, indicating persistence of the drug or its metabolites in the tissue. The unchanged drug represented only a small fraction of the total radioactivity excreted.

Two features of the design of this phase I study deserve comment. As part of our ongoing evaluation of the commonly used parameters for phase I study, we used somewhat unconventional approaches to starting dose and escalation.

The starting dose used was higher than is customary, namely 1/12 of the LD_{50} found in animal toxicology. This higher than usual starting dose was based on the observation that the toxicity was uniform across species (mouse, rat, and dog showed the same LD_{50} of 120–150 mg/m^2 when the drug was given daily for 5 days). Moreover, the pharmacokinetics in two species, the rat and the dog, are similar. This gave rise to an expectation that the human toxicology would be quantitatively similar to that in animals. From these animal studies, a maximum tolerated dose of approximately 60 mg/m^2 daily for 5 days was predicted. This was very close to the value of 90 mg/m^2 actually determined.

The escalation (100% until minimal biological effect, 50% to grade 1 toxicity, 33% for grade 1 toxicity, no escalation for grade 2 toxicity, and deescalation for > grade 2 toxicity) has been used in several of our recent phase I studies. The traditional Fibonacci search, still used by many investigators, would have reached a dose of 100 mg/m^2 in seven dose escalations, quite similar to the result produced in the present study (a dose of 90 mg/m^2, the maximum tolerated dose, in five dose escalations, followed by deescalation to 75 mg/m^2). The major advantage of the more rapid escalation is seen when the starting dose is too low, which, as noted above, was not the case in the present study.

The starting dose of 8 mg/m^2 per hour for schedule II, a total dose approximately 50% of the total dose yielding moderate toxicity on schedule I, gave the moderately toxic dose on the first dose step. This was probably fortuitous and, in retrospect, a more conservative approach would probably have been advisable.

The recommended dose for phase II studies is 75 mg/m^2 per day for 5 days, with subsequent escalation to 90 mg/m^2 if 75 mg/m^2 produces no hematologic toxicity. Slow infusion of the drug is recommended, with careful observation for possible neurotoxicity. For 24-h continuous infusion, a dose of 192 mg/m^2 should probably not be exceeded. Patients with increased liability to seizures should be excluded from treatment with the drug.

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