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Pharmacokinetic and phase I study of intravenous DON (6-diazo-5-oxo-L-norleucine) in children*

Margaret P. Sullivan[1], J. Arly Nelson[1], Stuart Feldman[2], and Bay Van Nguyen[3]

[1] Division of Pediatrics, The University of Texas M.D. Anderson Hospital and Tumor Institute at Houston, Houston, TX, USA
[2] Department of Pharmaceutics, The University of Houston, College of Pharmacy, Houston, TX, USA
[3] 2622 NASA Road 1, Seabrook, TX, USA

Summary. DON (6-diazo-5-oxo-L-norleucine), a glutamine antagonist, has been subjected to limited clinical trials since 1957. Use of the drug in adults has been curtailed due to sparse reports of effectiveness as well as its dose-limiting toxicities, i.e., severe nausea, vomiting and mucositis. In earlier studies, children given DON orally in combination with 6-mercaptopurine had significant prolongation of remission of acute leukemias during maintenance therapy. As DON is acid-labile and relatively unstable in solution, oral administration does not appear to be ideal for DON. In the trial described in this report, i.v. DON therapy was studied, using i.v. chlorpromazine to control vomiting, in 20 children, 17 of whom were evaluable following treatment at DON dose levels ranging from 150 mg/m^2 to 520 mg/m^2. Nausea and vomiting, the dose-limiting toxicity for adults, was controlled with chlorpromazine. Mucositis, which has also been observed in adults, did not occur in the children given DON i.v. A maximum tolerated dose was not defined; however, the projected maximum tolerated dose appears to be in excess of 450 mg/m^2.

DON was measured in plasma using a rapid-sampling HPLC procedure. The total body clearance, plasma t½, and area under the plasma concentration curve (AUC) were calculated using a noncompartmental method. The drug is rapidly cleared from plasma (t½ = 3 h), and its volume of distribution is approximately twice that of total body water in children. These pharmacokinetic data, differ from that of adults reported by others. Specifically, the plasma t½ for children is longer: total body clearance (Cl), and volume of distribution at steady state (V_{ss}) are greater. In addition, no dose dependency of t½, Cl or V_{ss} was observed in this study, and the DON pharmacokinetics were linear and predictable. Five of nine children with acute leukemia showed improvement, though insufficient for classification as partial response, and five of eight children with solid tumors also showed improvement. Further trials using DON in combination with thiopurines or other agents appear indicated.

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Offprint requests to: M. P. Sullivan, U.T. M. D. Anderson Hospital & Tumor Inst., Division of Pediatrics, 1515 Holcombe Boulevard, Houston, TX 77030, USA

Introduction

DON (6-diazo-5-oxo-L-norleucine) was initially isolated from a *Streptomyces* broth in 1956 [7]. It is structurally related to glutamine [4] and possesses the properties of a glutamine antagonist. These properties include inhibition of the biosynthesis of D-glucosamine phosphate [7], purines [12], pyrimidines [6], nicotinamide adenine dinucleotide (NAD^+) [1], L-asparagine synthetase [10], and proteins in general [23]. Antimicrobial [5], cytotoxic [21], teratogenic [9], and antitumor [14] activity have been demonstrated in preclinical studies. Toxicity was manifested primarily in the gastrointestinal tract and bone marrow of beagle dogs, with lesser effects on the liver, kidneys, heart, and lungs [16]. Azotomycin, a structurally related compound that contains 2 mol DON per mol, produced toxic reactions in mice, involving the brain, liver, and intestines [16].

Limited studies conducted in humans from 1957 to 1981 indicated occasional responses in adult patients with lymphoma, cancer of the breast, lungs, colon, and testicles [13, 15, 27]. Orally administered DON in combination with 6-mercaptopurine for maintenance therapy in childhood acute lymphocytic leukemia significantly prolonged the remissions obtained [26]. However, since DON is acid-labile and relatively unstable in solution, it appears more rational to administer the drug i.v. [10]. Recently, phase I studies in adults with i.v. DON at doses of 480 and 500 mg/m^2 indicated the dose-limiting toxicity to be nausea with vomiting [11, 25]. These findings prompted a formal phase I appraisal of i.v. DON in children.

Materials and methods

Patients. Twenty pediatric patients were entered in this phase I study of 6-diazo-5-oxo-L-norleucine (DON), conducted under an NCI Contract from 28 January 1981 through 21 July 1983. All patients were under the care of the Pediatrics Department, The University of Texas MD Anderson Hospital and Tumor Institute at Houston. Each had a tissue diagnosis of malignant disease prior to their 16th birthday. Eligibility for the study was based on the following: (1) the malignant disease was resistant to conventional therapy; (2) the clinical condition of the patient was of sufficient stability to permit completion of the study; (3) there was no evidence of drug toxicity from prior therapy; and (4) there was no impairment of renal or hepatic function. In patients with solid tumors there had been no involvement of bone marrow in the malignant

process. In all patients, the WBC count was greater than or equal to 3200/mm^3, the absolute granulocyte count was greater than or equal to 1500/mm^3, and the platelet count was greater than or equal to 75000/mm^3. All patients were less than 18 years of age when they entered the study. Informed consent was given by parents or guardians and by patients if they were over 7 years of age.

The dose escalation plan for successive groups of patients was as follows: initial dose, 150 mg/m^2; first escalation (50%), 225 mg/m^2; second escalation (approximately 30%), 300 mg/m^2; third escalation (25%), 375 mg/m^2; fourth escalation (20%), 450 mg/m^2; and fifth escalation (15%), 520 mg/m^2. DON was administered i.v. as a 15-min infusion twice weekly every 2 weeks for a total of eight treatments provided prohibitive toxicity did not occur. There was no restriction regarding the use of antiemetics. Upon the request of the parents and with the consent of the Project Officer, patients could continue with the therapy beyond eight doses.

Patients were considered fully evaluable when the course of therapy at a given dose was completed in 4 weeks and the patient was reevaluated 4 weeks later for delayed toxicity. Patients treated for less than 2 weeks were nonevaluable; those receiving therapy for more than 2 but less than 4 weeks were partially evaluable. Patients lost to follow-up within the first 2 weeks following drug administration were considered nonevaluable.

The pretreatment evaluation included the following: physical examination, measurement of tumor masses, complete blood count with differential and platelet counts, a 12-component chemical survey, radiograph of the chest, bone marrow aspiration, electromyocardiogram, urinalysis, serum electrolyte analysis, guaiac testing of the stool, and cell count, protein, and sugar determinations from the cerebrospinal fluid. During the study, the complete blood count was obtained three times each week. Other studies (excluding chest radiograph, bone marrow aspiration and lumbar puncture) were repeated weekly. All studies except lumbar puncture and aspiration of previously uninvolved marrow were repeated in the post-treatment period.

The degree of toxicity manifest in each organ or system was determined in accordance with the Toxicity Criteria of the Pediatric Division of the Southwest Oncology Group (now the Pediatric Oncology Group). Hematologic toxicity gradings on a scale of 0, 1, 2, 3 and 4 were as follows:

Hb (g%)	0 ≥10	1 = 9.0–9.9
WBC cells (per /mm^3)	0 ≥4K	1 = 3K–3.9K
Pl per /mm^3	0 ≥100K	1 = 75K–99.9K
2 = 7.0–8.9	3 = 5.0–6.9	4 ≤5.0
2 = 2K-2.9K	3 = 1K–1.9K	4 ≤1K
2 = 50K–74.9K	3 = 25K–49.9K	4 ≤25K

The following standard criteria of response were employed for solid tumors: complete remission, disappearance of all evidence of tumor as a result of the therapy employed; partial remission, tumor regression insufficient for classification as complete remission but exceeding 50% (as determined by the sum of the products of the greatest diameters of the measurable tumor masses); stable disease, no regression and no progression of tumor; and treatment failure, a lesser response than stable disease. The criterion of response employed for acute leukemia was in accordance with the Definition of Response – Acute Leukemia published by the Pediatric Division of the Southwest Oncology Group [8].

Methods. DON was administered by i.v. infusion in 50 ml dextrose (5% in water) over a 15-min interval. Plasma samples were obtained immediately prior to the start of the infusion and at 15 min, 30 min, and 1, 2, 3, 6, 9, 12, and 24 h after the infusion. Samples of protein-free plasma were analyzed by high-performance liquid chromotography as applied by Nelson and Herbert [18]. Protein was removed by membrane filtration and samples were generally measured within 1 h after blood withdrawal. The 280 nm absorbing peak corresponding to DON was confirmed (when necessary) by heating samples in a boiling water bath for 5 min [18]. Patients were not given acetaminophen during this time, since a metabolite of this substance interferes with the DON assay.

DON plasma concentration-time data were evaluated by noncompartmental methods of analysis and statistical moment theory. Calculations were performed using the LAGRAN [22] computer program. The clearance (cl) of the drug was obtained using the following equation:

$$cl = \text{Dose}/\text{AUC} \quad (1)$$

where AUC is the area under the plasma concentration-time curve from time zero to infinity. The volume of distribution at steady state (V_{ss}) was obtained using the following relationship:

$$V_{ss} = \frac{\text{Dose} \times \text{AUMC}}{(\text{AUC})^2} - \frac{\text{Dose} \times T}{2\,\text{AUC}} \quad (2)$$

where AUMC denotes the total area under the first moment of the drug concentration-time curve from time zero to infinity and T is the infusion time. The half-life ($t_{1/2}$) of DON was calculated using the following relationship:

$$t_{1/2} = 0.693/\beta \quad (3)$$

where β represents the rate constant obtained from the terminal log-linear phase of the plasma concentration-time curve.

Results

Of the 20 children entered in the DON phase I study, 17 (9 with hematologic malignancies and 8 with solid tumors) received four or more treatments and were evaluable for toxicity and therapeutic effects. All had measurable disease at the time of study entry, as shown in Table 1. Escalating doses starting at 150 mg/m^2 were given to groups of 3 patients. The "over-age" patient at the 300 mg/m^2 level was replaced in the study by another patient at that level. At the 450 mg/m^2 level, a replacement entry was made for 1 patient who died prior to receiving the four scheduled doses of DON. Only two entries were made at the 520 mg/m^2 dose level.

Toxicity

Nausea and vomiting occurred in all patients. Premedication with i.v. chlorpromazine (Thorazine), 15–25 mg depending on weight, either prevented nausea and vomiting completely or markedly reduced the severity of the symptoms. Gastrointestinal toxic effects were limited to nausea and vomiting directly related to drug administration. Clinically, vomiting was not comparable in severity to that seen with *cis*-platinum therapy.

Table 1. Pediatric phase I DON study: description of patients entered

Patient no.	Age (years)/ Sex	Dose (mg/m^2)	Diagnosis	Sites of measurable disease
1	13/M	150	ALL	Bone marrow, peripheral blood
2	9/M	150	ALL	Bone marrow, peripheral blood, spleen, pleural and conjunctival masses
3	7/F	150	Pontine tumor	Pontine mass, ventricular dilatation
4	4/F	225	Rhabdomyosarcoma	Nasal tumor mass, subcarinal mass
5	16/M	225	Osteosarcoma	Pulmonary metastases
6	10/M	225	ALL	Bone marrow, peripheral blood
7	14/F	300	Neuroblastoma	Mass, right upper arm; epidural mass T5–T10
8	5/F	300	ALL	Bone marrow, peripheral blood
9	21/F	300	Ewing's sarcoma (over-age)	Left femur, sacrococcygeal and soft tissue masses
10	5/M	300	Melanoma	Tumor nodules, right upper eyelid; facial swelling
11	16/F	375	ALL, lymphoma	Bone marrow, peripheral blood
12	3/M	375	ALL	Bone marrow, peripheral blood
13	13/M	375	Malignant fibrous histiocytoma (prior retinoblastoma)	Temporal mass
14	12/F	450	AML	Bone marrow, peripheral blood
15	6/M	450	ALL (not evaluable, early death)	Bone marrow, peripheral blood
16	14/M	450	Carcinoma of colon	Abdominal mass
17	14/M	450	Brain tumor	Vertebrae; paraspinal soft tissue
18	8/M	520	ALL	Bone marrow, peripheral blood, liver, spleen, lymph nodes
19		520	Withdrawn	
20	6/F	520	ALL	Bone marrow, peripheral blood, liver, spleen

Table 2. A Hematologic toxicity and tumor response to DON in children

Patient no.	DON dose (mg/m^2)		Hematologic toxicity[a] after dose no.								Bone marrow or clot section cellularity[e]		Remarks
			1	2	3	4	5	6	7	8	Pre-DON	Post-DON × 3	
Solid tumor patients: no radiotherapy													
3	150	Hb	2	2	2	3	2[b]	1	1	0	BMS: N	BMS: N	
		WBC	1	1	2	3	0	1	1	2			
		PL	0	0	0	0	0	0	0	0			
4	225	Hb	0	0	0	0	0	0	0	0	BMS: N	BMS: N	
		WBC	1	0	1	0	1	2	?	0	CS: N	CS: N	
		PL	0	0	0	0	0	0	0	0			
5	225	Hb	0	0	0	1	2	1	2	2	BMS: N	BMS: N	
		WBC	1	1	3	3	3	2	3	3	CS: D	CS: D	
		PL	0	0	0	0	3	3	3	4			
10	300	Hb	0	0	0	0	0	0	0	0	BMS: N	BMS: N	Reduction of orbital swelling;
		WBC	0	0	0	0	0	0	0	0	CS: I	CS: I	no new tumor nodules.
		PL	0	0	0	0	0	0	0	0			Continuation of DON (22 doses) at parents' request
13	375	Hb	2	1	1	1	0[b]	2	1	2	BMS: N	BMS: N	Decrease in left facial swelling
		WBC	0	0	0	1	0	0	1	0	CS: D	CS: D	and soft tissue mass
		PL	0	0	0	0	0	0	0	0			
16	450	Hb	–	0	0	1	0	1	0	0	BMS: N	–	Decrease in abdominal pain
		WBC	–	0	0	0	1	0	0	0			
		PL	–	0	0	0	0	0	0	0			
Solid tumor patients: radiotherapy													
7[c]	300	Hb	0	0	0	0	0	0	2		BMS: N	–	Disappearance of left humeral
		WBC	0	0	0	2	2	3	4		CS: N	–	lesion
		PL	0	0	1	2	4	2[b]	3				
17[d]	450	Hb	3	1	0	2					BM: N	–	Decrease in bone pain,
		WBC	2	2	2	3					CS: I	–	improved neurologic status
		PL	2	3	2	4							

[a] Hb, hemoglobin; WBC, white blood cells; PL, platelets. The numerical scores (0–4) indicate increasing toxicity (see Methods)
[b] Transfusion of appropriate blood product(s)
[c] XRT (cranium, spine, femur)
[d] XRT (spine)
[e] BMS: N, BM smear: normal cellularity; CS: N, clot section: normal cellularity; CS: D, clot section: decreased cellularity; CS: I, clot section: increased cellularity

Table 2. B Hematologic toxicity and tumor response to DON in children

Patient no.	DON dose (mg/m^2)		Hematologic toxicity[a] after dose no.								Bone marrow (percent blasts)		Remarks
			1	2	3	4	5	6	7	8	Pre-DON	Post-DON × 3	
Leukemia/lymphoma patients													
1	150	Hb	2	0[b]	0	1	1	0[b]	2	0	93%	59%	Continuation of DON therapy (25 doses), on request, with decrease in marrow blasts to 5%
		WBC	3	4	4	4	4	3	3	3			
		PL	4	3	4	2[b]	4	0[b]	4	1			
2	150	Hb	2	3	2[b]	3	2[b]	3	2	3	60%	57%	Reduction in size of pleural and conjunctival masses; reduction in spleen size from 5.5 cm below RCM to non-palpable. WBC count decreased from 5.6K (88% blasts) to 1.1K (57% blasts). Continuation of DON therapy (3 doses) on request
		WBC	0	0	0	0	0	0	1	3			
		PL	4	3	4	2[b]	4	4	4	4			
6	225	Hb	0	2	0[b]	0	0	2	0[b]	2	86%	78%	
		WBC	0	2	1	2	3	3	4	3			
		PL	3	4	4	4	3	0	3	0[b]			
8	300	Hb	2	0[b]	1	2	0[b]	0	1	0[b]	84%	98%	
		WBC	0	0	0	1	2	1	2	2			
		PL	0	0	1	4	0[b]	2	0[b]	1			
11	375	Hb	0	0	0	0	0	0	0	0	34%	27%	Continuation of DON therapy (8 doses) on request
		WBC	1	0	0	1	2	2	3	2			
		PL	0	0	0	0	0	0	0	0			
12	375	Hb	0	0	0	0	0	0	1	1	84%	–	
		WBC	3	1	2	2	0	0	0	0			
		PL	0	0	0	3	4	2	4	4			
14	450	Hb	–	0	0	0	1[b]	2	3[b]	4	95%	–	
		WBC	–	0	0	0	0	0	0	0	(WBC 31.2K)	(WBC 162K)	
		PL	–	0	0	0	0	0	0	0			
18	520	Hb	–	0	0	0					87%	Autopsy; BM replaced	
		WBC	–	–	–	–							
		PL	–	0	0	0							
20	520	Hb	0	0	0	0	0	0			82%	–	Transient decrease in WBC count from 104K to 39.2K
		WBC	0	0	0	0	0	0					
		PL	0	0	0	0	0	0					

[a] Hb, hemoglobin; WBC, white blood cells; PL, platelets. The numerical scores (0–4) indicate increasing toxicity (see Methods)
[b] Transfusion of appropriate blood product(s)
[c] XRT (cranium, spine, femur)
[d] XRT (spine)
[e] BMS: N, BM smear: normal cellularity; CS: N, clot section: normal cellularity; CS: D, clot section: decreased cellularity; CS: I, clot section: increased cellularity

In solid-tumor patients not receiving radiotherapy, hematologic toxicity was marked (scale 3 or higher) in 2 of 6 patients (Table 2). In this population, hematologic toxicity did not clearly show a dose-related effect. In two patients receiving radiotherapy, hematologic toxicity after i.v. DON appeared to be enhanced and to occur rapidly. In leukemic patients, hematologic toxicity due to DON administration did not appear to be clearly related to dose in the range studied (Table 2). The need for whole blood products correlated roughly with the extent of marrow replacement. The general clinical impression was that of mild hematologic toxicity. Infection associated with myelosuppression necessitated intensive care for the two patients who had been extensively irradiated. Each patient recovered fully from the infection.

Urinary tract toxicity was not significant; however, microscopic hematuria occurred in 8 patients, 2 of whom had myelosuppressed solid tumors and 2 had leukemia. Hepatic toxicity was not severe. Grade 1–2 SGOT elevations occurred in 11 patients and grade 3 elevations in 1. Bilirubin was transiently elevated in only 1 patient. Skin toxicity was suspected in 1 patient who developed an erythematous macular papular rash during DON therapy. Other skin changes in this patient were subsequently shown to be related to T-cell disease activity.

Therapeutic responses

Of the 9 evaluable children with leukemia, 5 showed some improvement while receiving DON (Table 2). The degree of response, however, was insufficient to qualify as partial

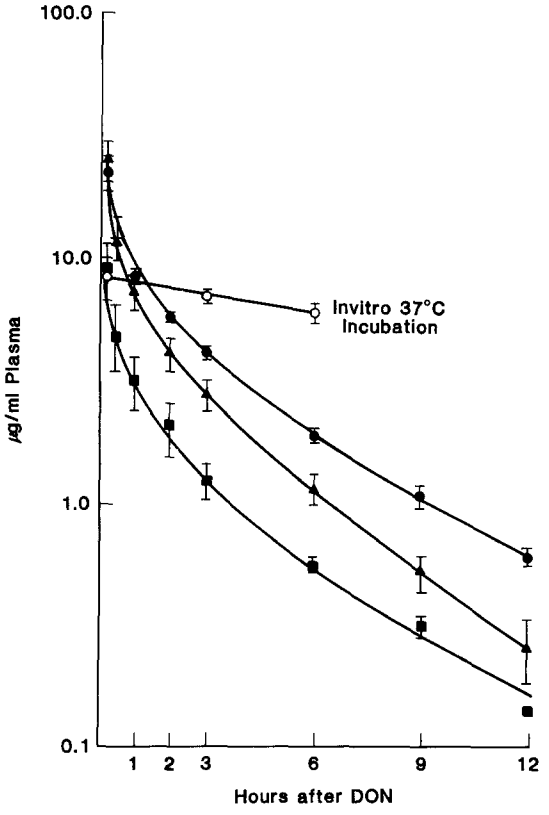

Fig. 1. 6-Diazo-5-oxo-l-norleucine: Plasma clearance I (disappearance) curves: ■, 150 mg/m^2; ▲, 300–375 mg/m^2; ●, 450 mg/m^2; ○, in vitro 37 degrees C incubation

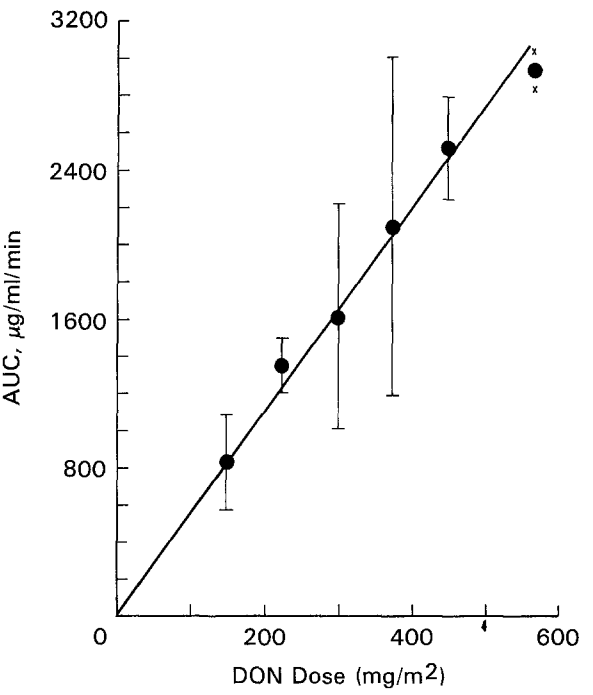

Fig. 2. Correlation of 6-Diazo-5-oxo-l-norleucine dose with area under the plasma concentration-time curve (AUC) in children with neoplasia

remissions. With continued therapy, marrow blasts in 1 patient with ALL eventually fell to 5% from a starting level of 93% at a DON dose of 150 mg/m^2. There were 8 patients with solid tumors; 5 of these showed some improvement, also insufficient to qualify as partial remission. In 1 adult patient with Ewing's sarcoma there was a striking disappearance of a large soft tissue mass when DON was given at a dose of 300 mg/m^2 (Table 1).

Pharmacokinetics

The plasma concentrations of DON as a function of time and dose are presented in Fig. 1. As reported in adults [11], decline of DON plasma levels appears to follow a biexponential pattern. The rapid plasma disappearance of DON is not due to rapid metabolism or degradation in whole blood; the rate of disappearance from whole blood incubated in vitro was markedly less than that observed in vivo (Fig. 1). This implies that sites other than blood cells or plasma are responsible for the rapid plasma disappearance, i.e., hepatic or extrahepatic metabolism or distribution to other tissues.

Mean pharmacokinetic parameters for DON at each dose level are listed in Table 3. The clearance values ranged from 163 to 222 ml/min (5.5–7.7 ml/min per kg), with a mean value for all doses of 202 ml/min (6.8 ml/min per kg). The volume of distribution at steady state (V_{ss}) ranged from 25 to 53 liters or 0.75 to 1.48 l/kg; the mean value for all doses was 1.20 l/kg. An examination of the influence of dose on the area under the plasma concentrationtime curve (AUC) of DON revealed no dose dependency, i.e, a plot of AUC versus dose was linear within the dose range studied (Fig. 2).

The relationships of half-life, clearance, and volume of distribution to patient age are illustrated in Fig. 3. Linear regression of these data indicates a positive correlation be-

Table 3. Pharmacokinetic parameters of DON in children[a]

Dose (mg/m^2)	Number of patients	t$^1/_2$ (min)	Cl (ml/min)	Cl (ml/min per kg)	V_{ss} (l)	V_{ss} (l/kg)
150	3	177±20	215± 73	7.1±2.4	44±23	1.44±0.68
225	3	121±15	170± 75	5.5±1.2	25±12	0.81±0.15
300	4	160± 9	207±125	7.7±2.2	40±26	1.48±0.48
375	3	126±47	222± 34	7.4±5.7	28±12	0.75±0.12
450	4	212±69	218± 58	6.2±0.7	53±17	1.48±0.10
520	2	150	163	6.6	26	1.01
All values	19	161±47	202± 71	6.8±2.4	38±19	1.20±0.45

[a] The values given are means ± SD

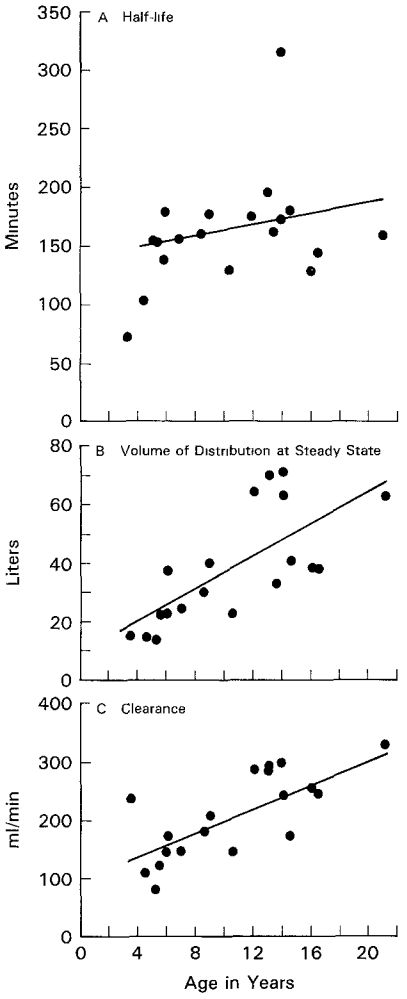

Fig. 3. Relationship of 6-diazo-5-oxo-l-norleucine pharmacokinetic parameters with age in children with neoplasia

Table 4. Comparison of present study pharmacokinetic parameters with values in literature

	Dose 300 mg/m²		Dose 450 mg/m²	
	Present study	[11]	Present study	[11]
t½	160	76	212	106
Cl, ml/min per kg	7.74	3.39	6.16	3.56
V_{SS}, l/kg	1.48	0.45	1.48	0.77

tween Cl and age ($r = 0.714$; $P < 0.01$), and between V_{ss} and age ($r = 0.715$; $P < 0.01$). A similar positive correlation was found between Cl ($r = 0.57$; $P < 0.05$) and V_{ss} ($r = 0.58$, $P < 0.01$) and patient weight. When these parameters were adjusted for patient weight, no correlation between the parameters and age was found.

Discussion

In 5 of 9 children with hematopoietic malignancies and 5 of 8 children with solid tumors clinical benefit from DON administration was recorded. The beneficial changes in the dose range used were not clearly dependent on the drug dose administered. Therefore, the maximum tolerated DON dose for children, as yet undefined, is probably not required to achieve results in some patients or disease states.

Neither the recommended dose for therapeutic trials nor the maximally tolerated dose was precisely defined by this study. The major toxic effects on the gastrointestinal tract, namely nausea and vomiting, were minimized by pretreatment with i.v. chlorpromazine. Mucositis, a very troublesome toxic effect in adults [8, 11, 25], was not seen. The accumulated data indicate that the maximum tolerated dose in children is in excess of 450 mg/m² twice weekly every 2 weeks for eight doses (possibly as high as 520 mg/m²), provided radiotherapy is not administered to a large portion of the bone marrow. When intensive or extensive radiotherapy had been given, DON produced a marked myelosuppressive response in one patient at 300 mg/m².

Results from the present investigation indicate that DON pharmacokinetics are linear and predictable at doses from 150 to 520 mg/m². The calculated volume of distribution reflected extensive extravascular distribution of DON, the mean value representing a volume approximately twice that of total body water. This would appear to reflect significant tissue uptake of DON. Pharmacokinetic parameters from our investigation differ from those reported by Kovach et al. [11] in that our values for t½ are longer and DON clearance and volume of distribution are larger. In addition, we observed no dose dependency of t½, Cl or V_{ss} as reported by Kovach et al. The previously reported data, however, were obtained from three adults receiving doses of 300 or 450 mg/m² and one patient receiving a dose of 550 mg/m². A comparison of the findings of the present study with those of Kovach and associates at the 300 and 450 mg/m² dose level are presented in Table 4. The bases for these differences may relate to the techniques used to measure the drug and/or to calculate the pharmacokinetic parameters.

DON (<0.2 μg/ml) was not detectable in the cerebrospinal fluid (CSF) of 2 patients, specifically a 9-year-old leukemic child given 150 mg/m² who had six CSF samples analyzed for DON from 15 min to 6 h after infusion, and a 14½-year-old brain tumor patient given 450 mg/m² of DON 6 h prior to CSF sampling. DON apparently does not readily cross the CSF-blood barrier in children.

DON was virtually undetectable by its absorbance at 280 nm [22] in urine samples obtained 1–24 h after infusion, i.e., less than 1 mg appeared in urine after total doses greater than 100 mg. The drug is apparently very effectively reabsorbed or the chromophore is degraded following renal filtration. DON is not significantly bound to human plasma proteins [22] and is, therefore, available for glomerular filtration.

The encouraging results obtained with DON as a single agent lead us to suggest that the drug be studied in combination with other chemotherapeutic drugs. Glutamine antagonists were among the first agents to be tested in combination trials in animals [24]. As single agents, azaserine and DON have been unimpressive in several murine models. However, in combination with 6-thiopurines (which also are often marginally active in model systems), highly effective and sometimes curative therapy has been noted [2]. The biochemical bases for the effectiveness of such combinations (i.e., purine de novo biosynthesis inhibitor with

purine analogues) have been studied in L5178Y [20] and sarcoma 180 cells [19]. Treatment of tumor cells with inhibitors of de novo purine biosynthesis enhances the salvage of preformed purines and their analogues. Alternatively, the effectiveness of DON alone may be enhanced when coadministered with an inhibitor of purine salvage such as dipyridamole [17]. In their review of clinical activity of the diazo analogue (azaserine, DON and azotomycin), Catane et al. [3] also concluded that further clinical trials of the agents were warranted.

References

1. Barclay RK, Phillips MA (1966) Effects of 6-Diazo-5-oxo-l-norleucine and other tumor inhibitors on the biosynthesis of nicotinamide adenine dinucleotide in mice. Cancer Res 26: 282–286
2. Bennett LL Jr (1975) Glutamine antagonists. Hdb Exp Pharmacol 38(2): 484–522
3. Catane R, Von Hoff DD, Glaubiger DL (1979) Azaserine, DON, and azotomycin: three diazo analogs of l-glutamine with clinical antitumor activity. Cancer Treat Rep 63: 1033–1038
4. Dion HW, Fusari SA, Jakubowski ZL (1965) 6-Diazo-5-oxo-l-norleucine, a new tumor-inhibitory substance: 11. Isolation and characterization. J Am Chem Soc 78: 3075–3077, 1965
5. Ehrlich J, Coffey GL, Fisher MW (1956) 6-Diazo-5-oxo-l-norleucine, a new tumor-inhibitory substance. 1. Biologic studies. Antibiot Chemother 6: 487–497
6. Eidinoff ML, Knoll JE, Marano B (1958) Pyrimidine studies: 1. Effect of DON (6-Diazo-5-oxo-l-norleucine) on incorporation of precursor into nucleic acid pyrimidines. Cancer Res 18: 105–109
7. Ellis DB, Sommar KM (1972) Biosynthesis of respiratory tract mucins: 11. Control of hexosamine metabolism by l-glutamine: d-fructose 6-phosphate aminotransferase. Biochim Biophys Acta 276: 105–112
8. Hewlett JS, Battle JD Jr, Bishop RC (1964) Phase II study of A-8103 (NSC-25154) in acute leukemia in adults. Cancer Chemother Rep 42: 25–28
9. Jackson D, Robson JM, Wander ACE (1959) The effect of 6-Diazo-5-oxo-l-norleucine (DON) on pregnancy. J Endocrinol 18: 204–207
10. Jayaram HN, Cooney DA, Milman HA (1976) DON, CONV and DONV: I. Inhibition of l-asparaginase synthetase in vitro. Biochem Pharmacol 25: 1571–1582
11. Kovach JS, Eagen RT, Powis G (1981) Phase I and pharmacokinetic studies of DON. Cancer Treat Rep 65: 1031–1036
12. Levenberg B, Melnick I, Buchanan JM (1957) Biosynthesis of the purines. XV. The effect of aza-l-serine and 6-Diazo-5-oxo-l-norleucine on inosinic acid biosynthesis de novo. J Biol Chem 225: 163–176
13. Li MC (1961) Management of choriocarcinoma and related tumors of uterus and testis. Med Clin North Am 45: 661–676
14. Livingston RB, Venditti JM, Cooney DA (1970) Glutamine antagonists in cancer chemotherapy. In: Garattini S, Goldin A, Hawking F, Kopin IJ (eds) Advances in pharmacology and chemotherapy. Academic Press, New York, pp 57–120
15. Magill GB, Myers WPL, Reilly MC (1957) Pharmacological and initial therapeutic observations on 6-Diazo-5-oxo-l-norleucine (DON) in human neoplastic disease. Cancer 10: 1138–1150
16. National Cancer Institute (1979) Clinical brochure. DON. NSC 7365. Investigative Drug Branch, Cancer Therapy Evaluation Program, DCT, NCI, Bethesda, Md
17. Nelson JA, Drake S (1974) Potentiation of methotrexate toxicity by dipyridamole. Cancer Res 44: 2492–2496
18. Nelson JA, Herbert B (1981) Rapid analysis of 6-Diazo-5-oxo-l-norleucine (DON) in human plasma and urine. J Liquid Chromatogr 4: 1641–1649
19. Nelson JA, Parks RE Jr (1972) Biochemical mechanisms for the synergism between 6-thioguanine and 6-(methylmercapto) purine ribonucleoside in sarcoma 180 cells. Cancer Res 32: 2034–2041
20. Paterson ARP, Wang MC (1970) Mechanism of the growth inhibition potentiation arising from combination of 6-mercaptopurine with 6-(methylmercapto) purine ribonucleoside. Cancer Res 30: 2379–2387
21. Potter M (1958) Variation in resistance patterns in different neoplasms. Ann NY Acad Sci 76: 630–642
22. Rocci ML, Vusko WJ (1983) LAGRAN program for area and moments in pharmacokinetic analysis. Comput Prog Biomed 16: 203–216
23. Rosenbluth RJ, Cooney DA, Jayaram HN (1976) Inhibition of l-asparaginase synthetase in vivo. Biochem Pharmacol 25: 1851–1858
24. Sartorelli AC (1965) Approaches to the combination chemotherapy of transplantable neoplasms. Prog Exp Tumor Res 6: 228–288
25. Sklaroff RB, Casper ES, Magill GB (1981) Phase I Study of 6-Diazo-5-oxo-l-norleucine (don). Cancer Treat Rep 64: 1247–1251
26. Sullivan MP, Beatty EC Jr, Hyman CB (1962) A comparison of the effectiveness of standard dose 6-mercaptopurine, combination 6-mercaptopurine and DON, and high-loading 6-mercaptopurine therapies in treatment of the acute leukemias of childhood: results of a cooperative study. Cancer Chemother Rep 18: 83–85
27. Veterans Administration Cancer Chemotherapy Study Group (1959) A clinical study of the comparative effect of nitrogen mustard and DON in patients with bronchogenic sarcoma, Hodgkin's disease, lymphosarcoma, and melanoma. J Nat Cancer Inst 22: 433–439

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