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

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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/m² to 320 mg/m². 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/m².

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 (CI), and volume of distribution at steady state (Vss) are greater. In addition, no dose dependency of t½, CI or Vss 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.

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/m² 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
In all patients, the WBC count was greater than or equal to 3200/mm³, the absolute granulocyte count was greater than or equal to 1500/mm³, and the platelet count was greater than or equal to 75000/mm³. 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 phase I trials of patients was as follows: initial dose, 150 mg/m²; first escalation (50%), 225 mg/m²; second escalation (approximately 30%), 300 mg/m²; third escalation (25%), 375 mg/m²; fourth escalation (20%), 450 mg/m²; and fifth escalation (15%), 520 mg/m². 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, electromyogram, 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 /mm³)**
  - 0 ≥ 4K
  - 1 = 3K–3.9K

- **Pl per /mm³**
  - 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 chromatography 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:

\[
cl = \frac{Dose}{AUC}
\]  

(1)

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

\[
V_{ss} = \frac{Dose \times \text{AUMC}}{(\text{AUC})^2} = \frac{Dose \times T}{2 \text{ AUC}}
\]  

(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 (t½) of DON was calculated using the following relationship:

\[
t_{\frac{1}{2}} = 0.693/\beta
\]  

(3)

where \(\beta\) 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/m² were given to groups of 3 patients. The “over-age” patient at the 300 mg/m² level was replaced in the study by another patient at that level. At the 450 mg/m² 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/m² 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.