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Pharmacokinetic Study of Indicine *N*-Oxide in Pediatric Cancer Patients

Matthew M. Ames[1], James S. Miser[2], William A. Smithson[3], Peter F. Coccia[4], Carla S. Hughes[2], and Dianne M. Davis[2]

[1] Division of Developmental Oncology Research, Department of Oncology, Mayo Clinic, Rochester, MN 55905
[2] Department of Pediatrics, Ohio State University and Columbus Childrens Hospital, Columbus, OH 43205
[3] Department of Pediatrics, Mayo Clinic, Rochester, MN 55905
[4] Department of Pediatrics, Rainbow Babies and Childrens Hospital, Case Western Reserve University, Cleveland, OH 44106, USA

Summary. *Pharmacokinetics of the experimental antitumor agent indicine N-oxide were investigated in a group of 23 pediatric cancer patients. Plasma elimination of indicine N-oxide was best described by a two-compartment open model. The mean plasma distribution phase half-life, plasma elimination phase half-life, and plasma clearance were 8 min, 84 min, and 62 ml/min/m² (2.1 ml/min/kg), respectively. One patient with renal impairment had an abnormally long plasma elimination phase half-life (275 min) and reduced plasma clearance (17 ml/min/m²). Plasma elimination phase half-life values increased and plasma clearance values decreased with increasing age of the pediatric patients. Plasma elimination of indicine N-oxide was more rapid in this group of children than in adults who had previously received the drug.*

Introduction

Indicine *N*-oxide (NSC 132319, INO) is an investigational antitumor agent of the pyrrolizidine alkaloid family [7]. Many of the more than 150 naturally occurring pyrrolizidine alkaloids have anti-mitotic, mutagenic, and anti-tumor activity [2, 4, 10]. Ingestion of plants containing certain pyrrolizidine alkaloids may cause severe acute hepatotoxicity in the form of veno-occlusive disease in humans, and an unusual chronic liver disease in grazing animals [2, 4, 10]. Hepatotoxicity appears to be associated with metabolism of the alkaloids to reactive dehydropyrrolizidine metabolites [2]. Indicine N-oxide was selected for clinical trials on the basis of anti-tumor activity in experimental tumor systems (Walker 256 carcinoma, murine leukemia P-388, murine leukemia L1210 and B-16 melanoma) [7, 9] and because it did not produce significant hepatotoxicity in dogs and monkeys [3, 14]. Toxicities seen in large animal trials included bone marrow hypoplasia, nephrosis, emesis, bloody diarrhea, and reversible changes in hepatic function without histologic evidence of liver damage [3, 14].

In phase I clinical trial of patients with advanced solid tumors, the dose-limiting toxicity was myelosuppression, manifested as both leukopenia and thrombocytopenia [6]. Other toxicities included mild nausea and vomiting and reversible increases in serum creatinine and serum glutamic-oxaloacetic transaminase [7]. Cumulative myelosuppression was seen with repetitive courses. Although no therapeutic responses have been seen in adults with solid tumors [13], complete remissions have been seen in both acute lymphoblastic and nonlymphoblastic leukemia [8]. As part of a phase I and pharmacokinetic study in children with malignant solid tumors and leukemia, we report pharmacokinetics of indicine *N*-oxide following IV administration in 23 pediatric patients.

Reprint requests should be addressed to M. M. Ames

Materials and Methods

Patients. Blood was taken from 23 of 51 patients on the phase I study for pharmacokinetic analysis; one patient was sampled twice at different dose levels for 24 patient studies. Patient characteristics are summarized in Table 1. Of the 23 patients, 15 children had acute leukemia and eight children had malignant, solid tumors.

Entry criteria for the study included:
1. Evaluation of hepatic and renal function,
2. Documented diagnosis of an active malignant disease, and
3. Written, informed consent, obtained from the parent or legal guardian prior to administration of indicine *N*-oxide.

Administration of Indicine N-Oxide and Sample Collection. Indicine *N*-oxide was provided by the Division of Cancer Treatment, National Cancer Institute. Each 1-g vial was reconstituted with 9.2 ml sterile water and the total dose per day was administered as a 3-min, rapid, IV infusion on each of 5 consecutive days. Indicine *N*-oxide was administered on day 1 at doses ranging from 1,500 to 4,000 mg/m² (the patient receiving 4,000 mg/m² had reduced doses on days 2 through 5, with an average daily dose of 3,750 mg/m²).

Blood samples were withdrawn from an indwelling catheter into a heparinized glass tube prior to administration of drug on day 1 and at specific times following administration. Single blood samples were obtained on days 2 through 6, 24 h following the previous dose. Plasma was separated by centrifugation and stored at −70° C until analysis.

Table 1. Summary of patient characteristics

	Age (yrs)	Weight (kg)	BSA (m²)	Dose (mg/m²)	Dose (mg)
Range	3–19	14–72.5	0.57–1.77	1,500–4,000	1,035–6,400
Mean value	11.4	40.9	1.24	3,031	3,697

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Determination of Indicine N-Oxide and Indicine. Indicine *N*-oxide and indicine were measured in plasma and urine by an electron-capture gas chromatographic method previously reported [1].

Pharmacokinetic and Statistical Analysis. Analyses of pharmacokinetic data were conducted using the NONLIN least-squares regression analysis program [11] on a CDC Cyber 170–720 computer with interactive graphic analysis. Pharmacokinetic parameters were calculated with allowance for a 3-min infusion. The biexponential decline in plasma concentrations of indicine *N*-oxide was fitted to the equation $C = Ae^{-\alpha t} + Be^{-\beta t}$ with a weighting factor of 1/Y. C is the plasma concentration of indicine *N*-oxide at time *t* after administration of indicine *N*-oxide; A and B are the intercepts at $t = 0$, and α and β are the fast and slow disposition rate constants. Correlation coefficients were determined by linear least-squares regression analysis on a Hewlett-Packard 9785 desk-top calculator. Significance of the correlation between age and $t_{1/2}\beta$ was determined from a table of levels of significance of correlation coefficients reported by Snedecor and Cochran [18].

Results

A summary of pharmacokinetic parameters for individual patients is shown in Table 2. The disappearance of indicine *N*-oxide from plasma following infusion on day 1 of treatment was best described by a two-compartment open model. Plasma time-concentration curves for a representative patient with normal renal function receiving 3,000 mg/m^2 and for a patient with abnormal renal function receiving 2,000 mg/m^2 are shown in Fig. 1. The patient with renal impairment had an abnormally long plasma elimination phase half-life (275 min) and a reduced clearance (16.6 ml/min/m^2) of indicine *N*-oxide (Table 2). While plasma concentrations increased with increasing dose, there was significant variation of plasma indicine *N*-oxide concentrations among patients receiving the same dose. This may be seen by plotting plasma concentrations for each patient 60 min following infusion as a function of dose administered (Fig. 2). Plasma samples obtained just prior to administration of drug on days 2 through 6 did not contain significant quantities of indicine *N*-oxide. Indicine, a metabolite of indicine *N*-oxide, was detected in the plasma of only a few patients receiving high doses (3,375–4,000 mg/m^2) of indicine *N*-oxide; furthermore, the concentrations of indicine were close to the limits of detection (0.5 μg/ml).

There was no indication of dose-dependent pharmacokinetics in this group of 23 pediatric patients, although it should be noted that 15 patients received doses of 3,000–3,375 mg/m^2, and only nine patients received higher (3,500–4,000 mg/m^2, 4 patients) or lower (1,500–2,000 mg/m^2, 5 patients) doses (1 patient was treated at 1,500 and 3,750 mg/m^2). Rates of elimination of indicine *N*-oxide from plasma were correlated to the age of the patient. When patients are divided into three age groups (3–6, 8–14, and 15–19), with six to eight patients per group. Clear differences in the mean values of pharmacokinetic parameters are observed for the three groups (Table 3). There is a marked increase in the elimination phase half-life ($t_{1/2}\beta$) and a decrease in clearance (calculated both by body weight or surface area) as a function of age (Table 3). The positive correlation between increasing patient age and increasing elimination phase

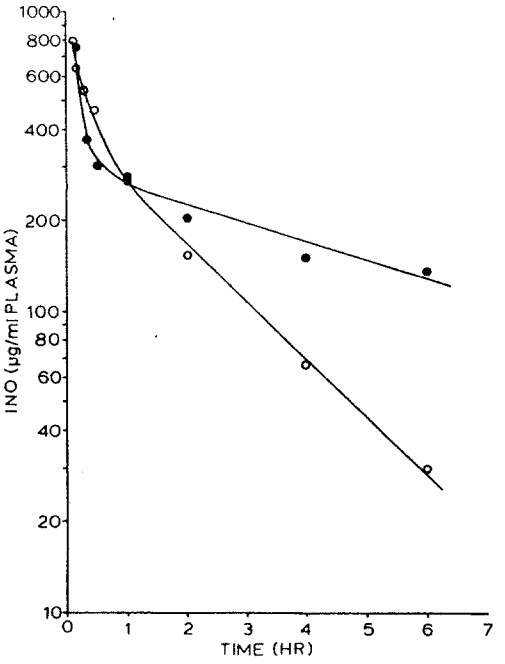

Fig. 1. Plasma indicine *N*-oxide time-concentration curves for a patient with normal renal function who received 3,000 mg/m^2 (○, ○, ○) and for a patient with impaired renal function who received 2,000 mg/m^2 (●, ●, ●)

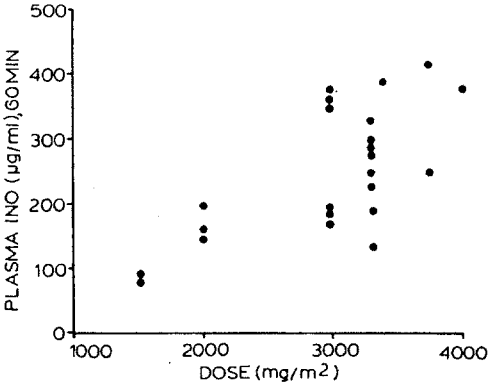

Fig. 2. Plasma concentration of indicine *N*-oxide 60 min after drug administration, plotted as a function of patient dose. Data from patient with renal impairment (patient 1) are not included

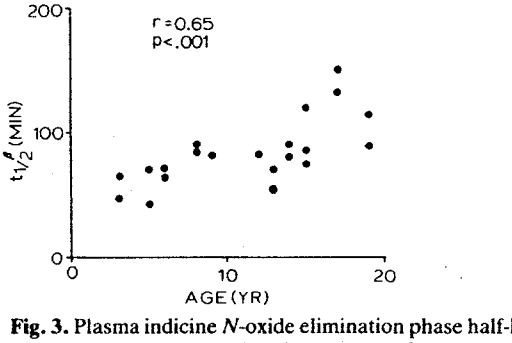

Fig. 3. Plasma indicine *N*-oxide elimination phase half-life ($t_{1/2}\beta$) values plotted as a function of patient age. $t_{1/2}\beta$ values were taken from Table 2, omitting value for patient with renal impairment (patient 1)

Table 2. Summary of pharmacokinetic parameters[a]

Patient study number	Age (yrs)	Dose (mg/m^2)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	Total clearance (ml/min)	Clearance ($ml/min/m^2$)	Clearance (ml/min/kg)	V_1 (ml/m^2)	V_2 (ml/m^2)
5	3	3,077	8.6	62.9	45.4	69.85	3.24	2,990	4,720
11	3	3,375	9.3	48.2	71.8	126.3	5.44	4,374	7,197
10	5	3,375	10.5	69.9	44.3	53.9	2.10	2,868	3,548
24	5	1,500	9.6	39.3	74.8	111.8	4.82	4,054	3,685
3	6	4,000	11.4	71.2	41.5	55.3	2.34	4,567	1,389
7	6	3,000	3.5	64.5	29.6	39.5	1.56	2,078	1,762
2	8	3,571	2.5	91.0	44.8	42.7	1.43	1,883	4,049
12	8	3,750	5.5	88.7	41.3	41.3	1.45	3,152	2,379
8	9	3,000	11.2	84.7	45.3	41.2	1.27	2,871	2,817
13	12	3,375	10.4	79.0	82.6	55.7	1.37	3,673	3,473
6	13	3,000	4.1	54.7	119.8	89.4	3.15	3,689	3,933
20	13	3,375	2.1	71.3	85.6	62.8	1.71	1,048	6,576
9	14	2,000	8.5	81.0	93.1	54.8	1.49	2,892	4,571
19	14	3,375	9.4	90.7	59.4	55.7	2.00	4,859	2,867
1	15	2,000	3.9	275.0	27.3	16.6	0.50	2,213	4,563
4	15	3,000	17.2	74.4	106.4	79.8	2.37	4,815	6,361
14	15	3,375	11.4	86.1	92.2	51.9	1.36	3,309	4,223
22	15	3,000	2.4	122.2	40.4	28.9	0.94	2,838	2,334
17	17	1,500	2.4	151.4	96.8	51.0	1.56	869	12,861
18	17	3,750	7.4	132.4	100.7	59.0	1.62	6,440	5,333
15	19	3,375	10.6	114.7	96.9	58.4	1.75	3,228	8,901
23	19	3,000	9.7	87.8	117.9	73.7	1.63	4,736	5,879
Mean ± SEM	11.4	3,081	8.0 ± 0.9	84.1 ± 6.0	72.9 ± 6.3	62.1 ± 5.2	2.1 ± 0.3	3,392 ± 290	4,707 ± 579

[a] Data from two patients (patients 16 and 21) were omitted because sampling was completed for only 2 h following administration of drug. Data from the patient with renal impairment (patient number 1) was omitted from determination of mean values of pharmacokinetic parameters

Table 3. Variation in pharmacokinetic parameters with patient age[a]

Number of patients	Age (yrs)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)	Total Cl (ml/min)	Cl ($ml/min/m^2$)	Cl (ml/min/kg)	V_1 (ml/m^2)	V_2 (ml/m^2)
6	4.7 (3–6)	8.8 ± 1.1	59.3 ± 5.2	51.2 ± 7.4	76.1 ± 14.3	3.3 ± 0.6	3,489 ± 404	3,717 ± 864
8	11.4 (8–14)	6.7 ± 1.3	80.1 ± 4.3	71.5 ± 10.0	55.5 ± 5.6	1.7 ± 0.2	3,008 ± 411	3,833 ± 469
7	16.7 (15–19)	8.7 ± 1.9	109.9 ± 10.6	93.0 ± 9.3	57.5 ± 6.3	1.6 ± 0.2	3,747 ± 671	6,556 ± 1,295
21	11.4	8.0 ± 0.9	84.1 ± 6.0	72.9 ± 6.3	62.1 ± 5.2	2.1 ± 0.3	3,392 ± 290	4,708 ± 580

[a] Pharmacokinetic parameters were taken from Table 2; data from the patient with renal impairment (patient 1) were omitted in calculation of mean values

half-life can be seen in Fig. 3 and is significant at the $P < 0.001$ level.

Discussion

Plasma elimination of indicine *N*-oxide in children participating in this study was best described by a two-compartment open model. When data obtained in the study were analyzed according to age, younger children had decreased plasma elimination half-lives and increased rates of plasma clearance compared with older children. In our pharmacokinetic study of indicine *N*-oxide in adults [6], plasma elimination of indicine *N*-oxide was also best fitted to a two-compartment open model. Comparison of data from the two studies reveals that the mean plasma elimination half-life was greater in adults than in children in this study (114 min vs. 84 min), and the mean clearance was reduced in adults compared with the children (2.1 ml/min/kg vs. 4.2 ml/min/kg) [6].

Effects of age on pharmacokinetics of drugs have been reviewed elsewhere [12, 15, 16, 19]. In general, it has been noted that children may require or tolerate greater doses of drugs than adults, probably because of increased rates of absorption, metabolism, and excretion [16, 17, 19]. Factors suggested to be related to differences in these rates include differences in tissue mass, volume of water compartments, and differences in ability to excrete drug via renal and hepatic mechanisms [12, 15, 16, 19]. Limited information is available in the literature on the pharmacokinetics of antitumor agents in pediatric patients compared with adult patients. Greater rates of plasma elimination in children as against adults have been reported for cyclophosphamide [17], methotrexate [20, 21], and more recently, VP16-213 [5].

Age-dependent differences in pharmacokinetics of drugs may play a role in the therapeutic and toxic activities of those drugs in specific age groups [12, 15, 16]. However, in this phase I study of indicine *N*-oxide in 51 pediatric patients (of whom 23

participated in the pharmacokinetic studies), toxicity and clinical responses were not related to age of the 51 patients or to any pharmacokinetic parameters of the 23 children participating in both portions of the study. Although there was no evidence of dose-dependent pharmacokinetics, there was greater toxicity at higher doses. Higher doses of indicine *N*-oxide were significantly correlated with occurrence of hepatoxicity ($P < 0.01$, J. S. Miser et al., unpublished work). The role of hepatic metabolism in the toxicity of indicine *N*-oxide observed at higher doses is not known. However, indicine, the reduced base metabolite of indicine *N*-oxide required for formation of the potentially toxic dehydropyrrolizidine metabolite dehydroindicine was observed only in the plasma of patients receiving high doses (3,375–4,000 mg/m^2) of indicine *N*-oxide. Details on the toxicity and clinical responses of indicine *N*-oxide in this pediatric study will be reported in a separate communication (J. S. Miser et al., unpublished work).

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