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Phase I trial of indicine-*N*-oxide in children with leukemia and solid tumors: a Pediatric Oncology Group study*

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Summary. A phase I trial of indicine-*N*-oxide was carried out in 12 children with solid tumors and in 16 with leukemia. Doses of 5, 6, and 7.5 g/m^2 were given parenterally as a 15-min infusion every 3 weeks. The maximum tolerated dose in patients with solid tumors was 7.5 g/m^2 and the dose-limiting toxicity was myelosuppression. In leukemia, the maximum tolerated dose was 6.0 g/m^2 and hepatotoxicity was dose-limiting. Half of the children with leukemia showed elevations in transaminase levels and one child died of massive hepatic necrosis. This hepatotoxicity limits the use of indicine-*N*-oxide in children with leukemia. Antineoplastic activity was limited to a transient reduction in the numbers of circulating leukemic cells.

Introduction

Indicine-*N*-oxide (NSC 132319, INO) is a naturally occurring, water-soluble pyrrolizidine alkaloid isolated from the plant *Heliotropium indicum*. It was selected for phase I study based on its activity against B16 melanoma, P388 leukemia, Walker 256 carcinoma, M5076 sarcoma, and the MX-1 mammary xenograft [2, 6, 12]. Seven phase I trials have been completed, of which two involved children [6–8, 10, 11, 13, 14]. Responses were obtained in both adult and pediatric patients with refractory leukemia using a daily ×5 schedule [8, 10]. The present study evaluated INO given as a 15-min infusion every 3 weeks to children with solid tumors and leukemia. Hepatotoxicity, which has previously been encountered [6, 13], was limited to children with leukemia in this study and resulted in one fatality [3].

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Treatment plan

This limited phase I pediatric trial started at an INO dose of 80% of that recommended for adults [11, 13], which was subsequently increased by 20% and 25%. At least two evaluable patients with solid tumors (ST) and two with leukemia were studied before the dose was increased. To enable the assessment of cumulative toxicity, the dose was not increased for individual patients.

Children were eligible whose cancer or leukemia was unresponsive to standard therapy and whose life expectancy was >6 weeks. Chemotherapy must not have been received in the 2 weeks prior to treatment. Evidence of recovery from prior toxicity was required. Excluded were patients with uncontrolled infection or with serum creatinine and/or bilirubin levels of >1.7 and >1.2 mg/dl, respectively. A neutrophil count of $>1.5 \times 10^9$/l and a platelet count of $>100 \times 10^9$/l were required, unless the bone marrow was diseased. Written informed consent was obtained.

INO was given intravenously as a single dose over 10–15 min every 3 weeks. Patients who showed improvement or stable disease received additional doses. Toxicity was monitored weekly or more frequently and its severity was graded according to Pediatric Oncology Group (POG) criteria.

Results

A total of 28 children were studied, including 12 with ST who received 15 courses of INO; of these, 2 received 5 g/m^2 (1 child underwent 2 courses), 2 were given 6 g/m^2, and 8 received 7.5 g/m^2 (2 children completed 2 courses). Three patients had Ewing's sarcoma, three had neuroblastoma, and one each had fibrosarcoma, rhabdomyosarcoma, synovial-cell sarcoma, yolk-sac tumor, embryonal-cell carcinoma, and Burkitt's lymphoma.

No severe toxicity occurred in the four patients with ST who were treated with 5 or 6 g/m^2 INO. Myelosuppression was dose-limiting in patients who were given 7.5 g/m^2, with thrombocytopenia being more severe than granulocytopenia (Table 1). Seven patients experienced a fall in hemoglobin levels. In two, the anemia was associated with thrombocytopenia and gastrointestinal (GI) hemorrhage. A 13-year-old boy with neuroblastoma developed hypertension [blood pressure (BP), 160/130 mm Hg] 21 days following treatment. A rogitine test was positive, indicating high levels of circulating catecholamines. Hepatic

Table 1. Toxicities of INO in patients with ST

	Mild	Moderate	Severe	Life-threatening
Anemia	1	4	1	1
Granulocytopenia	0	2	1	1
Thrombocytopenia	0	0	0	3
Pancytopenia	0	1	1	1
Hepatic enzymopathy	2	1	0	0
Nausea/vomiting	0	2	1	0
Hemorrhage	0	0	2	0
Abdominal pain	0	0	2	0
Hypertension	0	0	0	1

Table 2. Toxicities of INO in patients with leukemia

	Mild	Moderate	Severe	Life-threatening	Fatal
Hepatic enzymopathy	2	4	0	2	0
Hemorrhage	0	1	1	2	1
Nausea/vomiting	0	4	1	0	0
Diarrhea	0	2	0	0	0
Abdominal pain	0	0	0	2	0
Hepatic necrosis	0	0	0	0	1

enzymes were elevated mildly in two patients and moderately in a third. No increase in bilirubin values was seen.

In all, 16 children with leukemia received 21 courses of INO; of these, 8 had non-T-, non-B-cell acute lymphoblastic leukemia (ALL), 4 had T-cell ALL, 3 had acute myeloblastic leukemia (AML), and 1 had acute undifferentiated leukemia. Three patients received 5 g/m^2 INO (one underwent three courses), eight were given 6 g/m^2 (one child had two courses), and five received 7.5 g/m^2 (two children completed two courses).

Children with leukemia experienced more frequent and more severe toxicities than did children with ST. Myeloid failure with cytopenia was common, reflecting disease in the bone marrow. Thrombocytopenia was more frequent and more severe than was granulocytopenia. In all, 12 patients experienced a fall in hemoglobin levels, which was associated with GI or intraabdominal hemorrhage in 5 cases (Table 2). Eight patients with acute leukemia experienced hepatotoxicity with elevated SGOT and/or SGPT levels at all INO doses studied. The bilirubin value was elevated in the single patient with fatal hepatic necrosis (see below).

Excessive toxicity was seen in the five patients who received INO at a dose of 7.5 g/m^2. Four children suffered GI or intraabdominal hemorrhage, and three patients died within 3 weeks of treatment. A 5-year-old black boy with refractory AML developed fulminant hepatic failure beginning 3 days following a dose of 7.5 g/m^2 INO and died 6 days later. Autopsy showed retroperitoneal hemorrhage and extensive hemorrhagic panlobular hepatic necrosis. Full details of this case have been published elsewhere [3].

A 5-year-old boy with refractory ALL received two courses of INO at 7.5 g/m^2. Mild emesis and a transient fall in circulating lymphoblasts followed the first dose. After the second dose, the patient vomited three times. Abdominal distention and pain developed and his hemoglobin levels fell, suggesting hemorrhage. SGOT values increased to 580 units/dl. Severe pancytopenia developed with disappearance of circulating lymphoblasts. This patient died of a subdural hematoma 19 days after the second dose of INO. A third patient developed abdominal pain and a fall in hemoglobin levels and died 9 days after treatment.

Eight children with leukemia were treated at a dose of 6 g/m^2 INO. These patients experienced much less toxicity than did those treated at 7.5 g/m^2.

There was no evidence of renal or central nervous system toxicity. Patients with ST or leukemia who received a second or third dose of INO at 6 or 7.5 g/m^2 experienced more severe myelotoxicity, usually accompanied by GI hemorrhage. This suggested that cumulative toxicity may occur with this dosing schedule.

Four of ten evaluable patients with ST who were treated with INO had stable disease. There were no complete or partial remissions in patients with leukemia. At 3–7 days following treatment, ten patients experienced a transient decrease in or disappearance of circulating leukemic cells that lasted 1–2 days.

Discussion

The chemical structure of INO is different from that of other antineoplastic agents, and this drug showed a broad spectrum of antitumor activity in preclinical evaluation [2, 6]. Phase I NCI-sponsored studies of INO have been carried out using three different dose schedules: every 3–4 weeks, weekly, and daily ×5 [6]. A pediatric study used the 5-day schedule [10], and another study of the 5-day schedule in leukemic patients included both adults and children [8]. This is the third NCI-sponsored pediatric study, and it is the only one that tested INO given every 3–4 weeks.

In patients with ST, severe toxicity was seen only at a dose of 7.5 g/m^2. At this dose, myelosuppression was the dose-limiting toxicity and appeared to be cumulative in two of the three patients who received a second course. Hepatotoxicity was not seen in patients with ST.

All patients with leukemia had peripheral cytopenia of variable degrees prior to therapy. Thrombocytopenia was more severe after INO than was granulocytopenia. Excessive hepatic and hemorrhagic toxicities were seen in patients treated with 7.5 g/m^2 INO; however, these side effects were much less severe in those who received 6.0 g/m^2, which appeared to be the maximum tolerated dose of INO in children with leukemia.

Although INO is a member of a family of known pyrrolizidine alkaloid hepatotoxins, no significant liver injury has been seen in animal studies [4]. However, a number of patients, both adults and children, have experienced hepatotoxicity in this and previous studies [3, 6, 9, 14]. In most cases, this was limited to elevated transaminase and/or bilirubin levels. However, seven patients have died of liver failure [3, 6, 14], including the patient in the present study [3]. A common finding at autopsy has been centrilobular congestion and necrosis in a pattern compatible with veno-occlusive disease (VOD). VOD is a known complication following intense chemotherapy and radiotherapy [1, 5]; it is also caused by alkaloids found in various species of *Heliotropium* [6]. The hepatic injury seen in leukemic children in the present study probably resulted from a previous combination of intense and hepatotoxic therapy, which made their livers more sensitive to the hepatotoxicity of INO and/or its metabolites.

Four patients with leukemia and two with ST developed severe abdominal problems, including pain, distention, and retroperitoneal hemorrhage. In those with ST, this appeared to be related to extensive intraabdominal tumor. However, this finding in children with leukemia suggests a specific toxicity of INO. In dogs, intestinal mucosal atrophy, degeneration, and necrosis developed following INO [2], and this may occur in patients as well. An additional side effect of INO was a fall in hemoglobin levels, which was seen in patients with ST and in those with leukemia. In several cases, there was no evidence of hemorrhage. This has also been noted in previous studies [11]. The mechanism by which INO or its metabolites destroys red blood cells is unknown.

No clinical responses were noted in patients with a variety of ST who received INO every 3 weeks; however, most patients received only a single course of therapy. Results were equally disappointing in children with leukemia as compared with those obtained using the 5-day schedule [6, 8, 10]. The severe hepatotoxicity associated with INO has led to a suspension of clinical interest in the drug. Attempts are being made to develop INO analogs that are less toxic but retain antitumor activity [6].

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