Phase I Study of a Five-Day Dose Schedule of 4-Ipomeanol in Patients with Non-Small Cell Lung Cancer


Medicine Branch [V. K. K., M. P. D., E. K. R., B. E. J., M. J. K.], Clinical Center Pharmacy Department [S. C. P.], and Investigational Drug Branch, Cancer Therapy Evaluation Program [M. C. C.], National Cancer Institute, and Department of Medicine, National Naval Medical Center [G. G. S., K. O.], Bethesda, Maryland 20889-5105; and Midwest Research Institute, Kansas City, Missouri 64110 [G. A. T., T. L. M.]

ABSTRACT

The mammalian pulmonary toxin 4-ipomeanol (IPO) is activated by the cytochrome P450 system in bronchial Clara cells in animals. The resulting metabolites bind rapidly to macromolecules, producing localized cytotoxicity. IPO has in vitro and in vivo antitumor activity in non-small cell lung cancer (NSCLC) and thus was proposed as a lung cancer-specific antitumor agent. We have completed a directed Phase I trial in patients with NSCLC. Forty-four patients (34 men and 10 women) with NSCLC were treated with IPO. All but two patients had an Eastern Cooperative Oncology Group performance status of 0 or 1. They received 91 courses of therapy with 1.v. IPO; 82 courses were administered daily for five days, and 9 were single bolus doses. The dose-limiting toxicity of elevated serum transaminases was observed in three of seven patients at 922 mg/m²/day. The maximum tolerated dose was 693 mg/m²/day on 5 consecutive days every 3 weeks. One patient developed grade 4 pulmonary toxicity at 167 mg/m²/day. There was no significant hematological or renal toxicity. No objective antitumor responses were observed. Pharmacokinetic analysis of 39 patients from day 1 of IPO administration showed biexponential elimination with mean half-lives of 8.6 (α half-life) and 76 min (β half-life). There was a linear relationship between the area under the plasma drug concentration-time curve and the dose of IPO. There was no significant difference between the pharmacokinetic parameters measured on day 1 and day 5. Using a 4-day in vitro cytotoxicity assay, two tumor cell lines established from patients treated at 693 mg/m²/day had IC₅₀ of ~6 mm, a concentration more than 75-fold higher than the plasma levels measured in these patients. Thus, although the total amount of drug administered per cycle on a daily times five dose schedule is more than 25-fold higher than the recommended single daily dose, IPO is unlikely to be a useful drug for patients with lung cancer.

INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths in the United States (1). Several strategies have been used to develop novel antineoplastic agents effective against lung cancer and other human solid tumors. Until the mid-1980s, the NCI* used compound-oriented preclinical screening systems using a small panel of transplantable rodent tumors and human tumor xenografts (2). Drugs identified by this process were most effective against hematopoietic malignancies (2). Another screening technique used at this time used a human tumor colony-forming assay to screen compounds for in vitro antitumor activity against cultures of fresh human tumors (3). However, two of the drugs selected by this human tumor colony-forming assay for clinical trials have little activity against NSCLC (4, 5). Subsequently, the NCI has continued high-throughput in vitro empiric drug screening using 60 human tumor cell line panels representative of common solid tumor types in an attempt to identify agents with better solid tumor activity (6–8).

The development of IPO (Fig. 1) as a potential anticancer agent arose outside of these screening strategies. IPO was identified as the agent in fungus (Fusarium solanae)-infected sweet potatoes (Ipomoea batatas) responsible for severe pulmonary toxicity and death in cattle (9–11). IPO is an inert substance that is metabolized to a highly reactive compound by a cytochrome P450-dependent monoxygenase system in most mammalian pulmonary Clara cells (12, 13), which share certain histological and biochemical features with bronchogenic carcinomas (14). The reactive metabolite of IPO, speculated to be a furan epoxide (15, 16), binds covalently to tissue macromolecules preceding the appearance of organ damage or death (12, 17). The degree of covalent binding correlates directly with organ-specific toxicity.

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1 Portions of this work were performed under NCI Contract NO1-CM-27748 to the Midwest Research Institute (Kansas City, MO).
2 Present address: 4120 W. Gazebo Hill Blvd., Mequon, WI 53092.
3 To whom requests for reprints should be addressed, at Medicine Branch, Building 8, Room 5101, National Naval Medical Center, 8901 Rockville Pike, Bethesda, MD 20889-5105. Phone: (301) 496-0901; Fax: (301) 496-0047; E-mail: mk4m@nih.gov.

4 The abbreviations used are: NCI, National Cancer Institute; NSCLC, non-small cell lung cancer; MTD, maximum tolerated dose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; ECOG, Eastern Cooperative Oncology Group; AUC, area under the concentration-time curve; IPO, 4-ipomeanol.
Five-Day Dose Schedule of IPO in NSCLC

**Fig. 1** Chemical structure of IPO [1-(3-furyl)-4-hydroxyl-1-pentanone; NSC 349438]. Molecular formula, C₉H₁₂O₃; molecular weight, 168.2.

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**in vitro** (18). It has also been noted that IPO has greater activity against NSCLC cell lines than against small cell lung cancer cell lines (19). Thus, clinical development of IPO as a lung cancer-specific antineoplastic agent was based on its organ-specific toxicity in nonhuman mammals (17).

Preclinical toxicology studies of IPO showed dose-dependent toxicity with a steep dose-response curve (20). In mammals, the predominant sites of binding of IPO are the Clara cells and the proximal renal cortical tubules (14, 21). In male Sprague Dawley rats treated with radiolabeled IPO, tissue distribution revealed the greatest concentration of radioactivity in the lung, followed by the gut, liver, and kidney (22). It was also observed that IPO could induce tolerance to its own toxic effects on normal tissues. In mice, resistance to toxicity was most rapidly induced by daily administration of IPO for 7 days (20, 23), suggesting an improved tolerance of subsequent drug dosages, presumably through the induction of cytochrome P450 activity.

A Phase I trial of IPO in 55 lung cancer patients using a single bolus dose of IPO in treatment cycles of 21 days has been reported (24). The dose-limiting toxicity in this trial was hepatocellular toxicity with reversible hypertransaminasemia. The MTD was 1290 mg/m², and pulmonary toxicity was observed in a single patient treated with 826 mg/m². No objective tumor responses were seen. Pharmacokinetic studies showed biphasic elimination with α and β half-lives of 6.7 and 114.5 min, respectively, at the MTD. Because the toxicity of IPO in animals is not cumulative (rodents) or less than cumulative (dogs), we conducted a Phase I trial of IPO to determine whether the total dose of drug that could be administered using a daily times five schedule is significantly higher than the single-dose MTD.

**MATERIALS AND METHODS**

**Patients.** Patients with histologically proven NSCLC who were not candidates for potentially curative surgery or radiation therapy were eligible for treatment with IPO, regardless of prior treatment status. Patients who were previously treated with bleomycin were excluded because of its potential to cause pulmonary toxicity. A minimum of 3 weeks since the last course of chemotherapy or radiotherapy (6 weeks for nitrosoureas or mitomycin C) was required to have elapsed. Other eligibility criteria included an ECOG performance status of 0–2, an absence of brain metastases, a life expectancy in excess of 8 weeks, a WBC count of >3,000/µL, a platelet count of >75,000/µL, bilirubin ≤ 1.5 mg/dL, serum creatinine ≤ 1.5 mg/dL or creatinine clearance ≥ 60 mL/min, and adequate pulmonary function (forced expiratory volume in the first second ≥ 50% of that predicted and arterial oxygen saturation ≥ 90%). No drugs known to interact with microsomal enzymes, such as cimetidine, barbiturates, alcohol, or dexamethasone, were administered concurrently with IPO. Written informed consent was provided by all patients. The trial was approved by the Institutional Review Boards of the NCI and the National Naval Medical Center.

**Drug Preparation and Administration.** IPO [1-(3-furyl)-4-hydroxyl-1-pentanone; NSC 349438] was supplied by the Division of Cancer Treatment, NCI (Bethesda, MD) and infused i.v. over 15–20 min. Patients were treated between 10 a.m. and noon to avoid potential variability due to circadian fluctuations in microsomal P450 enzymes and tissue glutathione that are involved in the metabolic activation and detoxification, respectively, of IPO.

The first nine patients received a test dose (equivalent to 2.5 times the daily dose) on day 1 as a single i.v. bolus dose over 30 min, followed 21 days later by five daily doses of the drug. Thereafter, five daily doses were repeated at 21-day intervals. The starting dose was 3.5 mg/m²/day, the equivalent of one-tenth of the LD₅₀ in murine toxicology studies (20). The 5-day treatment regimen was selected to determine whether cumulative toxicity would occur in humans. The initial patients received the test dose as a control, based on tolerance data from the murine toxicology studies described above (23). To eliminate the potential of encountering dose-limiting toxicity from the test dose before that from the five daily doses, the protocol was amended after the ninth patient enrolled to eliminate the test dose and institute the five daily doses every 21 days for all subsequent courses.

Doses were escalated using the modified Fibonacci schedule. Three patients at each dose level were fully evaluated for acute toxicity (i.e., one 21-day cycle) before IPO-naïve patients were entered at the next highest dose level. If a patient experienced no toxicity during a cycle of IPO, the dose could be escalated to the next highest level, up to a maximum of two escalations for that patient. The patients were hospitalized for the first cycle of treatment with IPO and for the first 5 days of treatment at an escalated dose. If dose escalation did not occur, and no significant toxicity was observed, subsequent cycles could be administered on an outpatient basis. If grade 2 toxicity was observed among the first three patients at a dose level, a total of six patients were entered at that level. If no patients had grade 3 or greater toxicity, the dose level was escalated in subsequent patients to the next highest dose level. If a patient developed reversible grade 3 toxicity, three additional patients were enrolled at that dose level, and if any of these developed grade 3 toxicity, the escalations were terminated. At least six patients were treated at the MTD, at which acceptable and reversible toxicity was observed.

**Pretreatment and Follow-Up Studies.** A history and physical examination; laboratory studies including complete blood cell and differential WBC counts, electrolytes, urea, creatinine, glucose, total protein, albumin, calcium, phosphate, uric acid, alkaline phosphatase, total and direct bilirubin, AST, and ALT; chest radiograph and chest computed tomography scan; electrocardiograph; pulse oximetry; and pulmonary function...
tests (including the diffusing capacity of carbon monoxide and arterial oxygen saturation) were performed before treatment. While the patient was hospitalized, vital signs and blood oxygen saturation (after an initial calibration by arterial blood gas and followed by digital oximetry) were recorded every 8 h. For outpatients, a daily history and physical examination and digital pulse oximetry were done for the first 5 days of each cycle. A chest radiograph was obtained every other day for a minimum of three per week. Full pulmonary function tests were obtained on day 9, and serum chemistries (including liver function tests) were obtained three times a week while IPO was administered. Toxicity was graded using the NCI Common Toxicity Criteria (25). Tumor responses were assessed using previously defined standard criteria (26).

In Vitro Drug Sensitivity Assay. Lung cancer cell lines were established from tumor samples obtained within 2 weeks of study entry as described previously (27-29). Single-cell suspensions were obtained from these lung cancer cell lines by trypsinization of monolayer cultures and manually counted with a hemocytometer. Cells were then plated at a seeding density of 20,000 cells/well in fresh medium (RPMI 1640 with 10% fetal bovine serum) into 96-well microtiter plates and incubated for 16 h at 37°C. IPO was then added at concentrations ranging from 0.032-32 μM, and incubation continued for 4 days, at which time the MTT assay was performed as described previously (30, 31), and absorbance was measured at 540 nm using an EL 312 microplate spectrophotometer (Bio-Tek Instruments, Winooski, VT). The experiment was performed twice for each lung cancer cell line.

Plasma and Urine Levels of IPO. All but the first two enrolled patients had plasma and urine samples collected during the first cycle of IPO. Venous blood was collected in heparinized tubes before the start of the infusion and at scheduled intervals (0, 5, 10, 20, 30, and 60 min and 4, 8, 24, and 48 h) during and after the infusion of IPO on days 1 and 5 of the five daily doses of drug. Plasma was separated by centrifugation and stored at −20°C until analyzed. Aliquots (25 ml) of 24-h urine collections collected each day over the 5 infusion days were frozen at −20°C until analyzed. Plasma and urine IPO concentrations were determined by a capillary gas chromatographic assay (32). Spiked standards and the plasma or urine samples were prepared by transferring an aliquot of standard and/or internal standard (1-decanol) in benzene to glass vials. Aliquots of untreated human plasma or urine or the patient’s serum or urine were added to each vial and mixed for approximately 5 min. The human clinical samples and standards were derivatized by the addition of 20 μl of heptfluorobutyrylimidazole (Pierce Chemical Co., Rockford, IL) and incubated for 15 min in a shaking water bath at 50°C. The samples were cooled to room temperature, and excess derivatizing reagent was reacted with water. The samples were then extracted with a 5% ammonia solution, and the organic layer was clarified by centrifugation before gas chromatographic analysis (Varian Model 3700 GC). All samples were prepared and analyzed in duplicate.

Pharmacokinetic Studies. Concentration-time data of IPO were fit to a two-compartment model using weighted non-linear iterative least squares regression with PCNONLIN (Statistical Consultants, Inc., Lexington, KY). Model selection was guided by Akaike’s information criterion and the examination of observed and model-fitted concentrations (33). The parameters determined included α and β half-lives, maximum concentration, volume of distribution, and total body clearance. AUC and mean residence time were calculated using standard noncompartmental equations (34). The percentage of drug recovered in the urine was calculated by multiplying the concentration of the aliquot by the total urine collected during that period and comparing that quantity with the dose administered.

RESULTS

Patient Characteristics and Treatment. Forty-four patients with advanced NSCLC were entered onto this study between February 1989 and November 1995. Their characteristics are listed in Table 1. Most patients were male and had an ECOG performance status of 1. The median age was 56.5 years, and the most common histological subtype was adenocarcinoma. A majority of patients had received prior chemotherapy and/or radiotherapy, although 17 patients were chemotherapy naïve, including 8 patients who had received no prior therapy (including surgery). Two patients had a history of prior Hodgkin’s disease; one had been treated with nitrogen mustard and mantle-field irradiation, and the other patient had received chemotherapy with nitrogen mustard, vincristine, procarbazine, and prednisone. The 25 patients who had received chemotherapy for lung cancer had been treated with a median of one regimen (range, one to three regimens).

The dose escalation schema is summarized in Table 2. Aside from the first enrolled patient, who received only a single
test dose of IPO at 22 mg/m², the next eight patients were given test doses equivalent to 2.5 times the daily (times five) dose three weeks before the five daily doses (Table 2). After the protocol was modified to eliminate the test dose, patients were enrolled beginning at a dose of 125 mg/m²/day for 5 consecutive days. Ninety-one courses of IPO were administered, with a median of two courses/patient (range, one to six courses). One 5-day course was terminated on day 3 at the 922 mg/m²/day dose level due to grade 4 hepatotoxicity. Of the 91 total courses, 5 were administered at dose reductions due to grade 3 toxicity at the prior level, and 9 were single bolus dose courses, 1 each to the first 9 patients on the study.

**Dose-Limiting Toxicity.** Toxicity was evaluable in each of the 82 five-day courses administered. Nine of the 91 treatment courses were single test doses, none of which resulted in hepatotoxicity. Dose-limiting hepatotoxicity was encountered at 922 mg/m²/day, and further accrual of patients was then made at 693 mg/m²/day dose level due to grade 4 hepatotoxicity. Of the 91 total courses, 5 were administered at dose reductions due to grade 3 toxicity at the prior level, and 9 were single bolus dose courses, 1 each to the first 9 patients on the study.

**Table 2** Dose escalation schema

<table>
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<th>Test dose (mg/m²)</th>
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<th>No. of patients escalated to level</th>
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* The number of cycles administered daily for 5 days. Nine additional cycles consisted of the test dose alone given to patients on the first four dose levels.

grade 3 hepatotoxicity. Thus, the MTD in this trial for IPO given on a daily times five schedule is 693 mg/m²/day.

Reversible hepatotoxicity, characterized by elevations of the serum transaminases, AST and ALT, was the dose-limiting toxicity on this schedule of IPO administration (Table 3). Grade 1 or greater hepatotoxicity with elevation of both AST and ALT was observed in 37 treatment courses; an additional 5 courses resulted in isolated elevations of AST. The median day of onset of elevations in serum transaminases was day 4 (range, 3–8 days). The median day of peak elevation was day 7 (range, 4–20 days), and the median day of resolution of the abnormalities was day 22 (range, 13–33 days). Peak ALT elevations were typically one-half to twice that of peak AST elevations. Successive courses of therapy in the same patient did not result in higher elevations of these enzymes; thus, hepatotoxicity was not cumulative. Grade 1 elevations of alkaline phosphatase were observed in nine cycles administered to seven patients, three of whom had known bone metastases. No bilirubin elevations were noted.

**Prothrombin Time Prolongation.** Prolongation of the prothrombin time was observed only in patients receiving the three highest dose levels of IPO and was associated with elevations in the liver enzymes. The patient with grade 4 hepatotoxicity abbreviating his first cycle after the third day had a prolongation of his prothrombin time to 17.5 s, with normal partial thromboplastin time, fibrinogen, d-dimer, and platelet levels. The prothrombin time corrected to normal in a 1:1 mixing study with normal plasma, indicating a probable factor deficiency.

**Pulmonary Toxicity.** A 48-year-old female with previously untreated adenocarcinoma of the lung treated at the 167 mg/m²/day dose level was the only patient to develop pulmonary toxicity. This patient had a prior history of Hodgkin's disease 26 years earlier, which had been treated with nitrogen mustard and mantle-field irradiation. She developed progressive dyspnea on the eleventh day of her initial course of therapy and was noted to be hypoxic (pO₂...
Table 3 IPO toxicity by dose level and grade

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>No. of courses</th>
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<th>Hypertransaminasemia</th>
<th>Prothrombin time prolongation</th>
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* No toxicity was observed among the first 23 courses (including test doses) administered to 12 patients (see the text for details).

Fig. 2 Correlation between the administered dose of IPO and the AUC on day 1 of treatment in 32 patients. The Pearson correlation coefficient is shown.

= 57 mm Hg) and have bilateral fluffy infiltrates on chest radiograph. An evaluation for an infectious etiology was unremarkable. The patient was treated with i.v. methylprednisolone and antibiotics, and by day 19, she had resolution of her symptoms and radiographic infiltrates. She had greater than 50% decrements in forced expiratory volume in the first second, forced vital capacity, and the diffusing capacity of carbon monoxide and was not retreated with IPO.

**Other Toxicities.** Nausea and/or vomiting was noted in 20 courses of therapy and was more severe at the higher dose levels (Table 3). Prophylactic antiemetics were instituted at the 693 mg/m²/day dose level after one patient treated at 521 mg/m²/day experienced grade 3 nausea. Nausea and vomiting occurred during and up to 6 h after drug administration. Both patients with grade 3 nausea experienced it on the third day of drug administration only.

Microscopic hematuria was seen in patients at all dose levels. It generally began on day 4 of treatment and resolved by day 10. Five patients had grade 2 proteinuria, one patient each at the 521, 693, and 922 mg/m²/day dose levels and two patients at the 222 mg/m²/day dose level, all of which was associated with concurrent microscopic hematuria. No gross hematuria or renal insufficiency was noted. One patient developed a rash 3 weeks after receiving a test dose of 75 mg/m² that was thought to be due to concurrent doxycycline therapy. No other clinically significant toxicities including myelosuppression were noted with this schedule of IPO administration.

**Response to Treatment.** Forty-two patients completed at least one full 21-day cycle of IPO administered daily for 5 consecutive days and were assessable for tumor response. One patient received only a test dose of the drug, whereas another patient received only three of his five daily doses due to the development of grade 4 hepatotoxicity during the administration of his first cycle of IPO, and these patients were not evaluable for response. No objective responses were observed in the evaluable patients. Seventeen patients had progressive disease, and 25 patients had stable disease for periods ranging from 6–28 weeks. The median survival of all patients was 28 weeks. Seven patients with bronchioloalveolar carcinoma had a median overall survival of 51 weeks as opposed to 35 weeks for those with adenocarcinoma. One patient with bronchioloalveolar carcinoma treated with four courses of IPO (125 and 167 mg/m²/day) in 1992 is the sole surviving patient with asymptomatic bilateral lung metastases 6 years after starting treatment.

**Pharmacokinetics.** Pharmacokinetic studies were undertaken on days 1 and 5 of the first treatment cycle. Pharmacokinetic data were obtained for both the test dose and the first 5-day course of IPO for the initial nine patients. Only two of these nine patients had IPO levels obtained on day 5 of their first 5-day cycle.

The pharmacokinetics of IPO was characterized by biexponential elimination with mean ± SD α and β half-lives of 8.6 ± 4.7 and 75.0 ± 26.6 min, respectively. IPO seemed to follow linear pharmacokinetics, as evidenced by a dose-proportional increase in AUC with increasing dose (Fig. 2) and a clearance that did not change significantly over the dosage range studied. There were no significant differences in any parameter between the day 1 and day 5 pharmacokinetics (Table 4), demonstrating the absence of time dependence in drug disposi-
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In Vitro Activity of IPO. Tumor cell lines (NCI-H2925, NCI-H2973, and NCI-H2830) established from three patients treated with IPO were available for in vitro drug sensitivity testing. All three patients had received chemotherapy before IPO treatment. Two other NSCLC cell lines (NCI-H322 and NCI-H358) that were not established from patients treated with IPO and had been used in prior in vitro drug sensitivity testing with IPO (19) were retested for their sensitivity to IPO. The IC50 of the five lung cancer cell lines continuously incubated with the drug for 4 days was measured by the MTT assay. The IC50 values of IPO determined in two separate experiments for NCI-H2830 and NCI-H2973 were approximately 6 mm (the greatest concentration used in our assay), whereas the IC50 values for NCI-H2925, NCI-H322, and NCI-H358 were greater than 6 mm. Both patients from whom NCI-H2830 and NCI-H2973 were established were treated at the MTD of 693 mg/m²/day, and the patient from whom NCI-H2925 was established was treated at 922 mg/m²/day.

DISCUSSION

IPO was developed as a lung-specific cytotoxic agent based on preclinical data that suggested preferential activation and cytotoxicity of this compound in both normal human lung tissue and lung cancers in vitro. The lung is the major target for IPO in at least seven mammalian species (17, 35). We conducted a Phase I trial of IPO given daily for 5 consecutive days to identify the MTD and dose-limiting toxicities on this schedule. IPO caused dose-limiting hepatotoxicity at the MTD of 693 mg/m² daily for 5 days. Thus, the total dose of IPO that can be administered in five daily doses per 3-week cycle (3465 mg/m²) is more than 2.5 times the MTD of a single dose per 3-week cycle (1290 mg/m²; Ref. 24).

As with the single-dose schedule, hepatotoxicity rather than pulmonary toxicity was the dose-limiting toxicity of IPO given by five daily doses, and it was characterized by reversible elevations in serum ALT and AST. This hepatotoxicity was also associated with prothrombin time prolongation at higher dose levels without clinical sequelae and was not cumulative. Nausea and vomiting were also prevalent at the higher dose levels, with grade 3 toxicity seen in one patient at each of the 521 mg/m²/day and 693 mg/m²/day dose levels. Only one patient had pulmonary toxicity possibly attributable to IPO, which occurred at a dose one-fourth that of the MTD. This patient had received mantle-field radiation for Hodgkin’s disease 26 years before treatment with IPO; hence, underlying chronic pulmonary changes could have potentiated the pulmonary toxicity of IPO. Patients included in this trial who had received prior chest radiotherapy for their lung cancers (n = 18) did not have any apparent clinical pulmonary toxicity. There was no identifiable hematological toxicity of IPO.

The preferential activation of IPO in Clara cells suggests that cell-specific isoenzymes of cytochrome P450 are involved in this process. Interspecies differences in cytochrome P450 isoenzymes as well as tissue-specific localization of the isoenzymes may explain the differences seen between the preclinical toxicology in animals and the observed toxicity profile in the two human studies of IPO. After the initiation of this trial, Czerwinski et al. (36) demonstrated 2–10-fold greater IPO activation by the hepatic cytochrome P450 isoenzymes CYP1A2, CYP3A3, and CYP3A4 compared with the pulmonary isoenzymes CYP2F1 and CYP4B1 (36). In contrast, the rabbit CYP4B1 isoenzyme had nine times the IPO activating activity of the most active human isoenzyme. Interspecies cytochrome differences may also explain the lack of induction of tolerance to IPO in humans, as evidenced in this trial by similar degrees of hepatotoxicity in patients treated with multiple cycles of the same dose. Thus, cognizance of the significant interspecies variation in cytochrome P450 isoenzyme activity is necessary in interpreting preclinical studies of agents requiring cytochrome activation.

As a result of the traditional Phase I trial design used in this trial and the ∼200-fold difference between the starting dose (the equivalent of one-tenth of the marine LD10) and the MTD, 31 of 44 (70%) patients were treated at dose levels below the MTD, decreasing the likelihood of therapeutic benefit in these patients and greatly increasing the time needed to complete the study. Several alternative Phase I trial designs incorporating pharmacokinetic (37), logistic (38), and Bayesian (39) dose escalation schemes have been proposed. An estimated 18–37 patients (compared with the 44 patients actually enrolled) would have needed to define the MTD if this trial used one of three accelerated escalation schemes (40). However, the exceedingly steep dose-response curve of IPO demonstrated in preclinical toxicology (12 and 30% differences between the LD10 and LD90 in male and female mice, respectively; Ref. 20) would have precluded the use of an accelerated dose escalation scheme for this drug.

Antitumor activity of IPO was measured in two of three NSCLC cell lines derived from the tumor material of patients enrolled in this trial by 4 days of continuous exposure to IPO in the MTT assay. The IC50 of IPO was approximately 6 mm for both cell lines. This compares with previous in vitro cytotoxicity testing using a colony-forming assay in which the IC50 for NCI-H322 (with Clara cell features) and NCI-H358 were 0.01–0.1 and 1–10 mm, respectively (19). The 100-fold difference in the sensitivity of H322 between these two studies may be due to changes in the cell line that occurred after years in culture or due to differences in assay conditions. The highest maximum plasma concentrations of IPO in the patients from whom these cell lines were derived in this study were 74 and 94 μM, respectively, and the average maximum plasma concentration for seven patients treated at the MTD of 693 mg/m²/day was 80 μM (range,
55–128 μM). Thus, peak IPO concentrations detected in the plasma were approximately 75-fold less than that necessary for in vitro antitumor activity against the tumor cell lines by continuous drug exposure in the MTT assay. If the in vitro assay is reflective of in vivo sensitivity, then it is unlikely that systemic administration of IPO will result in blood concentrations necessary to inhibit the growth of lung cancer cells. Regional therapy remains an untested route of IPO administration that, at best, would be palliative. Therefore, despite the rational pathway by which IPO came to clinical evaluation, further testing of this compound for lung cancer does not seem appropriate.

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