Phase I and Pharmacological Study of the Pulmonary Cytotoxin 4-Ipomeanol on a Single Dose Schedule in Lung Cancer Patients: Hepatotoxicity Is Dose Limiting in Humans

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ABSTRACT

4-Ipomeanol (IPO), a naturally occurring pulmonary toxin, is the first cytotoxic agent to undergo clinical development based on a biochemical-biological rationale as an antineoplastic agent targeted specifically against lung cancer. This rationale is based on preclinical observations that metabolic activation and intracellular binding of IPO, as well as cytotoxicity, occurred selectively in tissues and cancers derived from tissues that are rich in specific P450 mixed function oxidase enzymes. Although tissues capable of activating IPO to cytotoxic intermediates in vitro include liver, lung, and kidney, IPO has been demonstrated in rodents and dogs to undergo in situ activation, bind covalently, and induce cytotoxicity preferentially in lung tissue at doses not similarly affecting liver or kidneys. Although the drug was devoid of antitumor activity in the conventional marine preclinical screening models, cytotoxic activity was observed in human lung cancers in vitro and in human lung xenografts in vivo, adding to the rationale for clinical development.

Somewhat unexpectedly, hepatocellular toxicity was the dose-limiting principal toxicity of IPO administered as a 30-min infusion every 3 weeks to patients with lung cancer. In this study, 55 patients received 254 courses at doses ranging from 102 to 826 mg/m². Transient and isolated elevations in hepatocellular enzymes, predominately alanine aminotransferase, occurred in the majority of courses of IPO at 1032 mg/m², which is the recommended IPO dose for subsequent phase II trials. At higher doses, hepatocellular toxicity was more severe and was often associated with right upper quadrant pain and severe malaise. Toxic effects were also noted in other tissues capable of activating IPO, including possible nephrotoxicity in a patient treated with one course of IPO at 154 mg/m² and severe, reversible pulmonary toxicity in another patient who received nine courses of IPO at doses ranging from 202 to 826 mg/m². Although individual plasma drug disposition curves were well described by a two-compartment first order elimination model, the relationship between IPO dose and area under the disposition curve was curvilinear, suggesting saturable elimination kinetics. At the maximum tolerated dose, the mean half-lives (A1 and A2) were 6.7 and 114.5 min, respectively. Renal excretion of parent compound accounted for less than 2% of the administered dose of IPO. An unidentified metabolite was detected in the plasma of patients treated at higher doses. No objective antitumor responses were observed; however, stable disease persisted for at least eight courses in 27% of patients.

The preponderance of clinical toxicity observed in liver rather than lung suggests that IPO may be preferentially activated and bound in liver rather than lung or other tissues in humans or that human lung tissue is more effective at detoxifying and/or is more tolerant to activated IPO than other species. In any event, these observations suggest further that the rationale for the clinical evaluation of IPO should be extended to include liver cancers and possibly renal cancers, as well as lung cancers.

INTRODUCTION

IPO² (Fig. 1) is a naturally occurring furan isolated from common sweet potatoes (Ipomoea batatas) infected with the fungus Fusarium solani (1–3). It is the first agent to be developed under the auspices of the NC1 based on a biochemical-biological rationale (4) as an antineoplastic agent targeted specifically against lung cancer (reviewed in Ref. 5).

IPO was isolated and identified by Boyd et al. (1, 2) as the agent responsible for outbreaks of a lethal pulmonary disease in cattle. IPO was demonstrated to be a tissue-specific cytotoxin which requires metabolic activation by cytochrome P450 mixed function oxides to a highly reactive intermediate that covalently binds to macromolecules (4–24). In lower mammals, such as rabbits, rats, guinea pigs, female mice, and dogs, IPO is preferentially activated in the lung, specifically in bronchiolar Clara cells, and to a lesser extent in type II pneumocytes, which are rich in the specific cytochrome P450 isoenzymes required for IPO activation (4). The predominant toxicity in these species is pulmonary (4–27). IPO binds immediately to nucleophilic macromolecules at the site of activation (4), leading to bronchiolar epithelial necrosis preferentially involving the Clara cells (4, 5, 25, 26). Maximal expression of lung toxicity occurs between days 1 and 5 after treatment (12, 25, 26). In dogs treated with lethal doses, radiographic findings are consistent with an interstitial pneumonitis including severe acute inflammation with neutrophil and mononuclear infiltration of alveoli and bronchioles, a proteinaceous alveolar exudate, capillary congestion, and alveolar cell necrosis (5, 25–27). In lower mammals the slope of the dose-toxicity curves are steep, with small differences between doses associated with reversible pulmonary toxicity and death (12, 25, 26).

The relative tissue content and substrate specificities of various isomers of cytochrome P450 may vary considerably among different species, different organs within a given species, and among different types of cells within a given organ. Such differences may explain, at least in part, certain species differences in the relative tissue specificities of IPO. For example, histopathological evidence of toxicity has been documented not only in the lungs but also in other tissues, such as the liver (hamster) and the kidneys (male mice), which also possess cytochrome P450 isoenzymes capable of activating IPO to cytotoxic intermediates (14, 22). However, the only species in which pulmonary toxicity is entirely absent and in which hepatotoxicity predominates is in birds. Interestingly, avian lungs are devoid of P450 enzymes for metabolic activation of IPO and they lack terminal airways and both Clara and type II alveolar cells that typically line these structures in other species (23, 24).

Received 11/4/92; accepted 3/9/93.

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1 This study was supported by NIH Contract NO1-CM-57738. Presented in part at the annual meetings of the American Society of Clinical Oncology, San Francisco, CA, May 1989, and San Diego, CA, May 1992.

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3 The abbreviations used are: IPO, 4-ipomeanol; NC1, National Cancer Institute; IC₅₀, 50% inhibitory concentration; FEV₁, forced expiratory volume over 1 s; PO₂, arterial partial pressure of oxygen; DLCO, pulmonary diffusion capacity for carbon monoxide; DLCO/ALV, DLCO adjusted for alveolar volume; MTD, maximum tolerated dose; FVC, forced vital capacity; LCT, limited computerized tomography; AUC, area(s) under the curve; CT, computed tomography; ALT, alanine aminotransferase; AST, aspartate aminotransferase.
IPO was not active against the in vivo tumor murine models (e.g., L1210 and P388 leukemias) nor in the human tumor xenografts used in the NCI screen in the early 1980s (5, 27), and continuous treatment with high concentrations were required to inhibit colony formation of many types of human tumors in colony-forming assays (27). Several in the NCI screen in the early 1980s (5, 27), and continuous treatment L1210 and P388 leukemias) nor in the human tumor xenografts used and type II alveolar cells (28). In addition, IPO was demonstrated to (27-30). For example, IPO was active against several other human sustained in vivo (5, 27). Since many of the tumors used in the traditional screens were not likely to contain the metabolic apparatus necessary to activate IPO, the rationale to develop IPO was further supported by some observations of activity in novel screening systems (27-30). For example, IPO was active against several other human non-small cell lung cancers growing in vitro and in xenografts (27- 30). In vitro, drug sensitivity correlated with the ability of these cells to activate IPO, as well as their morphological resemblance to Clara and type II alveolar cells (28). In addition, IPO was demonstrated to inhibit the growth of non-small cell lung cancers implanted intrabronchially into athymic mice using a novel orthotopic xenograft model permitting cytotoxic testing in a milieu resembling the clinical setting (29, 30). Several lung cancer cell lines morphologically resembling small cell cancer are also capable of activating and covalently binding IPO (28). In addition, a diverse sampling of biopsied primary human lung carcinomas, as well as adjacent lung tissue from fresh surgical specimens, have been demonstrated to be capable of activating and binding IPO (31).

The decision to develop IPO as an anticancer drug was based on its unique mechanism of action, as well as its potential tissue specificity. These characteristics, as well as the steep dose-toxicity relationship of IPO, indicated that clinical trials should be performed cautiously, using such measures as stringent eligibility criteria, rigorous pulmonary monitoring, and a conservative dose escalation scheme. The purposes of this study were to: (a) determine the MTD of IPO given by a single, brief i.v. infusion repeated every 3 weeks; (b) recommend a dose for phase II trials; (c) characterize the toxicities associated with this schedule of administration; (d) seek preliminary evidence for antitumor activity; and (e) describe the pharmacology of IPO and determine whether it could be related to relevant clinical endpoints.

**MATERIALS AND METHODS**

**Eligibility.** Based on the lack of significant preclinical activity in extrapulmonary neoplasms, only patients with histologically documented advanced non-small and small cell lung cancers were candidates for this study. General eligibility criteria included ages >18 years; no major surgery within 14 days or weeks for those treated with a nitrosourea or mitomycin); adequate hematological (WBC count ≥ 4000/μl and platelet count ≥ 100,000/μl), hepatic (total bilirubin ≤ 1.2 mg/dl, and renal (creatinine ≤ 1.5 mg/dl) functions; and no other coexisting medical problems of sufficient severity to prevent full compliance with the study. Due to the potential pulmonary toxicity of IPO, eligibility was also restricted to patients with adequate pulmonary function as defined by: (a) a FEV₁ ≥ 1.5 liter; (b) a PO₂ ≥ 70 mm Hg; and (c) a DLCO/ALV ≥ 75% of the predicted value. All patients gave informed written consent.

**Dosage and Drug Administration.** The starting dose of IPO, 6.5 mg/m², was equivalent to 10% of the dose that was lethal in 10% of female mice. It was administered as a 30-min infusion every 3 weeks. Initially, doses were escalated using a modified Fibonacci search method to a dose of 104 mg/m². This was followed by 40% increments to 154, 216, 302, 422, 590, and 826 mg/m². Thereafter, a more conservative dosing scheme, in which doses were escalated in increments of 25%, was used due to the occurrence of pulmonary toxicity in one patient treated at the 826 mg/m² dose level. At least three IPO-naïve patients were treated at each escalated dose level. As the MTD was approached and potential dose-limiting toxicity was observed, at least six new patients were treated. The MTD was defined as one dose level below the dose that induced greater than NCI grade 3 toxicity in more than one-third of IPO-naïve patients. Initially, dose escalation was not permitted in the same patient. However, the protocol was subsequently amended due to the lack of toxicity in patients treated up to the 46 mg/m² dose level. Intrapatient dose escalation was then permitted if the patient received at least two courses at the lower dose level without toxicity and if two new patients had already been treated at the next highest dose. Dose escalation in the same patient proceeded until pulmonary toxicity was observed at 826 mg/m². Thereafter, intrapatient dose escalation was not permitted. The protocol permitted dose reductions by one to two dose levels for patients experiencing the following toxicities during a previous course: (a) NCI grade 2 pulmonary toxicity defined as moderate pulmonary symptoms; or (b) a decrease in FEV₁, FVC, PO₂, or DLCO/ALV of 25 to 50%. Dose modifications were not performed for other toxic effects.

IPO was supplied by the Division of Cancer Treatment, NCI (Bethesda, MD), in 2-ml vials containing a mixture of 10 mg IPO/ml in 0.9% sodium chloride injection, with sodium hydroxide added to adjust the pH to 6. IPO was initially reconstituted with 100 ml of 0.9% sodium chloride solution and then administered over 30 min, but IPO was administered undiluted at higher doses. The first dose was administered on the inpatient units of The Johns Hopkins Oncology Center, and all subsequent treatment was given in the clinic. Patients were treated at approximately 10 a.m. to avoid potential variability due to circadian fluctuations in microsomal P450 enzymes and tissue glutathione which are involved in IPO metabolic activation and detoxification, respectively. Medications, including steroids and H₂ antagonists, barbituates, and phenytoin that are known to modulate the activity of microsomal enzymes, were avoided.

**Pretreatment and Follow-up Studies.** Histories, physical examinations, and routine laboratory studies were performed prior to treatment and weekly during therapy. Routine laboratory studies included 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 (serum glutamic oxaloacetic transaminase), ALT (serum glutamic pyruvic transaminase), prothrombin time, and urinalysis. Liver function tests were also performed on days 2 and 4 after acute enzyme elevations were initially observed. In addition, pulmonary testing was performed at regular intervals including: (a) pulmonary function testing (spirometry, FVC, FÉV₁, lung volumes, vital capacity, alveolar volume, and single breath DLCO) prior to treatment and on days 2, 4, 8, 15, and 22 during course 1 and prior to treatment and on days 1, 2, 3, 4, 8, 15, and 22 during course 1 and weekly during subsequent courses; (b) arterial blood gases (pH, pO₂, and pCO₂) prior to treatment and on days 1, 2, 3, 4, 8, 15, and 22 during course 1 and weekly during subsequent courses; (c) chest radiographs prior to treatment and on days 1, 2, 3, 4, 8, 15, and 22 during course 1 and weekly during subsequent courses; and (d) LCT of the lungs prior to treatment and on day 2, 4, 8, 15, and 22 during course 1 and weekly during subsequent courses. LCT was obtained from a level just below the lung apex through the diaphragm at 15-mm intervals on either a Siemens Somatom Plus or DRH scanners (Siemens Medical Systems, Iselin, NJ); scan parameters were 1 s, 125 kVp, 250 mA, and 4-mm collimation or 4 s, 125 kVp, 250 mA, and 4-mm collimation, respectively. All scans were reconstructed with a high spatial frequency reconstruction algorithm to optimize definition of the lung parenchyma. The lung parenchymal density in Hounsfield units was recorded at each level. Formal tumor measurements were performed after every two courses, and patients were able to continue treatment if they did not develop progressive disease. A complete response was defined as complete disappearance of all
active disease and a partial response required a 50% or greater reduction in the sum of the product of the bidimensional measurements of all measurable lesions.

**Pharmacological Analyses.** Pharmacological studies were performed during the initial course of therapy in all patients. Blood samples in heparinized tubes were collected before the infusion, at 10 and 20 min during the infusion, and at the end of infusion. Samples were also collected at 1, 2, 5, 10, 20, and 30 min and 1, 2, 4, 6, 10, 24, and 48 h after the end of infusion. Urine was collected continuously for 48 h following drug administration. Plasma was separated by centrifugation and was stored at -20°C until analysis was performed. IPO is stable for at least 6 months under these conditions (data not shown).

IPO concentrations in plasma and urine were measured by gas chromatography using 1-undecanol as an internal standard. To each 1 ml plasma sample, 3 ml of benzene were added, along with 40 μl 1-undecanol 10 mmol/liter in methanol. After being vortexed for 30 s and centrifuged for 10 min, 2 ml of the benzene supernatant were removed and transferred to clean tubes. Next, 25 μl of heptfluorobutylimidazole were added to the supernatant, and the mixture was heated in a heating block at 50°C for 15 min. After 0.5 ml of water was added to neutralize the derivatizing agent, the mixture was mechanically shaken for 5 min, and 4 ml of 5% ammonium hydroxide were added. Forty μl of the sample were then diluted with 1 ml of benzene in an autosampler vial. A 1-μl sample was then injected into a Varian Model 3400 gas chromatograph (Varian, Palo Alto, CA) fitted with a Restek (Bellefonte, PA) RX-1 megabore 60 m (length) × 0.53 mm (inside diameter) × 1 μm (outside diameter) column. The GC was maintained at 90°C, while the column was maintained at 135°C for 7 min and increased at a rate of 25°C/min to 285°C which was held for 2 min. Nitrogen was the carrier gas at a flow rate of 30 ml/min and detection was accomplished by electron capture. The retention times of the fluorinated derivatives of IPO and the internal standard were 4.8 and 5.9 min, respectively. Peak areas were quantitated using a Nelson 3000 integration system (Perkin Elmer Nelson System, Cupertino, CA), and drug concentrations were determined from linear regression equations derived from calibration curves prepared with standards between 0.2 and 16 μmol/liter.

Individual plasma drug disposition curves were fit using a pharmacokinetic model characterized by two-compartment distribution of drug and first order elimination of drug from the central compartment. The values of the following kinetic parameters were estimated for each disposition curve: the disposition rate constants and associated half-lives, central volume of distribution, steady-state volume of distribution, AUC, and plasma clearance rate.

Pharmacokinetic modeling was performed by nonlinear regression analysis using PCNONLIN (Statistical Consultants, Lexington, KY). Correlations among kinetic parameter values and categorical toxicity data were calculated using Spearman’s rank-order correlation statistics.

**RESULTS**

Fifty-five patients received 254 total courses of IPO through 17 dose levels (Table 1). All courses were evaluable for toxicity. The median number of courses administered/patient was 3 and ranged from 1 to 18. Twenty-six patients received only 1 or 2 courses but 15 patients received more than 5 courses and 8 patients received more than 10 courses. The median cumulative dose of IPO was 826 mg/m² and ranged from 13 to 11,092 mg/m². Thirteen patients had IPO escalated due to minimal or no toxicity, including seven, two, one, two, and one patients who received IPO at two-, three-, four-, five-, and seven-dose levels, respectively. One patient was taken off study for severe, but reversible, pulmonary toxicity. Patient characteristics are listed in Table 2. Six patients had advanced small cell cancer that had progressed on or within 1 to 6 months of treatment with platinum-based therapy, and 49 had non-small cell cancer. No objective responses were observed; however, stable disease persisted for at least eight courses in 15 of the 55 patients (27%).

**Hepatotoxicity.** Hepatotoxicity was the dose-limiting toxicity of IPO on this schedule of administration. Hepatotoxicity was characterized by isolated elevations in the hepatocellular enzymes ALT and AST. Bilirubin and alkaline phosphatase levels remained normal even in courses associated with NCI grade 3 (5.1–20-fold above normal limits) and grade 4 (>20-fold above normal limits) hepatocellular enzyme elevations. Elevations in ALT were more pronounced than elevations in AST, with peak ALT levels generally greater than peak AST levels by 1.5–2-fold. The onset of hepatocellular toxicity was noted as early as day 2, and elevations in both AST and ALT were maximal on day 4. The enzyme abnormalities typically resolved by day 15, and values were rarely abnormal after day 22. Hepatotoxicity was not cumulative in that the magnitude of ALT and AST elevations were not generally more pronounced with successive courses of therapy.

Table 3 depicts the grade of hepatocellular toxicity as a function of IPO dose. Liver enzyme elevations were noted in only one patient during one course administered at IPO doses below 826 mg/m². However, reversible and asymptomatic elevations in hepatocellular enzymes were common at IPO doses ≥826 mg/m², with both the frequency and severity of IPO-induced hepatocellular enzyme elevations progressively increasing with higher drug doses. Grade 3 and 4 toxicities occurred in 3 of 7 courses involving 3 of 4 patients receiving 1230 mg/m² and in 2 courses involving a single patient treated at the 1612 mg/m² dose level. At IPO doses of 1230 and 1612 mg/m², right upper quadrant pain and/or moderate to severe malaise were associated with grades 3 and 4 hepatotoxicity during 4 of 5 courses involv-
Therefore, based on the lack of other hepatic and constitutional manifestations associated with hepatocellular toxicity at the 1032 mg/m² dose level, as well as the brief and noncumulative nature of IPO-induced hepatic dysfunction, 1032 mg/m² was determined to be the MTD for IPO on this schedule of administration.

**Pulmonary Toxicity.** Rigorous pulmonary monitoring by pulmonary function tests, arterial blood gases, and LCT revealed that IPO induced neither dose-dependent subclinical nor clinical pulmonary toxicity on this schedule of administration. The percentage of change in several spirometric (FVC and FEV₁) and gas exchange (DLCO and DLCO/ALV) parameters as a function of total cumulative IPO dose is depicted in Fig. 2, A-D. Although many patients developed cumulative decrements in these functions of at least 20% compared with baseline values, most decrements occurred in the setting of lung tumor progression and were attributed to progressive disease. In addition, decrements in these spirometric and gas exchange parameters were not related to cumulative dose. In fact, the magnitude of decrements in these parameters were similar among patients receiving cumulative doses which spanned four orders of magnitude (13 to 11,092 mg/m²).

Pulmonary toxicity was clearly documented in only one of 55 patients, a previously untreated 42-year-old male with adenocarcinoma of the lung. Neither clinical nor subclinical evidence of pulmonary toxicity were noted during his first three courses of IPO administered at doses of 202 and 422 mg/m². However, he developed moderate pleuritic chest pain and 15% decrements in FVC, FEV₁, and DLCO on day 22 of his fourth course at 422 mg/m². Additionally, despite a normal chest radiograph, CT scanning revealed a new pleural-based pulmonary paranchymal infiltrate which was contralateral to his primary neoplasm (Fig. 3, A-B). All symptoms, as well as pulmonary function and radiographic abnormalities, resolved by day 28, and the patient was subsequently treated with four additional courses of IPO uneventfully, including two courses each at 422 and 590 mg/m². However, 2 to 4 days after receiving a ninth course of IPO at 826 mg/m², he developed transient myalgias and progressive dyspnea. On day 8, hypoxia was noted (PO₂ = 50 mm Hg), and pulmonary function testing revealed 50% decrements in FEV₁, FVC, and DLCO. Diffuse pulmonary infiltrates were also evident on plain chest radiographs and on CT scan. His symptoms progressively worsened until day 13, at which time his PO₂ was 31 mm Hg, and panlobar infiltrates and bilateral effusions were noted on CT scanning (Fig. 3, C-D). Following an unremarkable bronchoscopic examination, transbronchial biopsy, and bronchoalveolar lavage, methylprednisolone, 125 mg i.v. every 6 h, was begun. Thereafter, his symptoms, as well as radiographic and spirometric abnormalities progressively improved, with complete resolution noted by day 35. IPO was not readministered.

**Renal Toxicity.** Possible renal toxicity occurred in a 48-year-old male with an adenocarcinoma of the lung and extensive pleural involvement. The patient developed gross, painless hematuria on days 16 and 22 following treatment with his first course of IPO at 154 mg/m². The hematuria lasted 24 to 48 h on both occasions. Urinalyses and microscopic examinations revealed proteinuria, glycosuria, and large numbers of RBC, RBC casts, WBC, and clumps of WBC. A urine protein electrophoresis revealed a glomerular pattern of proteinuria. Further studies included an abdominal CT scan which revealed normal kidneys and no evidence of intraabdominal disease; unremarkable bacterial, fungal and viral urinary cultures; and stable serum levels of creatinine and urea. All gross and microscopic abnor-

### Table 3 Hepatotoxicity

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<th>Dose (mg/m²)</th>
<th>Total courses</th>
<th>Hepatotoxicity Grade</th>
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<td>8</td>
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<tr>
<td>1612</td>
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<td>9</td>
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* Four courses in which hepatotoxicity was associated with reversible RUQ pain and severe malaise.

![Fig. 2. Changes in various pulmonary functions including FVC, FEV₁, DLCO, and DLCO/ALV as a function of the cumulative dose of IPO in all patients. There was no relationship between worsening of these parameters and cumulative IPO dose. Disease progression (P) accounted for the majority of episodes in which pulmonary function parameters decreased by at least 20% compared with baseline values. T, parameters of the single patient who developed severe pulmonary toxicity (see text).](image-url)
Fig. 3. CT lung scans of a 42-year-old male with metastatic adenocarcinoma of the lung. A. CT scan before course 4 of IPO administered at 422 mg/m². B. CT scan on day 22 of course 4 revealing a pleural-based infiltrate (arrows) associated with pleuritic chest pain and modest decrements in pulmonary function tests. C. CT before course 8 of IPO. D. CT on day 8 of course 8 revealing diffuse pulmonary infiltrate involving the left lung and a large pleural effusion. This was associated with substantial decrements in pulmonary function tests and severe hypoxia.

Malignancies resolved by day 35. The patient was not retreated with IPO due to tumor progression.

Miscellaneous Toxicities. Other toxicities, including nausea, vomiting, myalgias, malaise, and myelosuppression, were infrequent, clinically insignificant, and occurred at all dose levels. Modest leukopenia and thrombocytopenia were observed in two patients, including one minimally pretreated patient with nadir WBC counts of 3900/µl and 2900/µl, respectively, during her first and second courses of IPO at 422 mg/m² and a nadir platelet count of 81,000/µl during course 2. A second heavily pretreated patient developed mild leukopenia with a nadir WBC count of 3500/µl during his only course at 522 mg/m². Thirteen patients experienced mild to moderate nausea and/or vomiting. Nausea without vomiting occurred during 12 courses, while vomiting occurred during 10 courses. Nausea and vomiting were not dose related and generally occurred for 1 to 4 h immediately after drug administration. Symptoms were never protracted and antiemetics were rarely required. In addition, one patient complained of mild myalgias lasting 2 to 4 days after each of nine courses of IPO administered at doses of 202, 422, 590, and 826 mg/m². Two patients also complained of moderate malaise during two courses at 1290 mg/m² and severe malaise was experienced by one patient during two courses at 1612 mg/m².

Pharmacokinetics. Complete IPO plasma disposition curves were obtained on 44 subjects. Seven of the curves (doses 13 to 33 mg/m²) were generated using an early version of the IPO assay which did not yield values consistent with the final version of the assay. Those data were not included in the analysis. Individual plasma disposition curves are well described by a two-compartment, first order elimination model (Fig. 4). The mean kinetic parameter values obtained using that model are listed in Table 4. At the MTD, 1032 mg/m²/day, the mean half-lives, $\lambda_1$ and $\lambda_2$, are 6.7 and 114.5 min, respectively, and the plasma clearance rate is 1.16 liter/min/m².

While individual plasma disposition curves do not suggest the presence of nonlinear kinetics, inspection of the scatterplot of IPO dose versus AUC reveals a curvilinear relationship between the two (Fig. 5), suggesting the presence of saturable elimination kinetics. To explore that possibility, the average plasma concentration data of the subjects at three different dose levels (33, 826, and 1290 mg/m²) were simultaneously fit using a two-compartment pharmacokinetic model with elimination of drug treated as a Michaelis-Menten process. This model fit the disposition data well. In addition, the AUC generated by simulations using the model described the observed AUC versus dose relationship very well (Fig. 4). The parameter values for the model are: central volume of distribution, 27.5 liter/m²; $V_{max}$, 22.7 µmol/liter/min·m²; and $K_m$, 5.86 µmol/liter.
Urine IPO determinations in five subjects (doses 216 to 422 mg/m²) revealed minimal parent drug excretion equivalent to less than 2% of the administered dose. Subjects treated at higher doses (>590 mg/m²) exhibited an unidentified chromatographic peak with kinetic characteristics consistent with a metabolite (i.e., the peak was not present in predose plasma and increased, then decreased in magnitude roughly in parallel with the parent drug concentrations). Using IPO disposition curves as the input function and assuming one-compartment distribution and first order formation and elimination of the species, its pharmacokinetics were modeled. The mean elimination half-life for the species was determined to be 33 min (12 cases analyzed; range, 7–88 min). Structural identification of the metabolite is pending.

**Pharmacodynamics.** While liver toxicity was seen only at higher doses and larger AUC, hepatotoxicity grade did not correlate with either IPO dose or AUC in patients who manifested hepatotoxicity.

**DISCUSSION**

The original rationale (4) and subsequent impetus to develop IPO as an anticancer agent specific for lung cancer was based on the novel mechanism of action of the agent, as well as the potential of IPO as a unique tissue-specific cytotoxin (5–31). Additionally, the demonstration that both normal human lung tissue and lung cancers are capable of metabolically activating IPO in vitro and the documentation of pulmonary toxicity as the predominant toxicity of IPO in dogs and rodents in preclinical studies suggested that pulmonary toxicity would likely be the principal toxicity of IPO in humans. Similar to preclinical studies, toxicological effects in this study were observed in human lung, liver, and kidney, which presumably all contain the cytochrome P450 isoforms capable of metabolically activating IPO. However, hepatocellular toxicity, rather than pulmonary toxicity, was the principal toxicity in humans when the IPO was administered as a 30-min infusion every 3 weeks. At 1032 mg/m², the MTD and recommended dose for subsequent phase studies of IPO on this administration schedule, hepatic toxicity was characterized by isolated elevations in hepatocellular enzymes, particularly ALT. Although NCI grade 3 toxicity was noted in 5 of 26 courses and 3 of 7 patients at 1032 mg/m², an incidence which exceeded the criteria initially established for the MTD, liver enzyme abnormalities at this dose resolved completely within 1 to 2 weeks, and were neither cumulative nor associated with other toxic sequelae, such as severe malaise and right upper quadrant pain. These other toxic manifestations were commonly associated with hepatocellular enzyme elevations in patients receiving higher doses of IPO.

The explanation for the difference in target-organ-specific toxicity in humans versus other species is not known. While the lack of terminal airways containing Clara and type II alveolar cells or any other cells capable of metabolically activating IPO in situ has adequately explained the exclusive occurrence of hepatotoxicity, rather than pulmonary toxicity, in birds (23, 24), all mammalian lungs studied to date, including human lungs (31), possess these specialized cells. In addition to pulmonary toxicity, hepatic binding and occasional necrosis has been noted in hamsters (14) but liver toxicity has largely been subclinical in this species, and birds are still the only nonhuman species known in which hepatotoxicity predominates (23, 24). Although there may be substantial interspecies differences in the pharmacological behavior of IPO that may partially explain the widely disparate MTDs between species, significantly greater and not less pulmonary toxicity would be predicted in humans if the relative susceptibility of various species to lung toxicity were solely due to quantitative differences in pharmacological exposure to IPO. Actually, the mean AUC achieved in mice at the murine dose that was lethal in 10% of mice and in dogs at the highest safe dose during preclinical studies were 144 μg/ml/min (860 mmol/liter/min) and 55.4 μg/ml/min (330 mmol/liter/min), respectively, which are equivalent to only 13.4 and 5.1% of the mean AUC (6531 mmol/liter/min) achieved at the MTD (1032 mg/m²) in humans (32). It is possible that inherent interspecies differences in the IPO activating potential and/or protective mechanisms of lung tissue account for the different susceptibilities of human, dogs, and rodents to pulmonary toxicity. This may not be the case for hepatotoxicity since mild and subclinical elevations of hepatocellular enzymes were observed in dogs and rodents treated at IPO doses associated with severe pulmonary toxicity, and therefore, a comparable degree of drug-induced hepatic dysfunction might be anticipated to occur in humans, dogs, and rodents at similar IPO AUC if dosing in the lower mammals were not limited by severe pulmonary toxicity.

This study demonstrated that IPO administered as a 30-min infusion did not induce as a prominent feature either dose-related acute or cumulative pulmonary toxicity, as assessed by sequential monitoring of radiographic, functional, and clinical indices. However, severe pulmonary toxicity clearly occurred in one of 55 patients after the patient received several courses of IPO at doses ranging from 202 to 826 mg/m². Except for the development of transient pleural-based infiltrates on CT during a previous course, which were also associated with mild pleuritic chest pain and modest decrements in pulmonary function tests, no other historical or clinical characteristics were identified as possibly predisposing the patient to the subsequent episode of severe pulmonary toxicity. In addition, the pharmacokinetic profile of the patient did not significantly differ from that of the other patients. Even if the true incidence of IPO-induced pulmonary toxicity in humans is very low, as suggested by this study, the sporadic nature and severity of the toxicity may have several pertinent implications with respect to the design of subsequent clinical trials. First, the potential severity of the toxicity mandates that the eligibility criteria used during subsequent studies be somewhat restrictive to patients with significant pulmonary dysfunction who may not be able to tolerate further respiratory compromise. Second, it suggests that a combination of clinical, radiographic, and functional pulmonary monitoring should be incorporated into these investigations. The assessment of multiple pulmonary parameters (e.g., LCT, pulmonary function tests, physical examinations) should be continued during the next phase of
discrete necrosis in the proximal cortical tubules with sparing of the
nephrotic syndrome sufficiently different from that of other subjects treated at
higher IPO doses. In preclinical studies, pathological evidence of nephrotoxicity, such as striking and
disposable disease. In 27% of the 55 total lung cancer patients studied, no objective
antitumor responses were observed. Additionally, the eligibility re-
quirements for the study were uniquely restrictive, thereby limiting
accrual to patients expected to possess biologically favorable character-
istics, and hence, a longer survival. All patients had a good to
excellent initial performance status, and the group included 33 and 17
individuals with no prior chemotherapy and radiotherapy, respec-
tively. In essence, the large number of patients with non-small cell
lung cancer and an ideal performance status may inadvertently permit
a rough assessment of drug activity in non-small cell lung cancer. This
trial, albeit phase I in design, revealed little evidence of activity of IPO
on this particular schedule in non-small cell lung cancer. No objective
responses occurred in 12 patients with non-small cell lung cancer treated with IPO doses ≥1032 mg/m²; the recommended phase II dose,
nor in 15 patients with non-small cell lung cancer treated with IPO
doses ranging from 826 mg/m² (1 dose level below the MTD) to 1612
mg/m² (2 dose levels above the MTD).

The preponderance of clinical toxicity observed in the present study
in liver rather than lung suggests that, in vivo, IPO may be preferen-
tially activated and bound in liver rather than in lung or other tissues
in humans. However, as with the lungs of all other species except
avoins, human lung clearly has very substantial enzymatic IPO acti-
ving potential (31). Therefore, one alternative explanation for the
lack of lung toxicity in this study could be that human lung tissue is
relatively more effective at detoxifying IPO than is the liver in humans
or the lungs in other species. For example, it may be relevant that
any compromise or enhancement of this pathway may sen-
itize or densensitize, respectively, the lung tissue susceptibility to
IPO. Also, a more specific histological and/or biochemical subtyping
or selection of patients as to lung cancers that might be most likely to
respond to IPO was not attempted in this phase I study. However, such
subtyping is feasible using surgical specimens from individual pa-
tients (31) and may be a consideration for the design of future efficacy
studies.

Irrespective of the above considerations concerning possible factors
regulating the specificity of toxicity of IPO to normal tissues of
humans compared with other species, the relevance to any potential in
vivo cytotoxic activity or selectivity (or modulation thereof) of IPO
against tumors originating from lungs or other tissues is unknown.
However, it remains that many human lung cancers apparently do
possess at least one fundamental attribute necessary for susceptibility;
a diverse group of fresh human lung tumor biopsies were found to
have very substantial capacity to metabolically activate IPO, and
indeed some tumors were even more metabolically competent than
adjacent lung tissue from the same patients (31). Interestingly, a more

Table 4 Mean pharmacokinetic parameter values

<table>
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<tr>
<th>Model</th>
<th>Dose (mg/m²)</th>
<th>No. of patients</th>
<th>Vₐ₀ (liters/m²)</th>
<th>Vₘₜₙ (liters/m²)</th>
<th>t₁/₂λ₁ (min)</th>
<th>t₁/₂λ₂ (min)</th>
<th>Clearance (liters/min/m²)</th>
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Fig. 5. Relationship between IPO AUC and dose. •, individual subject data. Contin-
uous line, relationship predicted by a two-compartment model of IPO pharmacokinetics
with Michaelis-Menten type elimination. The Michaelis-Menten parameter values yielding
the fit shown are: Vₘₜₙ, 22.7 μmol/min·m²; and Kₘₜₙ, 5.86 μmol/liter.

devolutional clinical trials until adequate data concerning pulmo-
ary toxicity are collected and a scheme for optimal clinical monitor-
ing can be developed. Finally, the occurrence of a severe episode
during a latter course also suggests that pulmonary monitoring and
close patient follow-up continue beyond the initial one to two courses
of treatment.

Nephrotoxicity also occurred in only one patient who was treated at
a relatively low IPO dose. As with the patient who developed cli-
cally significant pulmonary toxicity, this patient did not have any
identifiable risk factors for nephrotoxicity, nor was his pharmaco-
ik profile sufficiently different from that of other subjects treated at
identical doses. Glomerular proteinuria, RBC, WBC, and WBC
clumps in the urine suggested a primary renal process, possibly af-
flecting the glomeruli, tubules, and/or interstitium. In preclinical stud-
ies, pathological evidence of nephrotoxicity, such as striking and
discrete necrosis in the proximal cortical tubules with sparing of the
adjacent tubules, glomeruli, and blood vessels, were limited to adult
male mice and could not be induced in either adult female or immature
mice (14, 25). Although nephrotoxicity was self limited and largely
inconsequential in this patient, tumor progression precluded retreat-
ment, and therefore, information concerning the effects of subsequent
therapy in patients who develop IPO-induced nephrotoxicity is not
available.

Although stable disease persisted for at least eight treatment courses in
27% of the 55 total lung cancer patients studied, no objective
antitumor responses were observed. Additionally, the eligibility re-
quirements for the study were uniquely restrictive, thereby limiting
recent study of fresh surgical specimens from diverse human lung cancers also revealed that such tumors contained glutathione levels at least equal to or exceeding adjacent normal pulmonary tissue (36).

Finally, the observations reported herein support the view that the rationale for clinical evaluation of IPO in humans should be extended at least equal to or exceeding adjacent normal pulmonary tissue (36).

PHASE I AND PHARMACOLOGICAL STUDY OF 4-IPOMEANOL

ACKNOWLEDGMENTS

The authors wish to thank Mary Duerr for assistance with data collection; Lisa Hurowitz, Chris Baba, and Tian-Ling Chen for technical assistance with pharmacological assays; and the referring medical staff and medical and nursing staffs of the Johns Hopkins Oncology Center for the care of the patients in this study.

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Phase I and Pharmacological Study of the Pulmonary Cytotoxin 4-Ipomeanol on a Single Dose Schedule in Lung Cancer Patients: Hepatotoxicity Is Dose Limiting in Humans

Eric K. Rowinsky, Dennis A. Noe, David S. Ettinger, et al.


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