A Phase I Study of Swainsonine in Patients with Advanced Malignancies

Paul E. Goss, Jose Baptiste, Berny Fernandes, Michael Baker, and James W. Dennis

ABSTRACT

Swainsonine, an α-mannosidase inhibitor which blocks Golgi oligosaccharide processing, represents a new class of compounds that inhibit both rate of tumor growth, and metastasis, in murine experimental tumor models. In this first phase I study, the quantitative and qualitative toxicities of swainsonine have been studied in patients given a continuous i.v. infusion over 5 days, repeated at 28-day intervals. Dose levels were escalated in increments of 100 µg/kg/day from 50–550 µg/kg/day. Nineteen patients with both solid tumor and hematological malignancies were given a total of 31 courses. Hepatotoxicity, particularly in patients with liver metastases, was the dose-limiting toxicity. The maximum tolerated dose (MTD) and the recommended starting dose (MTD –1 level) were 550 and 450 µg/kg/day, respectively. Common side effects included edema, mild liver dysfunction, a rise in serum amylase, and decreased serum retinol. Acute respiratory distress syndrome possibly precipitated by swainsonine resulted in a treatment-related death in a patient with significant pretreatment hepatic dysfunction. One patient with head and neck cancer showed >50% shrinkage of tumor mass for 6 weeks after treatment. Two patients with lymphangitis carcinomatosa on chest X-ray noted improvement in cough and shortness of breath during the infusion of swainsonine and for 1 week thereafter.

Clearance and serum half-life for swainsonine were determined to be approximately 2 ml/h/kg, and 0.5 day, respectively. Golgi oligosaccharide processing, a putative anticancer target for swainsonine was inhibited in peripheral blood lymphocytes as evidenced by a marked decrease in leuкоagglutinin binding after 5 days of treatment. Oligomannosides in patient urine increased 5- to 10-fold over the 5 days of treatment, indicating that tissue lysosomal α-mannosidases were also blocked by swainsonine. Urine oligomannoside accumulation reached steady state at 3 days, approximately 1 day after serum drug levels reached steady state. The fraction of HLA-DR-positive cells in peripheral blood lymphocytes increased following 5 days of swainsonine treatment, an effect similar to that observed for peripheral blood lymphocytes from normal subjects cultured with swainsonine. No significant changes in CD3, CD4, CD8, CD16, and CD25 were observed. Swainsonine produces minimal toxicity when administered i.v. to cancer patients at dosages that inhibit both Golgi α-mannosidase II and lysosomal α-mannosidases. Detection of hepatic metastases or liver enzyme abnormalities prior to treatment predict for more significant toxicity.

INTRODUCTION

Malignant transformation in rodent and human tumors is associated with increased branching at the trimannosyl core of complex-type asparagine (N)-linked oligosaccharides (1–3). We have shown that the degree of β1–6 branching in rodent tumor models (3, 4) and in human breast and colon carcinomas (5, 6) correlates with disease progression. More importantly, we have demonstrated that somatic mutations which inhibit oligosaccharide processing in the Golgi dramatically inhibit metastases and reduce solid tumor growth in mice (4, 7, 8).

Furthermore, inhibitors of N-linked oligosaccharide processing such as castanospermine and swainsonine have been shown to attenuate both metastasis and tumor growth in animal models (8–11).

Swainsonine is an indolizidine alkaloid found in Australian Swainsona canescens (12), North American plants of the genera Astragalus and Oxytropis (13), and also in the fungus Rhizoctonia leguminicola (14). The alkaloid is a potent inhibitor of the Golgi enzyme α-mannosidase II (15), an enzyme required for maturation of N-linked oligosaccharides on newly synthesized glycoproteins. Swainsonine also blocks lysosomal α-mannosidases causing the accumulation of oligomannoside chains in cells exposed to the drug (16). T24Hu-ras-transfected NIH 3T3 cells lose their ability to grow in an anchorage-independent manner when cultured in the presence of swainsonine (17), suggesting induction of a more differentiated phenotype. The autocrine-dependent growth rate of murine and human tumor cell lines in vitro is reduced by swainsonine (18, 19). Swainsonine-treated MDAY-D2 lymphoma tumor cells show reduced adhesion to endothe- thelial cells in vitro and in vivo (20), and swainsonine blocks invasion of murine and human tumor cells through extracellular matrix while enhancing cell adhesion to extracellular matrix proteins in vitro (21, 22).

In animals, swainsonine exhibits antitumor activity when given p.o. (8, 23, 24), by i.p. injection (25), and by systemic infusion (18). For example, swainsonine administered to athymic nude mice in drinking water inhibited the growth rate of human colorectal carcinoma and MeWo melanoma xenografts by 50% (18, 19). The growth of murine Sarcoma 180 ascites tumors was completely inhibited by 30 and 100 mg/kg/day administered p.o. for 5 consecutive days after tumor cell inoculation (25). Swainsonine p.o. at 10 µg/ml in the drinking water of athymic nude mice showed additive antitumor activity with α interferon, such that human HT29m colon carcinoma xenografts grew at one-tenth the rate of those in untreated mice (18).

The action of swainsonine as an immune modulator has been re- viewed (23, 26). When given p.o. to mice, the alkaloid alleviates chemically and tumor-induced immune suppression (25, 27, 28), stimulates lymphocyte proliferation (29) and NK cell activity (23), and activates macrophages (30, 31). The drug also stimulates LAK cell activity when added to cultures of human lymphoid cells (32).

Preclinical toxicology studies in rodents showed swainsonine to be well tolerated (18, 33, 34). No mortality occurred in young rats given swainsonine at doses up to 46 mg/kg/day by miniosmotic pump for periods of 3 weeks (34). The drug suppressed the growth rate of young rats apparently by suppression of appetite, and caused lysosomal storage of oligosaccharides in tissues including the central nervous system. However, no evidence of neurotoxicity was noted in this or other preclinical studies on rodents treated with swainsonine (19, 34).

In this first phase I study, cancer patients were given courses of continuous 5-day i.v. infusions of swainsonine in escalating doses from 50–550 µg/kg/day repeated every 28 days. The starting dose level of 50 µg/kg/day was an estimate of the p.o. dosage which had antitumor activity in mice (18, 19, 23). The schedule of a 5-day...
infusion given every 28 days was based primarily on the antitumor activity noted in mice given a 24-h exposure to swainsonine in an uninterrupted twice weekly schedule (24). The limitations of administering an in-hospital 5-day infusion to patients with advanced malignancies more often than once monthly were also taken into consideration. An in-hospital 5-day infusion to patients with advanced malignancies more often than once monthly were also taken into consideration. The data on toxicity, serum drug levels, Golgi and lysosomal α-mannosidase inhibition, and lymphocyte marker changes are included in this report.

PATIENTS AND METHODS

Patients. Nineteen patients were enrolled in the study between January 1992 and June 1993. The study was approved by the University of Toronto and the Toronto Hospital ethics committee. Patients aged 18–75 years with a life expectancy of at least 3 months, Eastern Cooperative Oncology Group performance status 0–3, and histologically confirmed diagnosis of metastatic carcinoma, leukemia, or lymphoma were eligible for enrollment on study. No recognized alternative therapeutic options were available for study patients. Exclusion criteria included significant cardiac (congestive heart failure or refractory edema), hepatic (bilirubin >34 µmol/liter or AST > 2× normal), renal (creatinine >175 µmol/liter), or neurological impairment. Screening prior to enrollment on study included: signed informed consent; history and physical and assessment of Eastern Cooperative Oncology Group performance status; chest X-ray, routine hematology, biochemistry, urinalysis and electrocardiogram; arterial blood gas determination and resting respiratory function testing were included in the pretreatment assessment after edema was noted in 2 of 3 patients treated at level 1. Base-line symptoms were recorded.

All patients were examined twice daily, including a neurological assessment, and all screening blood work was repeated daily. Arterial blood gas determination, respiratory function testing, and chest X-ray were repeated once between days 2 and 4 during the 5-day infusion. Patients were seen as outpatients 3 weeks after each course of swainsonine for a full assessment and decision regarding their next course of treatment.

Swainsonine Administration. Swainsonine was synthesized by Toronto Research Chemicals and purified by recrystallization to constant melting point, and purity was confirmed by proton magnetic resonance spectroscopic analysis to be greater than 98%.

Each course of swainsonine was administered to patients by pump as a continuous infusion via a peripheral vein over 5 days and repeated at 28-day intervals. The daily dose was prepared immediately prior to use in one liter of 0.9% saline solution and the bag was protected from light during the infusion. Blood for laboratory testing was taken from a heparinized indwelling catheter in the opposite arm. The dose levels were 50, 150, 250, 350, 450, and 550 µg/kg/day.

Dose Escalation. Three patients were treated at each dose level. Dose escalation occurred until dose limiting toxicity (MTD) was reached. MTD was defined as <3 of 3 patients experiencing grade 3, and 0 of 3 patients grade 4 nonhematological or hematological toxicity according to standard National Cancer Institute of Canada toxicity criteria after one course of treatment. The exception was grade 4 edema in patients with preexisting edema which was not considered a defining criterion for MTD. Intrapatient dose escalation was not allowed. In the event of MTD being reached, 3-further patients were to be treated at 1 level lower until no more than 2 of 6 patients experienced grade 3 or greater toxicity. This level would define the recommended starting dose for future clinical testing.

Serum Drug Levels. The method for extraction, acetylation, and measurement of serum swainsonine is described in detail elsewhere (35). Briefly, the internal standards methyl α-α-mannopyranoside and methyl β-D-galactopyranoside were prepared in H2O at 100 µg/ml, and 45 µl of each standard were added to 1.5 ml of serum in Kimax tubes (13 mm x 16 cm). Two ml of cold chloroform were added, samples were gently mixed to avoid foaming, followed by centrifugation at 2500 rpm for 10 min at 4°C. The supernatant was quantitatively transferred into clean Kimax tubes (13 mm x 16 cm) by using a Pasteur pipette, followed by the addition of 2 ml of cold acetone and vigorous mixing by vortex. The mixture was centrifuged at 2500 rpm, the supernatant was saved, and the protein pellet was washed twice with 2 ml of cold acetone. The combined supernatants were evaporated under a stream of nitrogen at 60°C. The residue was further dried in a desiccator over P2O5 at room temperature for 12 h.

To the dry samples were added 1 ml of dry acetonitrile, 15 µg of 4-dimethylaminopyridine, and 15 mg of sodium acetate. The samples were cooled to 0°C and 0.5 ml of acetic anhydride was added and left overnight at room temperature. The acetylated reaction products were then partitioned between 1 ml of chloroform and 1 ml of distilled water with gentle shaking of the samples. Polar contaminants dissolve in the aqueous layer, whereas the acetylated saccharides and swainsonine dissolve in the lower chloroform layer. The chloroform layer was washed with 1 ml of water, and traces of water and acetic anhydride were removed by passing the samples over 500-mg cartridges of basic alumina (Alltech Inc.). The solvent was evaporated at 60°C under a stream of nitrogen, the residue was dissolved in 30 µl of chloroform and injected in 1-µl aliquots into the gas-liquid chromatogram.

Gas-liquid chromatographic analysis of each derivatized sample was done by using a Model 3400 Varian Gas Chromatograph equipped with flame ionization detector and wax coated open tubular fused capillary column (15 m x 0.53 mm inside diameter; Megabore) coated with 1.2-µm thin layer of DB225. The column temperature was kept at 180°C for 22 min after the injection, then raised from 180 to 230°C at 1°C/min; the latter temperature was maintained for 30 min. The injector port temperature was 240°C, and the detector block was 280°C. Samples of 1 µl were injected by direct flash vaporization, and prepurified dry helium (Canox) was used as the carrier gas at a flow rate of 6 ml/min. Quantitative analyses were done by using a Model 3396B Hewlett-Packard integrator. Recovery of swainsonine was 90% and the standard deviation for reproducibility on 10 replicate samples was 5%.

Urine Oligomannosides. FACE from GLYKO was used for determining swainsonine-induced oligosaccharide storage. The reducing end of saccharides are subject to reductive amination with 7-aminonaphthale-1,3-disulfonic acid (36). Five-µl samples of urine were dried, and to each tube were added 5 µl of 0.2 w/v 7-aminonaphthale-1,3-disulfonic acid in 2.6 w/v acetic acid and 5 µl of NaCNBH3 in 1 w/v dimethyl sulphoxide, followed by incubation at 37°C for 2 h. After drying, samples were redissolved in 62.5 µl Tris-HCl, pH 6.8, containing 20% glycerol, and were applied to 30% polyacrylamide gels, precast by GLYKO. Gels were run at 15 mA constant current and cooled to 4°C for 2 h. The separated oligosaccharides were imaged with a high-sensitivity digital camera and computer system. This method allows less than 1 pmol of oligosaccharide to be detected and quantified by comparing test samples to standards run in parallel.

FACS Analysis of PBL. PBL were prepared and analyzed for CD3, CD4, CD8, CD14, CD16, CD25, CD57, and HLA-DR by FACS. Peripheral blood was collected in heparin-containing tubes, transported at room temperature, and analyzed within 48 h. Mononuclear antibodies conjugated with either FITC or phycoerythrin were purchased from Becton-Dickinson; FITC-labeled L-PHA was from E.Y. Labs. Aliquots of 100 µl of whole blood were incubated with anti-CD antibodies or 0.1 µg/ml of FITC-labeled L-PHA for 20 min followed by lysis of RBC, washing by centrifugation, and fixation of the cells by using the automated Q Prep system (Coulter). The lymphocyte population was gated, and the contaminating monocytes were assessed by staining with CD14 which was consistently below 1% of the gated population.

Cytokine Measurements. IL-6 and TNF-α levels in patient serum were measured by using enzyme-linked immunosorbent assay kits (R&D). The cytokine enzyme-linked immunosorbent assay test kits is a solid-phase enzyme immunoassay using capture and detection antibodies, and was used as described by the manufacturer.

Serum Retinol Measurements. Serum retinol levels were measured as previously described (37). Briefly, 1-ml serum samples were spiked with retinyl acetate and extracted with 0.4 ml of equal volumes of butanol/acetone, then 0.3 ml of 1.2 kg/liter of K,HPO4 was added, mixed, and samples were centrifuged at 8700 X g for 2 min. Retinolins were separated by injecting 80 µl of supernatant onto a 25- x 4.6-mm ODS-2 high-performance liquid chromatin column (Millipore-Whatman) and run in a mobile phase of acetic acid/water/acetone (0.5/20.0/79.5) at 3.0 ml/min. Retinol and retinyl acetate elute at 8 and 14 min, respectively, and were quantified by absorbance at 324 nm. Between-run error was estimated for the internal standard. The standard deviation about the mean was 2.2% (n = 5), and similar variation was observed for repeat runs of unknown serum samples.

Downloaded from cancerres.aacjrournals.org on April 14, 2017. © 1994 American Association for Cancer Research.
RESULTS

Treatment-related Effects and Patient Outcome. The characteristics of the patients treated at 6 drug levels (50–550 μg/kg/day) are summarized in Table 1. The major side effects observed with swainsonine treatment were edema and liver and pancreas dysfunction (Table 2). All patients showed treatment-related elevation of serum AST, while marginal increases in ALT were observed. Patients with normal serum AST levels on beginning treatment showed a rise of up to 4-fold during the 5 days of swainsonine infusion. The patients with most notable swainsonine-induced increases in AST/ALT were those who had elevated pretreatment levels. All but 1 of these patients (6 of 7) had known involvement of liver with malignancy. MDT (550 μg/kg/day) was reached in patient B. P. who developed grade 4 elevation of bilirubin. This patient had significant involvement of her liver with metastatic colon cancer and elevated transaminases and bilirubin prior to receiving swainsonine. Five of 6 patients treated at MTD –1 developed < grade 3 toxicity which defined 450 μg/kg/day as the recommended starting dose for future clinical testing.

A transient and asymptomatic rise in serum amylase was observed in 8 patients who began treatment with normal levels, and in 2 patients who started with elevated levels; 9 patients showed no change. These changes in serum enzyme levels occurred at all doses with no apparent increase in severity over the dose range used in the trial.

Edema was observed in 11 of 19 patients, and those patients who had multiple rounds of treatment showed reproducible levels of edema (Table 2). Of the 2 patients (VM/150 and VG/350) who developed gross (4+) edema, both had pretreatment edema (2+, 1+, respectively). Edema was not consistently associated with severity of liver dysfunction, although all but 1 patient with known liver involvement with disease developed edema. Serum IL-6 and TNF-α levels were measured in 10 patients. The two patients with 4+ edema on treatment (VG/350 and VM/150), showed significant elevation of IL-6, 38- and 8-fold, respectively (Table 2). The mean IL-6 levels in patients before treatment was 12.0 ±9.7 pg/ml (n = 10) and after 120 h of swainsonine treatment were observed to increase rapidly during the first 8–12 h of drug infusion, and approach steady state (C_{ss}) at 48–72 h. The serum C_{ss} values ranged from 100 to 400 times greater than the K_{f} for inhibition of Golgi α-mannosidase II (i.e., 20–30 ng/ml). Serum drug levels were measured in VM/150 and VG/350 at intervals after discontinuing swainsonine infusion and indicated a serum half-life of approximately 14 h (data not shown), consistent with the time required to reach C_{ss} (i.e., approximately 4 half-lives) (38). The C_{ss} values for 16 courses of treatment plotted as a function of dosage in Fig. 2B shows a linear relationship but the line does not intersect the origin. The apparent nonlinear pharmacokinetics at doses of drug below 50 μg/kg/day suggests two serum compartments; one with slow clearance (i.e., 0.5 ml/h/kg), and the other with a more rapid clearance rate of 3.8 ml/h/kg. The mechanisms underlying the two clearance rates for swainsonine may be due to a swainsonine-binding molecule in serum which is saturated above 50 μg/kg/day; and/or drug-induced activation of a clearance mechanism which enhances the clearance rate at higher dosages (38). The latter may be explained for example by conjugation of swainsonine, and will require further analysis of both swainsonine and its metabolites to address this question.

Inhibition of Golgi Oligosaccharide Processing α-Mannosidase. L-PHA lectin shows binding specificity for β1-6GlcNAc-branched complex-type oligosaccharides (39) and can be used to measure the activity of swainsonine as an inhibitor of Golgi oligosaccharide processing (i.e., α-mannosidase II). Glycoproteins synthesized in PBL during swainsonine treatment would be expected to bear hybrid-type oligosaccharides rather than the L-PHA-reactive complex-type structures. Five days of swainsonine treatment reduced expression of L-PHA-reactive complex-type oligosaccharides on the surface of PBL lymphocytes by 2- to 7-fold based on mean fluorescent intensity (Fig. 3). A reciprocal increase in Con A binding to PBL was observed consistent with the conversion of complex- to hybrid-type structures (data not shown). The FACS analysis showed decreased L-PHA reactivity in the total PBL population rather than evidence for multiple cell populations, suggesting that a significant fraction of surface glycoproteins on circulating PBL were replaced during the 5 days of drug treatment. As a control, MDAY-D2 murine lymphoma cells were cultured in 1 μg/ml of swainsonine for 48 h, to effect maximal swainsonine-induced inhibition of processing (8), and these conditions resulted in a 9-fold decrease in L-PHA binding to the cells (Fig.3).

Urinary Oligomannosides. In addition to Golgi α-mannosidase II, swainsonine also inhibits lysosomal α-mannosidases, and causes the accumulation of oligomannosides in tissues and body fluids in animals (15, 40). FACE was used to quantify the reducing oligosaccharides in patient urine (Fig. 4). Urine oligosaccharides migrating in the FACE gels in the region of Glc3 to Glc5 standards, accumulated rapidly over the first 72 h on swainsonine, and appeared to reach steady state by 72 h (Fig. 5). Since serum swainsonine C_{ss} is attained in 48 h, and with
Table 2 Summary of patient treatment, disease, edema, and changes in serum markers

<table>
<thead>
<tr>
<th>Patient /dose</th>
<th>Disease</th>
<th>Course</th>
<th>Edema (grade)</th>
<th>AST &lt;45</th>
<th>ALT &lt;40</th>
<th>Bilirubin &lt;20</th>
<th>Amylase &lt;115</th>
<th>Grade</th>
<th>IL-6</th>
<th>RA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TK/50</td>
<td>Breast</td>
<td>1</td>
<td>2</td>
<td>44</td>
<td>19</td>
<td>10</td>
<td>56</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>48</td>
<td>5</td>
<td>9</td>
<td>56</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td>83b</td>
<td>34</td>
<td>15</td>
<td>14</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>LG/50</td>
<td>Leukemia</td>
<td>1</td>
<td>0</td>
<td>90</td>
<td>61</td>
<td>10</td>
<td>239</td>
<td>(2,1,0,2)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>36</td>
<td>31</td>
<td>109</td>
<td>(1,0,5,0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>WH/50</td>
<td>Lymphomas*</td>
<td>1</td>
<td>2</td>
<td>90b</td>
<td>31</td>
<td>14</td>
<td>96</td>
<td>(2,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2</td>
<td>67b</td>
<td>26</td>
<td>15</td>
<td>103</td>
<td>(1,0,0,0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>VM/150</td>
<td>Breast</td>
<td>1</td>
<td>4b</td>
<td>239b</td>
<td>136</td>
<td>15</td>
<td>56</td>
<td>(3,2,0,0)</td>
<td>8X</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>72</td>
<td>31</td>
<td>20</td>
<td>225</td>
<td>(1,0,1,2)</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td>EG/150</td>
<td>Colon</td>
<td>1</td>
<td>0</td>
<td>171b</td>
<td>33</td>
<td>22</td>
<td>97</td>
<td>(2,0,1,0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
<td>180b</td>
<td>37</td>
<td>20</td>
<td>88</td>
<td>(3,0,1,0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LS/250</td>
<td>Kidney</td>
<td>1</td>
<td>0</td>
<td>49</td>
<td>8</td>
<td>9</td>
<td>142</td>
<td>(1,0,0,1)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>69</td>
<td>12</td>
<td>7</td>
<td>119</td>
<td>(1,0,0,1)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>AW/250</td>
<td>Breast</td>
<td>1</td>
<td>3</td>
<td>53</td>
<td>18</td>
<td>9</td>
<td>96</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>3</td>
<td>112b</td>
<td>14</td>
<td>11</td>
<td>171</td>
<td>(2,0,0,1)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>GB/350</td>
<td>Breast</td>
<td>1</td>
<td>0</td>
<td>39</td>
<td>9</td>
<td>18</td>
<td>84</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>44</td>
<td>14</td>
<td>21</td>
<td>(1,0,1,-)</td>
<td>NC</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>MJ/350</td>
<td>Lung</td>
<td>1</td>
<td>1</td>
<td>127b</td>
<td>17</td>
<td>15</td>
<td>165</td>
<td>(2,0,0,1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1</td>
<td>115b</td>
<td>26</td>
<td>19</td>
<td>111</td>
<td>(2,0,0,0)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>VG/350</td>
<td>Lung*</td>
<td>1</td>
<td>4b</td>
<td>995b</td>
<td>263</td>
<td>35*</td>
<td>97</td>
<td>(4,3,3,0)</td>
<td>38X</td>
<td>—</td>
</tr>
<tr>
<td>CV/450</td>
<td>Lymphomas*</td>
<td>1</td>
<td>1</td>
<td>123b</td>
<td>67</td>
<td>29</td>
<td>142*</td>
<td>(2,1,1,1)</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td>WL/450</td>
<td>Pancreas</td>
<td>1</td>
<td>0</td>
<td>59</td>
<td>17</td>
<td>12</td>
<td>226*</td>
<td>(1,0,0,2)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>RC/450</td>
<td>Pancreas</td>
<td>1</td>
<td>1</td>
<td>62b</td>
<td>44</td>
<td>17</td>
<td>45</td>
<td>(1,1,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>RB/450</td>
<td>Colon</td>
<td>1</td>
<td>2</td>
<td>60</td>
<td>15</td>
<td>10</td>
<td>77</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>AL/450</td>
<td>Head/neck</td>
<td>1</td>
<td>0</td>
<td>42</td>
<td>24</td>
<td>9</td>
<td>39</td>
<td>(1,0,0,0)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td>MS/450</td>
<td>Head/neck</td>
<td>1</td>
<td>0</td>
<td>63</td>
<td>12</td>
<td>11</td>
<td>265</td>
<td>(1,0,0,3)</td>
<td>NC</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>0</td>
<td>63</td>
<td>17</td>
<td>11</td>
<td>(1,0,0,-)</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>BP/550</td>
<td>Colon</td>
<td>1</td>
<td>1</td>
<td>316b</td>
<td>54</td>
<td>67*</td>
<td>155</td>
<td>(3,1,4,1)</td>
<td>—</td>
<td>↓</td>
</tr>
</tbody>
</table>

Upper limit for normals is indicated directly under the headings for AST, ALT (units/liter), bilirubin (μmol/liter), and amylase (units/liter). The values are those measured on completion of 5 days of swainsonine infusion. Increases in serum AST, ALT, bilirubin, amylase after 5 days of swainsonine are indicated by italics. RA, serum retinol levels; NC, no change; ↓, increase; ↓, decrease levels; —, not measured.

a Abnormal pretreatment.

b Patients with liver metastases pretreatment.

c This patient died from progressive disease and acute respiratory distress syndrome as suggested by autopsy.

* National Cancer Institute of Canada grade 4 bilirubin by day 3. Infusion stopped. MTD reached.

DISCUSSION

In this first phase I trial of swainsonine, we have measured serum C₈₀ and f₁/₂, which allowed an estimate of serum clearance. The drug showed a relatively long serum half-life of 0.5 day. The serum C₈₀ values were 100 to 400 times greater than the Kᵢ for inhibition of Golgi α-mannosidase II, suggesting that the therapeutic dose may be significantly lower than that used in our study. In cell culture, the 50% inhibitory dose for Golgi oligosaccharide processing by swainsonine is similar to the 50% inhibitory dose for α-mannosidases in vitro, suggesting that the drug has excellent cell membrane permeability.

Analysis of PBL Cell Surface Markers. White cell counts showed that swainsonine was neither lympho- nor myelosuppressive over the 5-day infusion. Swainsonine administered to mice has been shown to increase NK cell activity (23) and class II antigen expression in hematopoietic cell populations (30). Therefore, cell surface antigens including CD3, CD4, CD8, HLA-DR, CD25, CD16, and CD14 on the PBL of patients with nonhematological malignancies were examined in 7 patients (9 courses). Only HLA-DR showed a significant change with an increase of approximately 5–40% after 5 days of swainsonine treatment (P < 0.01) (Fig. 6). A similar increase in the percentage of HLA-DR-positive cells was observed for PBL from healthy donors cultured for 3 days in the presence of swainsonine.

Fig. 1. Time course for serum retinol measurements in patient VM/150. , period of swainsonine infusion; , minimum for serum retinol in healthy individuals.

an additional 24 h, a widespread effect of the drug also approaches steady state, it appears that the drug is rapidly distributed throughout the body. The swainsonine-induced oligosaccharides were susceptible to Jack bean α-mannosidase digestion, indicating that they were iso-mers of Man₂₅GlcNAc (Fig. 4B). Urine oligosaccharides from patients with hereditary α-mannosidosis showed a pattern and level of oligosaccharides by FACE gels similar, but not identical to that of swainsonine-treated patients (Fig. 4C).
Golgi α-mannosidase II was markedly inhibited in PBL as inferred by the loss of L-PHA-binding complex-type oligosaccharides after 5 days of treatment. Tissue lysosomal α-mannosidases were also inhibited by swainsonine, and saturation of this drug-related effect was observed within 24 h of reaching steady-state levels of drug in serum. Based on these measurements, dosages of 150 and 450 µg/kg/day showed indistinguishable levels of inhibition for Golgi and lysosomal α-mannosidases, which suggests that these biological effects are saturated above 150 µg/kg/day. This is supported by the observation that the amount of urine oligomannosides after 5 days of swainsonine treatment was similar to that observed in patients with the genetic disorder α-mannosidosis.

Although swainsonine was well tolerated in our patient group, definite treatment-related side effects included abnormal liver function tests, edema, and elevation of pancreatic amylase. These effects did not appear to correlate with one another or with drug dosage. Hepatic toxicity was reflected by transaminase elevation, and the time course suggests acute nonspecific hepatocellular damage. Significant elevation of liver enzymes only occurred in patients with known hepatic tumor involvement. In preclinical tests of swainsonine in two

Rhesus monkeys, a 5-fold increase in AST levels was observed when the drug was given p.o. at 8 mg/kg/day over 36 days. This is similar to the rise in AST and ALT observed in our patients treated with swainsonine, but at doses approximately 16 times greater than that used in our trial. MTD was defined in a patient with significant pretreatment hepatic dysfunction and it is possible that higher levels of

\[ \text{Fig. 3. L-PHA lectin binding to PBL of patients pre- and postswainsonine treatment. Two samples of PBL, at -24 h and at initiation of swainsonine treatment, were compared to samples taken at 120 h after treatment. Measurements for L-PHA binding were taken on 8 courses of treatment; WL/450, MSI/450, RC/450, RB/450, EG2/150, AL/450, SR/150, MS2/150. For comparison, MDAY-D2 murine lymphoma cells (O); were cultured in vitro absence (pre) and presence of 1 µg/ml of swainsonine for 48 h (post).} \]

\[ \text{Fig. 4. A, fluorescent image of FACE gel showing separation of oligosaccharides from urine of a patient treated with 150 µg/kg/day; EG1/150. B, Lane 1 is 5-day urine from a swainsonine-treated patient; Lane 2 is the same urine digested overnight with 50 milliunits of Jack bean α-mannosidase. (C). Lane I is 5-day urine from a swainsonine-treated patient: Lane 2 is urine from an α-mannosidosis patient.} \]

\[ \text{5 J. J. Lipman, Vanderbilt University, personal communication.} \]
values were normalized to pretreatment urine and expressed as fold increase. The creatinin
summed and expressed as a ratio of urine: creatinine levels. Patients were WL/450 (D),
are derived from densitometry reading of FACE gels where the densities of hands which
apparent effect on creatinine levels.

MSI/450 (A), RC/450 (•). RB/450 (•), EG1/150 (Y), EG2/150 (O), VM/150 (V). The
either 1 or 2 samples on day 5 following completion of swainsonine infusion. •, PBL from

been shown to manifest reversible liver vacuolization but without

swainsonine (post).

0.01. In comparison, analysis of this group of patient for CDI6 showed a tendency to

patients EG1/150; EG2/150; MSI/450; MS2/450; RC/450; RB/450; WL/450; AL/450, and
treatment (post). For each patient, two pretreatmcnt blood samples were analyzed, and

acute liver cell damage in animals, in sharp contrast to the pyrroliz-
does not appear to be mutagenic in cell culture and does not cause

Swainsonine is a potent immune modulator in mice, stimulating
lymphocyte proliferation (29), activating natural antitumor immunity
(23, 32), and enhancing T-cell stimulation by antigen (47). In mice
treated with chemotherapeutic drugs, swainsonine has been shown to
enhance bone marrow cellularity (27) (reviewed in Ref. 26). The basis
of immune cell activation and bone marrow proliferation may be
related to the observation that some cytokines and growth factors have
Carbohydrate-binding activities (48). IL-1, IL-2, and TNF bind to

swainsonine, the pyrrolizidine alkaloids have a double bond at 1,2 and
are bioactivated to pyrrolic dehydroalkaloids which are mutagenic and
hepatoxic (43). The pyrrolizidine alkaloids are found in tansy ragwort
(Senecio jacobaea), and their consumption by rats and chickens
results in hepatotoxicity associated with depletion of liver retinol
stores (44). In chickens fed a diet supplemented with tansy ragwort,
the liver histopathology could be prevented by a vitamin A supple-
ment, suggesting that its depletion was a significant factor in hepa-

totoxicity (44). Four of 9 swainsonine-treated patients examined showed
evidence of drug-related depletion of serum retinol. Therefore in
subsequent clinical studies of swainsonine, analysis of serum retinol
levels and hepatotoxicity must be examined and consideration be
given to intervention with vitamin A supplements.

Studies in mice suggest that swainsonine therapeutic effects are
related to inhibition of Golgi α-mannosidase II (19). Although re-
versible on withdrawal of drug, the accumulation of oligomann-
sides due to inhibition of lysosomal α-mannosidases is a side effect,
which on long-term administration of swainsonine, may become a
significant problem. The number of major oligomannoside species
observed in the urine of swainsonine-treated patients was greater
than that of an α-mannosidosis patient (Fig. 4C). α-Mannosidosis
patients are deficient in lysosomal α1-3 mannosidase, and for rea-
sons which are not entirely clear, accumulate three major storage
products in tissues and urine (i.e., Mana1-3Manβ1-4GlcNAc and
Mana1-2Manα1-3Manβ1-4GlcNAc, Manα1-2Manα1-2Manα1-3
Manβ1-4GlcNAc) (45). Swainsonine inhibits both α1-3 mannosi-
dase as well as the recently described α1-6 mannosidase (39, 46),
and therefore results in the accumulation of two major storage prod-

ucts; Manα1-6[Manα1-3]Manα1-6[Manα1-3]Manβ1-4GlcNAc and
Manα1-6[Manα1-3]Manβ1-4GlcNAc in fibroblasts (46). The more
heterogeneous mixture of oligomannosides in the urine of swain-
sonine-treated patients probably reflects incomplete inhibition of the
lysosomal α-mannosidases, which results in the accumulation of
Manα1-3Manβ1-4GlcNAc,Manα1-6[Manα1-3]Manβ1-4GlcNAc, as
well as isomers of Manα1-3GlcNAc (see Fig. 4A).

HLA-DR, a class II antigen found on monocytes, activated T-cells,
and NK cells, was elevated in PBL of swainsonine-treated patients,
similar to that observed for PBLs from healthy subjects cultured in the
presence of swainsonine for 3 days. In mice, thioglycollate-elicited
peritoneal macrophages were shown to be activated by culturing the
cells in the presence of swainsonine or by injecting swainsonine into
the peritoneal cavity (30, 31). The macrophages showed increased
tumoricidal activity, IL-1 production, induction of protein kinase C
activity, and increased cell surface class II histocompatibility antigen
IIa. In these studies, stimulation of tumoricidal activity by swainsonine
was found to be comparable to that of other activating agents includ-
ing Corynebacterium parvum, lipopolysaccharide and IFN-γ.

Swainsonine would be well tolerated in patients without liver metastases or
liver dysfunction secondary to other causes. It is worth noting that
there was no evidence of neurotoxicity in the two rhesus monkeys or
in the 19 patients in our trial, an effect which has been observed in
grazing animals that consume "loco weed," a natural source of swain-
sonine and several other alkaloids (13, 42).

Rats fed a diet supplemented with swainsonine have previously
been shown to manifest reversible liver vacuolization but without
oligosaccharide accumulation or hepatocyte death (33). Swainsonine
does not appear to be mutagenic in cell culture and does not cause
acute liver cell damage in animals, in sharp contrast to the pyrroli-
idine alkaloids, a structurally related class of compounds. Unlike

drug would be well tolerated in patients without liver metastases or
liver dysfunction secondary to other causes. It is worth noting that
there was no evidence of neurotoxicity in the two rhesus monkeys or
in the 19 patients in our trial, an effect which has been observed in
grazing animals that consume "loco weed," a natural source of swain-
sonine and several other alkaloids (13, 42).

Rats fed a diet supplemented with swainsonine have previously
been shown to manifest reversible liver vacuolization but without
oligosaccharide accumulation or hepatocyte death (33). Swainsonine
does not appear to be mutagenic in cell culture and does not cause
acute liver cell damage in animals, in sharp contrast to the pyrroli-
idine alkaloids, a structurally related class of compounds. Unlike

drug would be well tolerated in patients without liver metastases or
liver dysfunction secondary to other causes. It is worth noting that
there was no evidence of neurotoxicity in the two rhesus monkeys or
in the 19 patients in our trial, an effect which has been observed in
grazing animals that consume "loco weed," a natural source of swain-
sonine and several other alkaloids (13, 42).

Rats fed a diet supplemented with swainsonine have previously
been shown to manifest reversible liver vacuolization but without
oligosaccharide accumulation or hepatocyte death (33). Swainsonine
does not appear to be mutagenic in cell culture and does not cause
acute liver cell damage in animals, in sharp contrast to the pyrroli-
idine alkaloids, a structurally related class of compounds. Unlike
cytes showed no increase in IL-2 production or receptor number. These observations are consistent with the hypothesis that α-mannosidase inhibitors increase the sensitivity of LAK cells to IL-2 activation (32). Infiltrating lymphocytes and monocytes in tumors are largely inactive, but can be activated in vitro by IL-2 and other cytokines (50), suggesting that swainsonine might serve to sensitize LAK cells, NK cells, and macrophages to local cytokines in the tumor. The edema observed in swainsonine-treated patients was not generally associated with increased serum cytokine levels (i.e., IL-6 and TNF), but may result from a sensitization of vascular endothelium to endogenous cytokines, which may also be higher at sites of bulky tumor burden. The potential relationship between treatment-related severe edema and elevated IL-6 observed in patients will require further study.

The shortness of breath noted in 3 patients was mild and not dose dependent, and like the peripheral edema, may represent “capillary leak” in the pulmonary vasculature. One patient (VG/350), developed significant edema and liver dysfunction in association with respiratory failure. Postmortem findings indicated significant tumor within the lungs and liver, as well as bilateral pneumonia. It was thought at the time that the patient died of progressive disease, but in retrospect a capillary leak resulting in acute respiratory distress syndrome may have been caused by swainsonine. This patient has therefore been recorded as a possible treatment-related death. As suggested above, severe liver involvement with tumor may have provoked local cytoxic release in the lung, and sensitization by swainsonine may have provoked the syndrome.

Swainsonine appears to have several anticancer actions which may vary in importance for different types of tumors. These include: (a) inhibition of tumor cell metastasis and invasion by direct action on the tumor cells as an oligosaccharide-processing inhibitor; (b) inhibition of solid tumor growth, at least partially by direct action of drug on the tumor cells; and (c) augmentation of immune function including LAK, NK cell, and macrophage activities. The relative importance of the action of swainsonine on tumor cells and on host immunity is unclear. If stimulation of host immunity is the target, it may be best to use modest doses of swainsonine on a longer interval as per a study in mice by Humphries et al. (23, 24). This strategy could be used in combination with other immunostimulants such as IL-2 and IFN, which have been shown to have additive antitumor activity in mice (51). Ionizing radiation has been shown to induce cytokine production by tumor cells including TNF-α, which may contribute to radiation lethality (52). Therefore, swainsonine might be used to sensitize tumors to irradiation therapy, and at the same time provide bone marrow protection.

In summary, this first phase I trial of swainsonine administered i.v. at 50 to 550 μg/kg/day shows that the drug is well tolerated in cancer patients, particularly when the liver is not compromised by tumor involvement. The dosages used in this study appear to be saturating for 2 drug-related effects, inhibition of Golgi α-mannosidase II in PBL, and tissue lysosomal α-mannosidases. Increased levels of HLA-DR-positive lymphocytes, objective clinical response in 1 patient, and symptomatic improvement in another 2, suggest that swainsonine may have therapeutic activity in cancer patients. Further studies are under way to assess bioavailability and toxicity of swainsonine administered p.o. to cancer patients.

ACKNOWLEDGMENTS

We thank Dr. Frances Shepherd and Dr. Michael Crump for overseeing some patients during swainsonine infusion; Virgilio Deleon and Karen Paul for research assistance; Dr. Chris Starr, GLYCO, Inc., for FACE analysis of urine oligosaccharides and helpful comments; and Cathy Bedlington and Zofia Kryzek for secretarial assistance.

REFERENCES

23. Humphries, M. J., Matsumoto, K., White, S. L., Molyneux, R. J., and Olden, K. Augmentation of murine natural killer cell activity by swainsonine, a new antitumor- 

Downloaded from cancerres.aacrjournals.org on April 14, 2017. © 1994 American Association for Cancer Research.


30. Grzegorzewski, K., Newton, S. A., Akiyama, S. K., Sharrow, S., Olden, K., and White, S. L. Induction of macrophage tumoricidal activity, major histocompatibility complex class II antigen (Iaα) expression, and interleukin-1 production by swainso


33. Novikoff, P. M., Touster, O., Novikoff, A. B., and Tulsiani, D. P. Effects of swainso


A Phase I Study of Swainsonine in Patients with Advanced Malignancies

Paul E. Goss, Jose Baptiste, Berny Fernandes, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/6/1450

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.