A Phase I Study of Swainsonine in Patients with Advanced Malignancies

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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. More importantly, we have demonstrated that somatic mutations in peripheral blood lymphocytes from normal subjects cultured with swainsonine. No significant changes in CD3, CD4, CD8, CD16, and CD19 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).

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 leguminocola (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). T24H-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 culture is reduced by swainsonine (18, 19). Swainsonine-treated MDAY-D2 lymphoma tumor cells show reduced adhesion to endothelial 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 i.p. 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 reviewed (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 interrupted 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. 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 electrocardio; arterial blood gas determination and resting respiratory function testing were added to 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 nuclear 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-D-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 acetonitrile 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 acetonitrile. 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 mg 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 wall 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-aminoctanohexane-1,3-disulfonic acid (36). Five-μl samples of urine were dried, and to each tube were added 5 μl of 0.2 w/w 7-aminoctanohexane-1,3-disulfonic acid in 2.5 w/w acetic acid and 5 μl of NaCNBH3 in 1 w/w dimethyl sulfoxide, followed by incubation at 37°C for 2 h. After drying, samples were redissolved in 62.5 mM 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. Monoclonal 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 kit 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/acetonitrile, then 0.3 ml of 1.2 kg/liter of K2HP04 was added, mixed, and samples were centrifuged at 8700 g for 2 min. Retinols were separated by injecting 80 μl of supernatant onto a 25-× 4.6-mm ODS-2 high-performance liquid chromatin column (Millipore-Whatman) and run in a mobile phase of acetic acid/water/acetonitrile (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.
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. MTD (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 amylose 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, 8- 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 infusion it was 11.4 ± 6.2 pg/ml (n = 8). Serum TNF-α levels were low and remained unchanged following swainsonine treatment. Other side effects noted in our patients included shortness of breath (n = 3; National Cancer Institute of Canada grade <2), lethargy (n – 1; grade 1) and skin rash (n = 1; grade 1).

Serum retinol levels were measured in 9 patients (10 courses) who started with elevated levels; 9 patients showed no change. These increases in serum enzyme levels occurred at all doses with no apparent increase in severity over the dose range used in the trial.

Inhibition of Golgi Oligosaccharide Processing α-Mannosidase.

L-PHA lectin shows binding specificity for B1-6GlcNAc-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 activity of swainsonine as an inhibitor of Golgi oligosaccharide processing. 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).

Urine 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 quantitate the reducing oligosaccharides in patient urine (Fig. 4). Urine oligosaccharides migrating in the FACE gels in the region of Glc3 to Glc2 standards, accumulated rapidly over the first 72 h on swainsonine, and appeared to reach steady state by 72 h (Fig. 5). Since serum swainsonine Cmax is attained in 48 h, and with
Table 2 Summary of patient treatment, disease, edema, and changes in serum markers

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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.

* Abnormal pretreatment.

* Patients with liver metastases pretreatment.

* 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.

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

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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 isomers of Man_2-GlcNAc (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).

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 antibody 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.

DISCUSSION

In this first phase I trial of swainsonine, we have measured serum C_s and t_1/2, which allowed an estimate of serum clearance. The drug showed a relatively long serum half-life of 0.5 day. The serum C_s values were 100 to 400 times greater than the K_m 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.
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

Fig. 2. A, representative time course of swainsonine accumulation in the serum of patients receiving , 50; , 150; , 250; and , 450 μg/kg/day of swainsonine. B, mean serum Css values were calculated by averaging serum measurements at 72, 96, 120 h; SD, <15%. The Css values were plotted for 16 courses of swainsonine treatment. Clearance (i.e., CL = dose/Css) was significantly different for 8 patients receiving 50–250 μg/kg/day compared to the 8 patients who received 350–550 μg/kg/day (t test, P < 0.0002), which suggests nonlinear pharmacokinetics (38).

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, MS1/450, RC/450, RB/450, EG2/150, AL/450, SR/150, MS2/150. For comparison, MDAY-D2 murine lymphoma cells (○); were cultured in vitro absence (pre) and presence of 1 μg/ml of swainsonine for 48 h (post).

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 1 is 5-day urine from a swainsonine-treated patient; Lane 2 is urine from an α-mannosidosis patient. 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

J. J. Lipman, Vanderbilt University, personal communication.
Swainsonine, the pyrrolizidine alkaloids have a double bond at 1,2 and are bioactivated to pyrrolic dehydroalkaloids which are mutagenic and hepatotoxic (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 supplement, suggesting that its depletion was a significant factor in hepatotoxicity (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 reversible on withdrawal of drug, the accumulation of oligomannosides 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 α-1,3 mannosidase, and for reasons which are not entirely clear, accumulate three major storage products in tissues and urine (i.e., Manα1-3Manβ1-4GlcNAC and Manα1-2Manα1-3Manβ1-4GlcNAC, Manα1-2Manα1-2Manα1-3Manβ1-4GlcNAC) (45). Swainsonine inhibits both α-1,3 mannosidase as well as the recently described α-1,6 mannosidase (39, 46), and therefore results in the accumulation of two major storage products; 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 swainsonine-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 Manα1-6GlcNAC (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 levels. In these studies, stimulation of tumoricidal activity by swainsonine was found to be comparable to that of other activating agents including Corynebacterium parvum, lipopolysaccharide and IFN-γ.

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 marrowcellularity (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 oligomannose structures (49), which are made available on the cell surface when cells are grown in the presence of swainsonine (i.e., the Manα1-6[Manα1-3]Manα1-6Manβ portion of the hybrid-type structure). In this regard, human PBL cultured with swainsonine enhances LAK cell killing of human colon carcinoma cells (32). Similar results were obtained with 1-deoxynojirimycin, but castanospermine and 1-deoxynojirimycin had no effect. Antibodies to IL-2 abolished swainsonine-induced enhancement of LAK cell killing but lympho-
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 cytokine 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.

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