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ORIGINAL ARTICLE

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Phase I and pharmacokinetic study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) using a single intravenous dose schedule

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Abstract *Purpose:* To perform a phase I and pharmacokinetics study of 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) a new ribonucleotide reductase inhibitor using a single intravenous (2-h) schedule every 4 weeks. 3-AP was given at a starting dose of 5 mg/m^2 with escalation based on a modified Fibonacci scheme. *Patients and methods:* A total of 27 patients with advanced cancer were entered into the study. Doses of 3-AP ranged from 5 mg/m^2 to 105 mg/m^2. Blood and urine samples were collected and 3-AP was measured by HPLC. *Results:* A total of 46 courses were evaluable. One patient developed grade 4 thrombocytopenia at the lowest dose level, and one patient had grade 3 anemia. Two patients developed grade 3 coagulation abnormalities. The only other toxicities of more than grade 1 occurring in more than 10% of patients were fever and asthenia. No toxicities were observed at the highest dose level. Peak serum concentration of 3-AP increased linearly with dose. No tumor responses were observed in this heavily pretreated population, although eight patients had stabilization of their disease. *Conclusions:* Relevant tumor inhibitory concentrations were achieved without significant toxicity using doses up to 105 mg/m^2 on this single intravenous dose schedule. Prolonged administration schedules and combinations with other cytotoxic agents, strategies predicted to have greater antitumor efficacy according to preclinical studies, are under investigation.

Keywords Phase I · Pharmacokinetic · Thiosemicarbazone

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Introduction

3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP) is a new ribonucleotide reductase (RR) inhibitor. 3-AP belongs to the class of metal-binding site affecting RR inhibitors that are derivatives of α-heterocyclic carboxaldehyde thiosemicarbazone (HCT) [1]. The HCTs are the most potent RR inhibitors being 65–5000 times more potent than hydroxyurea, another RR inhibitor [7, 8].

The first HCT to undergo clinical trials was 5-hydroxy-pyridine-2-carboxaldehyde thiosemicarbazone. While this drug exhibited a broad spectrum of antitumor activity in transplanted animal models, clinical studies in patients showed no activity in solid tumors [2, 10]. A possible explanation for its lack of clinical antitumor activity may be due to rapid metabolism (via formation and subsequent elimination of the *O*-glucuronide conjugate) resulting in a short biological half-life of 2.5–10 min in patients [5]. 3-AP was designed to avoid this metabolic fate by minimizing the formation of the *O*-glucuronide conjugate. In addition, 3-AP has increased potency against RR enzymes.

The mechanism of action for the antitumor activity of 3-AP is believed to be inhibition of cytosine diphosphate (CDP) reductase, thus leading to inhibition of tumor growth. 3-AP has been shown to inhibit CDP reductase with average IC_{50} (concentration that induces 50% inhibition) values of 0.3 m*M* against a highly purified enzyme, and 0.82 m*M* against a partially purified enzyme from the Ehrlich carcinoma [1, 8]. 3-AP has also been shown to have antitumor activity against M109 and L1210 tumors, and the hydroxyurea-resistant

subline version of L1210 tumors [1]. The average IC_{50} values for the antitumor activities are 0.75–1.6 m*M*. The duration of inhibition of CDP reductase, as reflected by the duration of inhibition of DNA synthesis, induced by 3-AP has been found to be longer in tumor cells than in normal cells. The R_{50} (the time for 50% recovery from 3-AP-induced inhibition of DNA synthesis) value for L1210 tumor cells is approximately 10.1 h, whereas for normal cells from mice the R_{50} value is in the range 4.8–7.3 h. These differential durations of 3-AP-induced inhibition of DNA synthesis between tumor and normal cells may provide selective cytotoxic effects with a proper dosing schedule of 3-AP.

3-AP has also been shown to reduce tumor size in mice previously implanted at a stringent distal site with murine M109 lung carcinoma and the human 2780 ovarian carcinoma. 3-AP inhibited the growth of both types of carcinoma with the optimum treatment schedule being 6–10 mg/kg per administration twice (approximately 8 h apart) per day. Furthermore, 3-AP was almost as effective against the M109 carcinoma as Taxol (the positive control).

In a preliminary study in rats the distribution and elimination of 3-AP and elimination of 3-AP-derived residues were investigated following intravenous (i.v.) administration of 3 mg/kg [^{14}C]3-AP. The primary route of excretion of radioactivity over the 24 h after dosing was via the feces (43% of administered dose), suggesting the involvement of biliary excretion. Urine contained 20% of the dose, and no radioactivity was detected in expired air traps. The HPLC radioactivity profiles for feces extracts showed numerous components, one of which was tentatively identified as 3-AP. The urine HPLC radioactivity profiles were also complex, but 3-AP was detected in the 0–8 h sample (major component) and the 8–24 h sample (very minor component). The blood and serum radioactivity concentration-time profiles were largely similar, with blood having slightly higher radioactivity concentrations than serum in samples from the early time points. These findings suggest an even distribution of radioactivity between the intra- and the extracellular space. HPLC analysis of extracts of serum samples obtained at 0.5 h and 24 h in a quantitative whole-body autoradiography (WBA) study showed decreasing amounts of 3-AP with time (83% and 9% of serum radioactivity at 0.5 h and 24 h, respectively). The decrease in serum radioactivity concentrations over time observed in this study was at a slower rate than that for unchanged 3-AP observed in a previous study involving administration of non-labeled 3-AP. This slower rate in the reduction of radioactivity reflects unchanged 3-AP and 3-AP-derived residues.

A quantitative WBA distribution study has indicated widespread distribution of radioactivity to all organs except organs related to the central nervous system (CNS) at 0.5 h after dosing. Highest radioactivities were found in the urine, small intestinal contents, kidneys (suggesting elimination of 3-AP by the kidneys and gastrointestinal tract) and, to a lesser degree, the cecum contents, esophagus, liver, thyroid, lung, and pituitary gland. At 24 h after dosing, there was a marked reduction in radioactivity levels in all organs. The radioactivity in the CNS was low, suggesting that 3-AP did not pass through the blood-brain barrier significantly. An in vitro study has shown that approximately 60% of 3-AP binds to human serum protein [5].

Toxicology studies have been performed in rats and dogs. In the rat toxicology studies, i.v. doses ranged from 1 to 20 mg/kg. The no observable effect level (NOEL) was found to be 1 mg/kg, whereas minimal treatment-related clinical signs of pallor and wet and stained pelvis were seen beginning at 3 mg/kg, and mortality was observed at a dose of 10 mg/kg and higher. The incidence and type of clinical signs increased with increasing dose (5, 10, 15 and 20 mg/kg) and included discolored urine, pallor, prostration, reduced activity, salivation and stained/wet pelvis. Additionally, treatment-related effects were seen at doses of 5 mg/kg and higher, and these effects included decreased body weight, protein and albumin levels, red blood cells and related indices (hematocrit and hemoglobin), and white blood cells (primarily due to decreased neutrophils and lymphocytes). Several of the in-life findings seen at 5 mg/kg and higher were attributable to the vehicle, as some mortality, clinical signs, and decreased body weight, protein and albumin levels and numbers of red blood cells occurred in vehicle-treated rats. However, some findings such as decreased white blood cells and platelets did not occur in control animals.

In single-dose toxicity studies in dogs, 3 mg/kg of 3-AP was better tolerated when given i.v. (no animals with emesis) than orally (7 of 16 animals with emesis) and when given orally after feeding than before feeding. However, 19 of 20 dogs had emesis after treatment with a single i.v. dose of 3-AP at 10 mg/kg. Preadministration of an antiemetic to dogs given 10 mg/kg of 3-AP appeared to cause a slight delay in the onset of emesis, but did not prevent its occurrence. In multidose toxicity studies, clinical signs of diarrhea, emesis and inappetence were observed in dogs given 3 mg/kg i.v. over either 15 min or 2 h, but the total incidence of emesis and diarrhea was greater in the 15-min group than in the 2-h group. At 1 mg/kg, no treatment-related effects on clinical signs, body weight, clinical pathology parameters or gross necropsy findings were observed. Therefore, 1 mg/kg in dogs was considered to be the NOEL.

Due to promising preclinical results, we performed a phase I and pharmacokinetic study of 3-AP in cancer patients. A single i.v. infusion schedule was selected since this was the simplest dose schedule and 3-AP has not been tested in humans before. We report our results here.

Materials and methods

Patients with histological proof of solid tumor cancers who were not candidates for established regimens or protocol treatments of

higher efficacy or priority were entered into the study. Patient eligibility requirements included age 18 years or older, body weight >50 kg, Karnofsky performance status >70%, creatinine clearance >40 ml/min, serum creatinine <1.5 mg/dl, total bilirubin <1.5 mg/dl, ALT and AST less than twice the upper limit of the normal range except for patients with liver metastases for whom twice normal was allowed, white blood count >3000/mm^3, platelet count >100,000/mm^3, hemoglobin >10 g/dl, fewer than three prior chemotherapeutic regimen, absence of all previous chemotherapy for at least 4 weeks, absence of nitrosourea or mitomycin chemotherapy for at least 8 weeks, and absence of radiotherapy for at least 3 weeks. All patients were required to have either an evaluable or measurable area of known malignant disease to serve as an objective indicator of response to therapy. Patients were required to have no history of active heart disease including myocardial infarction within the previous 3 months, symptomatic coronary heart disease insufficiency or heart block, uncontrollable congestive heart failure or active infectious process. For females, adequate contraception or abstinence and a negative result of a pregnancy test performed within 2 weeks prior to study drug administration were also required.

Patients were excluded if they had received extensive radiotherapy such as total spinal radiation and pelvic radiation, or had known active central nervous system metastasis. Also, other eligibility requirements included no other investigational agents during the previous 6 weeks. No concurrent calcium channel blockers or cyclosporine A, which can cause p-glycoprotein inhibition, were allowed since it is not known whether 3-AP is affected by the p-glycoprotein pump mechanism. All patients signed an informed consent approved by the institutional review board prior to the start of treatment to acknowledge that they were aware of the investigational nature of the study.

Pretreatment evaluation included a complete history and physical examination including documentation of all measurable disease as well as signs and symptoms. Laboratory studies included a complete blood count, differential, platelet count, prothrombin time, partial thromboplastin time, urinalysis with microscopic examination, and SMA-17 (serum electrolytes and a biochemical profile, including blood urea nitrogen, creatinine, creatinine clearance, ALT, AST, lactate dehydrogenase, total bilirubin, alkaline phosphatase, calcium, phosphorus, magnesium, total protein, albumin, glucose and albumin/globulin ratio, and uric acid). Serum iron, total iron binding capacity (TIBC) and serum ferritin levels were determined since rat studies with 3-AP had shown anemia as one side effect of the drug. An electrocardiogram and chest radiograph were required. Serum pharmacokinetics and 8-h urine for 3-AP and possible metabolites were performed on consenting patients.

3-AP was supplied as a sterile solution for i.v. injection in l0-ml vials. Each vial contained 3-AP 50 mg, citric acid anhydrous USP 60 mg, L-ascorbic acid USP 10 mg, ethyl alcohol anhydrous USP 3 ml, and polyethylene glycol 300 USP 7 ml. Each milliliter in the vial contained 5 mg 3-AP. The volume for injection was determined by the following formula: volume of 3-AP injection (undiluted) = BSA×dose (mg/m^2)/5 mg/ml where BSA is body surface area. The drug was diluted with normal saline so that the total volume was 500 ml and was administered i.v. over 2 h. The diluted drug was administered to the patients using a polyethylene-lined infusion set within 8 h of the time of dilution. 3-AP has been found to be physically and clinically compatible when diluted to a final concentration of 0.01–2 mg/ml for up to 8 h. Patients were observed for at least 4 weeks from the start of therapy before another course was administered.

The initial dose of 3-AP was 5 mg/m^2. This dose represents less than one-tenth of the estimated 10% lethal dose in rodents. At least three patients were treated at each dose level and observed for 4 weeks prior to starting additional patients at the next higher dose level. Patients who had no toxicity (grade 0 toxicity) from their first course of therapy and had been observed for at least 4 weeks from treatment were eligible to receive a second course of the drug at the next higher dose level. Patients were treated on an outpatient basis. Evaluation during study included twice-weekly CBC, differential, platelet count and once-weekly blood chemistry which included SMA-17, magnesium level, serum iron, TIBC, and ferritin level. Patients were seen at least once a week while on study. Tumor measurements were recorded every 4 weeks by physical examination or every 8 weeks by CAT scan.

Dose-limiting effects were defined as grade 3 or 4 toxicities according to the National Cancer Institute common toxicity criteria. The dose that induced grade 3 or 4 toxicities in two out of not more than six patients was considered to be the maximum tolerated dose (MTD). The dose below the MTD was considered to be the dose for phase II studies.

The criteria for response were as follows. Complete remission was defined as disappearance of all clinical evidence of active tumor for a minimum of 4 weeks. The patient was required to be free of all tumor-related symptoms. Partial remission was defined as a decrease (by greater than 50%) in the sum of the product of the diameters (x, y, and z diameters of measured lesions). No simultaneous increase in the size of any lesion or the appearance of new lesions was allowed. A partial remission in the liver could also consist of either a 3-cm reduction in hepatomegaly or a 50% reduction in the summation of the liver enlargement below the costal margin in both midclavicular lines plus the epigastric line. This improvement must have continued for 4 weeks to be considered a partial remission. Increasing disease was defined as an unequivocal increase of at least 50% in the size of any measured lesion. The appearance of significant new lesions was also considered increasing disease. Stable disease was defined when the steady state of response was less than a partial remission or progression was less than increasing disease for a minimum of 8 weeks. There could be no appearance of new lesions.

Pharmacokinetic analysis

Blood samples (1–1.5 ml) were collected at baseline, at 2 h, 2 h 10 min, 2 h 20 min, 2 h 30 min, 2 h 45 min, and at 3, 4, 5, 6, 8 and 24 h after 3-AP infusion. Urine samples were collected during the periods 0–4 h and 4–8 h after 3-AP infusion. High-performance liquid chromatography (HPLC) with UV detection was used to analyze the serum and urine samples for 3-AP concentration. An Agilent Technologies 1100 series HPLC system was used. It consisted of a quaternary gradient pump, an autosampler, a column heater, and a multiwavelength or a photodiode array detector. Agilent ChemStation software was used for data acquisition and processing. The HPLC conditions were as follows: the column was a Supelco Discovery column (5 μm, 250×4.6 mm); the mobile phases were (A) potassium phosphate buffer (20 mM, containing 15 mM 1-hepatanesulfonic acid and 1 mM EDTA, pH 3.0), and (B) acetonitrile; the gradient program was 90% A/10% B at 0 min to 30% A/70% B at 15 min; the flow rate was 1 ml/min; the UV wavelength was 400 nm; the run time was 10 min; the equilibration delay was 6 min; and the 3-AP retention time was approximately 7.7 min.

Serum was separated and stored at –20ºC until analysis. Urine was collected for 8 h after administration during two periods: 0–4 h and 4–8 h. Aliquots were frozen until analysis. Serum or urine samples (0.5 ml) were extracted with 1.0 ml methanol (containing 4 mM EDTA). After centrifugation, the extract was concentrated to dryness and reconstituted with 0.25 ml of a solvent consisting of 10% acetonitrile and 90% mobile phase A. The reconstituted solution was then injected into the HPLC system. External calibration standards were prepared in pooled control human serum or urine and processed identically to the test samples. The validated assay had a nominal curve range of 0.02 to 10 μg/ml for serum, and 0.05–10 μg/ml for urine. Quality control samples for both serum and urine were prepared at various concentration levels, stored with the test samples in a freezer, and analyzed with each sample batch.

Pharmacokinetic modeling and pharmacokinetic parameter calculations were conducted using WinNonlin software with compartmental as well as noncompartmental methods. The following

pharmacokinetic parameters were computed: area under the serum concentration-time curve (AUC) from time zero to the last data point, peak serum concentration (C_{max}), elimination half-life ($t_{1/2}$), volume of distribution at steady state (V_{dss}), and total body clearance (Cl_{tot}). Descriptive statistics (mean and standard deviation) were calculated and used to characterize the pharmacokinetic parameters at each dose level. For urine samples, cumulative urinary recovery of unchanged drug was determined for the time period 0 (start of the infusion) to 8 h.

Results

A total of 27 patients were entered into the study. All patients were considered evaluable. The 27 patients received 46 evaluable courses of 3-AP. Their characteristics are outlined in Table 1. A majority of the patients had good performance status. The numbers of patients treated at their respective dose levels are shown in Table 2. The range of courses patients received was one to four (median two).

Hematological toxicity was uncommon. One patient at the lowest dose level of 3-AP (5 mg/m^2) developed grade 4 thrombocytopenia (Table 3). The patient had received interleukin-2 during the 4 weeks before study entry. The thrombocytopenia resolved after treatment with prednisone. One patient developed grade 3 anemia at the 60 mg/m^2 dose level. There was no evidence of cumulative toxicity (data not shown). The worst-grade non-hematological toxicities are shown in Table 4. Mild (grade 1) nausea and diarrhea occurred in a couple of patients at the highest dose levels (60 and 80 mg/m^2). Other toxicities were uncommon and were probably related to tumor progression.

Table 1. Patient characteristics

Age (years)	
Median	65
Range	30–78
Sex	
Male	21
Female	6
Performance status (%)	
Median	90
Range	70–100
Prior therapy	
Chemotherapy	23
Surgery	22
Radiotherapy	8
Immunotherapy	8
Tumor types	
Colorectal cancer	7
Lung cancer	8
Renal cell cancer	3
Melanoma	3
Other	8

Table 2. Dose schedule

3-AP dose level (mg/m^2)	Total no. of patients	Total courses delivered	No. of patients as initial dose
5	6	9	6
10	4	6	3
17	3	3	1
25	4	4	3
35	3	3	0
45	6	8	6
60	3	4	2
80	3	4	3
105	3	5	3

Pharmacokinetics were evaluated in a total of 27 patients who received a total of 39 treatments. Figure 1 presents the mean serum 3-AP concentration versus time profiles at selected doses. 3-AP was quantifiable in serum for up to 8 h after dosing, and for 24 h for some patients at higher doses (24-h samples were not collected for all patients). Serum concentrations of 3-AP exceeded the minimum inhibitory concentration for antitumor activity in vitro (0.2 μg/ml or 1 μ*M*) for approximately 5 h after dosing in the high-dose groups (>60 mg/m^2). Mean pharmacokinetic parameters of 3-AP at different doses are summarized in Table 1. AUC and C_{max} values versus dose were plotted to examine the linearity of 3-AP pharmacokinetics. As shown in Fig. 2, 3-AP appeared to show a linear pharmacokinetic behavior, although the occurrence of saturation could not be ruled out completely due to large variation in the high-dose groups. The elimination half-life ($t_{1/2}$) ranged from 30 to 120 min ($t_{1/2}$ 190 min in one patient) with a median value of approximately 1 h. In urine, 3-AP was detected during the 8 h following drug administration. Cumulative urinary recovery averaged 2–5% of the administered dose, suggesting that the elimination of 3-AP was primarily through metabolism. Pharmacokinetic data for the highest dose showed the following: $t_{1/2}$ 66 ± 32 min, Cl 0.79 ± 0.47 ml/min per m^2, V_{dss} 59.9 ± 6.6 l/m^2, C_{max} 0.97 ± 0.32 μg/ml and urinary excretion 1.4 ± 0.3% of dose.

No objective tumor responses were observed in this group of patients. Eight patients experienced stabilization of their disease for 2–4 months. The remaining 19 patients experienced disease progression.

Discussion

3-AP was brought into clinical trial because of promising preclinical results. The drug is active against selected cell lines resistant to hydroxyurea and gemcitabine [1, 3, 6, 7] which are RR inhibitors. In addition, 3-AP is active in several human and murine animal models and has been shown to increase the effectiveness of other antitumor agents such as cisplatin, cyclophosphamide and etoposide [3].

The initial dose of 5 mg/m^2 was chosen according to the following rationale. Traditionally, and according to the guidelines established by the FDA, the initial dose for the first clinical trial of a cytotoxic drug for oncology indications is one-tenth the dose (based on a single-dose toxicology study) that is lethal to 10% (or LD_{10}) of rodents on a BSA basis (milligrams per meter squared). This is provided that this dose does not cause serious

Table 3. Hematological toxicity

Dose (mg/m^2)	No. of evaluable courses	WBC		Granulocytes		Platelets	
		Median lowest recorded count ($\times 10/mm^3$)	Median day	Median lowest recorded count ($\times 10/mm^3$)	Median day	Median lowest recorded count ($\times 10/mm^3$)	Median day
5	9	5.5	27	67	21	118	27
10	6	5.7	1	58	11	239	23
17	3	4.0	10	65	21	230	22
25	4	3.5	3	64	22	238	20
35	3	3.9	4	64	14	330	28
45	8	7.2	3	63	28	218	29
60	4	8.2	20	64	20	183	31
80	4	7.4	13	43	17	249	27
105	5	6.5	7	60	6	243	29

Table 4. Non-hematological toxicity

Dose level (mg/m^2)	Toxicity (worst grade)	Grade 1	Grade 2	Grade 3	Grade 4
5 (n = 6)	Dyspnea	1	3	–	–
	Increased alkaline phosphatase	–	–	1	–
	Fever	1	2	–	–
17 ($n = 3$)	Increased alkaline phosphatase	–	–	1	–
	Anorexia	2	–	–	–
45 ($n = 6$)	Increased prothrombin time	–	–	1	–
	Pain	–	3	–	–
60 ($n = 3$)	Anemia	–	–	1	–
	Nausea and vomiting	2	–	–	–
80 ($n = 3$)	Increased partial thromboplastin time	–	–	1	–
	Diarrhea	2	–	–	–

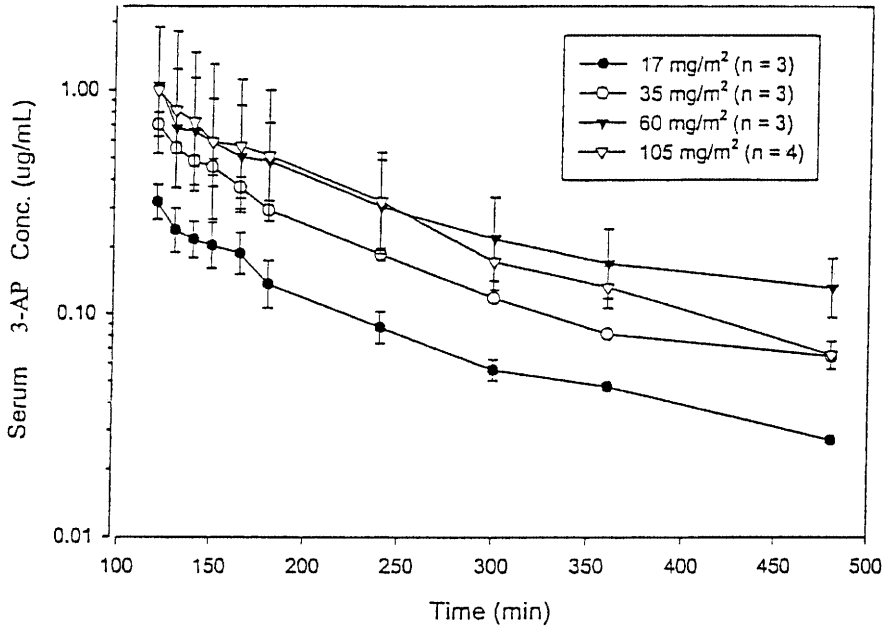

Fig. 1. Serum 3-AP concentration-time curves in cancer patients receiving a single 2-h infusion

irreversible toxicity in non-rodents (usually dogs). For 3-AP, the single-dose LD_{50} (dose that is lethal to 50%) in rats was 20 mg/kg, but at 10 mg/kg no death was observed. Therefore, the single-dose LD_{10} is between 10 and 20 mg/kg. In a 3-day single-dose toxicology study, 10 mg/kg induced death in one out of ten rats. In a 5-day single-dose toxicology study, 10 mg/kg did not induce any death in 26 rats, but 5 out of 16 rats died at 15 mg/kg. Therefore, multiple doses (i.e. 3–5 daily single doses) LD_{10} is between 10 and 15 mg/kg. In the calcu-

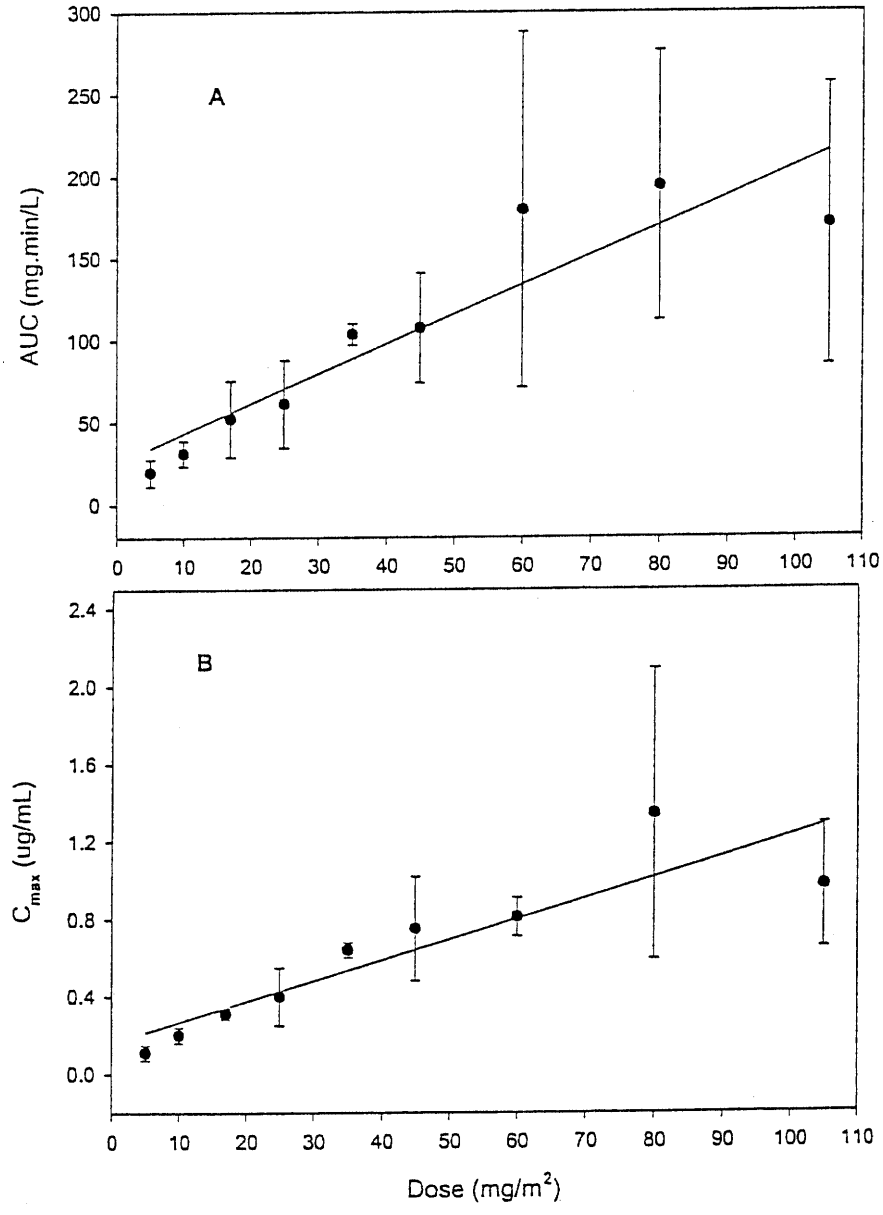

Fig. 2A, B. Mean AUC vs dose (**A**) and mean C_{max} vs dose (**B**)

lation of the initial dose for the phase I clinical trial, we conservatively considered 10 mg/kg to be the LD_{10}. At one-tenth of 10 mg/kg (i.e. 1 mg/kg) no toxicity was observed with a single or 5-daily doses in dogs. Therefore, the initial dose in this study was 1 mg/kg, which is equivalent to 5 mg/m^2.

In this study a 2-h infusion time was chosen. Since alcohol and citric acid are part of the 3-AP formulation, it was felt that a 2-h infusion might produce less risk of possible phlebitis or pain associated with the administration.

This is the first reported clinical trial of 3-AP in humans. This phase I study did not reach a maximum tolerated dose level using a single i.v. 2-h infusion schedule despite multiple (eight levels) dose escalations. Since after eight escalations of the dose, no dose-limiting toxicity was observed, it was decided to pursue other alternative schedules instead. Our study showed that single doses up to 105 mg/m^2 of 3-AP were generally well tolerated even in heavily pretreated cancer patients. Uncommon side effects including asthenia, fever, nausea, and anorexia were not dose-related and may have been related to disease progression. Serum concentrations of 3-AP did exceed the minimum inhibitory concentrations for in vitro activity in patients who received the highest dose level of 3-AP. While disease stabilization occurred in several patients, no documented tumor responses were observed.

It may be worthwhile to administer 3-AP in a prolonged infusion schedule for several reasons. In animal models, multiple doses or prolonged administration is required for antitumor activity [3]. In vitro studies with hydroxyurea have shown that cytotoxic

effects are highly dependent on time of exposure [11]. In addition, it seems reasonable to perform combination phase I trials with cisplatin and gemcitabine. In vitro studies have been conducted to examine the antitumor activity of 3-AP in combination with cisplatin. Several murine and human tumor cell lines were exposed to 3-AP for 24 h, then 3-AP and cisplatin for 24 h, followed by 3-AP alone for 24 h. Concentrations of 3-AP ranged from 0.3 to 1 μM (within the range obtained safely in phase I trials), and those of cisplatin from 3.125 to 25 μM. The effects on the majority of cell lines were consistent with additive or synergistic cytotoxicity. In mice implanted with the L1210 leukemia cell line into the peritoneal cavity, the combination of 3-AP (twice daily for 6 days) and cisplatin (daily for 6 days) administered by the intraperitoneal route was well tolerated and produced an improved outcome compared to the highest dose tested of either drug alone (data on file internally, Vion Pharmaceuticals, New Haven, Ct.). Inhibition of RR lowers cellular dNTP pools, which leads to increased salvage pathway activity and cellular uptake of the nucleoside analogues. Low cellular dNTP pools also favor the incorporation of the phosphorylated nucleoside analogues into DNA during synthesis or repair [4, 12], which supports 3-AP in combination with gemcitabine. Therefore, prolonged administration schedules with 3-AP alone and in combination with other chemotherapeutic drugs are currently underway [9].

In conclusion, this phase I trial demonstrated that 3-AP as a single 2-h i.v. infusion every 4 weeks was generally well tolerated in doses up to 105 mg/m^2. No dose-limiting toxicity was identified using this dose and schedule. Peak serum concentrations of 3-AP increased linearly with the dose. Relevant tumor inhibitory concentrations at the highest doses were achieved in the serum without significant toxicity, although no antitumor responses were observed in these heavily pretreated cancer patients. Since 3-AP has shown promising preclinical results, including activity in hydroxyurea-resistant tumors, further clinical trials using alternative prolonged infusion schedules with 3-AP alone or in combination with other agents are recommended.

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