A Phase I and Pharmacological Study of the Platinum Polymer AP5280 Given as an Intravenous Infusion Once Every 3 Weeks in Patients with Solid Tumors

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ABSTRACT

Purpose: **This Phase I study was designed to determine the maximum tolerated dose, profile of adverse events, and dose-limiting toxicity of AP5280 in patients with solid tumors. Furthermore, the platinum (Pt) pharmacokinetics after AP5280 administration and preliminary antitumor activity were evaluated. AP5280 is a Pt agent linked to the water-soluble, biocompatible copolymer *N*-(2-hydroxypropyl)methacrylamide, which potentially increases Pt accumulation in tumors via the enhanced permeability and retention effect. In this way, it is anticipated that a higher activity of therapeutic Pt can be reached. The pharmaceutical product contains approximately 8.5% of Pt by weight and has a molecular weight of approximately 25,000.**

Experimental Design: **Adult patients with solid tumors received AP5280 as a 1-h i.v. infusion every 21 days. Pharmacokinetics of total and unbound Pt were determined during the first treatment course and before the start of each new cycle using noncompartmental pharmacokinetic analysis. Pt-DNA adduct concentrations in WBCs and, if available, in tumor tissue were quantified using a sensitive ^{32}P postlabeling assay.**

Results: **Twenty-nine patients were treated at eight dose levels (90–4500 mg Pt/m^2). The dose-limiting toxicity was Common Toxicity Criteria grade 3 vomiting and was experienced at 4500 mg Pt/m^2 in two of six patients. The maximum tolerated dose on this schedule was therefore 4500 mg Pt/m^2, and the recommended dose for a Phase II study is 3300 mg Pt/m^2. Renal toxicity and myelosuppression, toxicities typically observed with cisplatin and carboplatin, were minimal for AP5280. The area under the curve of total Pt increased with increasing AP5280 dose. Plasma clearance of total Pt was 644 ± 266 ml/h, and the terminal half-life was 116 ± 46.2 h. After AP5280 administration, Pt-guanine-guanine DNA adduct concentrations in WBCs ranged from 70 to 1848 amol/μg DNA, concentrations that were substantially lower than concentrations measured after administration of therapeutic doses of cisplatin.**

Conclusions: **AP5280 can be administered safely as a 1-h i.v. infusion at a dose of 3300 mg Pt/m^2 once every 3 weeks and produces prolonged plasma exposure compared with any of the free Pt-containing drugs. However, it remains to be determined whether AP5280 can actually increase Pt delivery to the DNA of tumor cells in man as has been shown in experimental models.**

INTRODUCTION

Since the discovery of cisplatin, platinum (Pt) agents have had a major impact on cancer chemotherapy and are often widely prescribed for the treatment of solid tumors. Pt compounds are routinely used to treat lung, head and neck, ovarian, and testicular cancers (1–6). Although numerous Pt analogs have undergone preclinical and clinical testing, only cisplatin, carboplatin, and oxaliplatin have been approved for clinical use (7, 8).

Conventional Pt agents have well-recognized side effects that limit the frequency of dosing and the amount of drug that can be given per course of treatment. Although the dose-limiting toxicity (DLT) differs for each drug, the major adverse events produced by these agents are myelosuppression, nausea and vomiting, nephrotoxicity, and peripheral neuropathy (7, 8). This necessitates the search for new analogs with a broader spectrum of antitumor activity and reduced side effects. An alternative strategy is to improve drug delivery selectively to tumor tissue. The polymer platinate AP5280 was designed to remain inactive while in the plasma but to be passively concentrated in the tumor extracellular volume via the enhanced permeability and retention effect and subsequently activated to a cytotoxic form by either extracellular or intracellular proteases.

As shown in Fig. 1, AP5280 consists of a cytotoxic Pt complex linked to a water-soluble, biocompatible, nontoxic polymer backbone consisting of poly-*N*-(2-hydroxypropyl)-methacrylamide. The linker consists of a tetrapeptide [GPLG (glycine-phenylalanine-leucine-glycine)] spacer (9) with the COOH-terminal glycine bound to an aminomalonic acid chelating agent that binds the bioactive Pt complex. The molecular weight is approximately 25,000, and the pharmaceutical product contains approximately 8.5% Pt by weight (w/w).

As growing tumors establish their own blood supply, they

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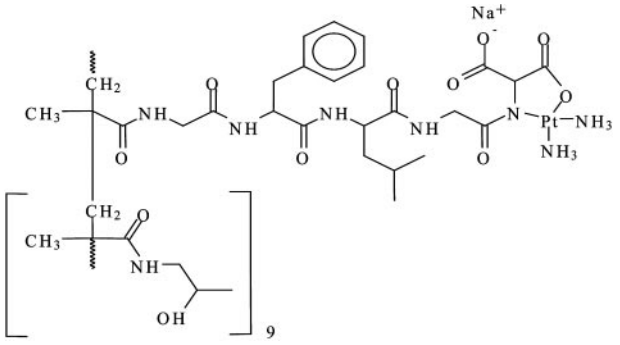

Fig. 1 The structural formula of AP5280.

develop a distinct neovasculature with blood vessels that are frequently hyperpermeable to circulating macromolecules and small particles. In addition to this enhanced permeability, tumor tissue often has limited lymphatic and/or capillary drainage. Therefore the macromolecules can be trapped and concentrated in the tumor (10). If a chemotherapeutic agent is coupled to a suitable polymer via a biodegradable linker, then such carriers have the potential to increase the concentration of the chemotherapeutic agent within the tumor tissue. As a result of these characteristics, concentrations of polymer-drug conjugates in tumor tissue can reach 10–100 times higher levels than those produced after administration of the free drug (11). This effect has been termed the enhanced permeability and retention effect (12–14). AP5280 was developed to function as a long-circulating, target-selective agent capable of making use of the enhanced permeability and retention effect in tumors (12, 13). The design goal was to produce an agent with very limited systemic toxicity that could increase the delivery of a cytotoxic Pt moiety to tumors relative to what can be attained with conventional Pt-containing drugs. Release of the cytotoxic moiety can occur intratumorally through the action of extracellular and lysosomal thiol-dependent proteinases known to be elevated in human tumors (9).

AP5280 was tested preclinically in the B16 melanoma and Lewis lung murine tumor models and the UMSCC10b squamous cell head and neck and 2008 ovarian carcinoma xenograft models. The maximum tolerated dose (MTD) of AP5280 was 6-fold greater than that of carboplatin *in vivo*. AP5280 was active in all four tumor models, and it displayed a higher therapeutic index than carboplatin in each of these tumor models. The antitumor effect of AP5280 given at 16% of its MTD was equivalent to that produced by a MTD of carboplatin.[6] All of the treatment-related toxicities observed in these studies were dose dependent. Based on the changes observed in blood urea nitrogen, serum creatinine, and hematological parameters, AP5280 appeared to be capable of producing nephrotoxicity and myelosuppression at high doses. It also produced changes in hepatic enzymes consistent with hepatotoxicity at high doses (15). The MTD of 698 mg Pt/m^2 was identified in mice as the most sensitive species. On this basis, the starting dose for this study was established at 90 mg Pt/m^2, which was 10% of the MTD in mice.

A Phase I dose-escalating study of AP5280 was performed at The Netherlands Cancer Institute (Amsterdam, the Netherlands) and the Centre Leon Berard (Lyon, France). The primary objectives of this study were (*a*) to determine the MTD of AP5280, (*b*) to identify a safe dose for Phase II evaluation, and (*c*) to determine the profile of adverse events. The secondary objectives were (*a*) to study the Pt pharmacokinetics of AP5280 and Pt-DNA adduct concentrations in WBCs and tumor, when available, and (*b*) to document any antitumor activity.

PATIENTS AND METHODS

Patient Eligibility Criteria. Patients were eligible if they had a histologically or cytologically confirmed diagnosis of a solid malignant tumor not amenable to established forms of effective therapy. Other eligibility criteria included a WHO performance status of 0–2, anticipated life expectancy of at least 3 months, and age ≥18 years. Previous anticancer chemotherapy had to be discontinued for at least 4 weeks before entry into the study or 6 weeks before entry into the study in case of pretreatment with nitrosourea, melphalan, or mitomycin C. Patients must have recovered from adverse events associated with prior chemotherapy. Prior exposure to cisplatin, carboplatin, or oxaliplatin was allowed. Radiation therapy had to have been completed at least 4 weeks before study entry and at least 8 weeks before study entry in case of extensive radiotherapy. All patients had to have acceptable bone marrow function, serum bilirubin within 1.5× the normal upper limit, normal serum creatinine, and aspartate aminotransferase and alanine aminotransferase ≤3 times the normal upper limit. Patients were excluded if they had clinical signs of brain tumor and/or leptomeningeal evidence of tumor, neuropathy [≥Common Toxicity Criteria (CTC) grade 2], or hearing loss (≥CTC grade 2). Additional exclusion criteria were pregnancy and signs or symptoms of an active uncontrolled infection. The Medical Ethics Committee of both institutes (The Netherlands Cancer Institute and the Centre Leon Berard) approved the study protocol, and all patients gave written informed consent.

Treatment Plan and Study Design. AP5280 was administered once every 3 weeks or after full recovery from adverse events associated with the prior cycle. The starting dose was 90 mg Pt/m^2 given as an i.v. infusion over 1 h in a vehicle consisting of 1 liter of 5% dextrose. Doses were escalated in decreasing increments that depended on the clinical judgement of the investigators. Dose escalation was scheduled as 180, 360, 720, 1440, 2200, 3300, and 4500 mg Pt/m^2. If no adverse events occurred at the preceding dose level, the dose was escalated by 100%. If grade 1 nonhematological (except alopecia and untreated nausea and vomiting) or grade 2 hematological toxicity (except anemia) occurred at the preceding dose level, the dose was escalated in 50% increments. If ≥grade 2 nonhematological toxicity (except for those mentioned above) or ≥grade 3 hematological toxicity occurred, the dose was escalated in increments of 20–33%. Initially, 1 patient/dose level was treated. When a dose that produced a grade 1 nonhematological or grade 2 hematological toxicity was reached, 3 patients/dose level were

[6] X. Lin *et al.*, submitted for publication.

treated. If a DLT occurred in one of the three patients within one cohort during the first cycle of treatment, then up to three additional patients were treated at that level. The MTD was defined as the dose producing DLT in at least two of six patients. Patients were to receive two cycles of AP5280 and were eligible to receive additional cycles if no tumor progression or DLT was observed.

Drug Product. AP5280 (containing approximately 8.5% Pt by weight) was provided by Access Pharmaceuticals, Inc. (Dallas, TX). It was initially supplied as a lyophilized powder in a 30-ml vial containing 200 mg Pt/vial (approximately 2.4 g of AP5280). This was modified during the course of the study, when AP5280 was manufactured and supplied in 50-ml vials containing 400 mg Pt/vial (approximately 4.8 g of AP5280). To prepare the drug for i.v. infusion, the required volume of stock solution was diluted to a final volume of 1 liter in sterile 5% dextrose in water. Because of the possible release of toxic Pt species from the polymer carrier of AP5280 in the presence of chloride ions, care was taken to be sure that AP5280 did not make contact with chloride-containing solutions (*e.g.*, normal saline) before infusion into patients. The pharmaceutical development has been reported elsewhere (16, 17).

Patient Evaluation. A complete medical history and physical examination were completed before registration. Before each cycle, the physical examination was repeated, and hematology and serum chemistry values were measured. A urine analysis was performed, and 24 h creatinine clearance was measured. Hematology and serum chemistry values were measured weekly. Tumor evaluations were performed by computed tomography scan every other cycle. Although assessment of the antitumor activity was not a primary objective of this study, patients with measurable disease were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (18). Patients were considered evaluable for response if they had measurable disease that met the RECIST criteria, received at least one complete infusion of drug, and had repeat measurements of tumor. All toxicities were graded according to the National Cancer Institute CTC (19). DLT was defined as any $\geq$grade 2 neurotoxicity, $\geq$grade 3 nonhematological toxicity (with the exception of inadequately treated nausea and vomiting), any grade 4 neutropenia lasting >5 days or complicated by fever or infection, or any grade 4 thrombocytopenia. A decrease in creatinine clearance was used as a parameter for evaluation of nephrotoxicity. Creatinine clearance during treatment (before every cycle) as well as at the end of the treatment (after the last administration) was considered, to detect any acute and cumulative renal toxicities.

Pharmacokinetic Studies. Pharmacokinetic studies were performed in all patients. Blood samples were obtained from patients at 14 time points for up to 2 weeks after the first administration: preinfusion; at the end of the infusion; 15 and 30 min after the end of the infusion; and 1, 2, 4, 7, 10, 24, 48, 96, 192, and 336 h after the end of the infusion. Before the start of every subsequent cycle, one blood sample was taken for measurement of trough drug level. Samples of 7 ml each were collected in heparinized tubes from the arm contralateral to that receiving the drug infusion. The sample was immediately centrifuged at 4°C for 5 min at 2500 $\times$ *g*. The resulting plasma layer (approximately 3 ml) was then removed, and two 1.0-ml aliquots were transferred to a YM-3 Centricon filtration system equipped with M_r 3000 cutoff filters. The loaded filter systems were then immediately centrifuged at room temperature for 30 min at 4100 $\times$ *g*. This yielded approximately 80 μl/filter system or a total of 160 μl of plasma ultrafiltrate for the analysis of unbound Pt. The plasma ultrafiltrate was immediately stored at −20°C until analysis. Plasma (approximately 1 ml) was also stored at −20°C until the analysis of total Pt as described previously (20).

The patient was also asked to void just before the start of the infusion. Thereafter, the total urine output was collected during hours 1–8 after the start of infusion and then over hours 8–24 and 24–48. The urine collected during each time interval was thoroughly mixed, the total volume was recorded, and two duplicate samples of 10 ml were stored at −20°C. All Pt analyses were performed using graphite flameless atomic absorption spectrometry. Total Pt concentrations were determined in diluted plasma samples. Urine samples and ultrafilterable Pt were determined in plasma ultrafiltrate samples, and for these measurements, the calibration curves were constructed with drug spiked in plasma ultrafiltrate (20).

Before the infusion and at 1, 24, and 96 h after the end of the infusion during the first cycle, whole blood samples of 16 ml were collected in heparinized tubes for determination of Pt-DNA adducts in WBCs. Immediately after withdrawal, the samples were centrifuged for 5 min at 4°C and approximately 2500 $\times$ *g*. Subsequently, the WBC fraction was isolated by adding ice-cold PBS to a final volume of 10 ml and lysing the contaminating RBCs by incubation in 30 ml of 0.83% ammonium chloride, 0.1% potassium bicarbonate, and 1 mM EDTA for 20 min at 4°C. After centrifugation for 5 min at 4°C and approximately 2500 $\times$ *g*, the WBCs were collected and washed with PBS and a Tris-EDTA buffer [10 mM Tris, 2 mM EDTA (pH 7.35)]. The WBCs were resuspended in 9 ml of the Tris-EDTA buffer and stored at −80°C until analysis (21). When a patient had a tumor that was easily accessible for a small true cut biopsy, a biopsy was performed during the first cycle on the day after the infusion to quantitate the level of Pt-DNA adducts in the tumor. The sample was taken as close as possible to 24 h after the end of the infusion. Pt-DNA adducts were quantified in WBCs and tumor tissue using a sensitive and validated ^{32}P postlabeling assay that enabled the selective determination of intrastrand cross-links between guanine-guanine groups (GG) and between adenine-guanine (AG) groups, the two major Pt-DNA adducts (22).

Pharmacokinetic Analysis. The pharmacokinetic parameters were estimated by applying a noncompartmental analysis using the pharmacokinetic computer program WinNonlin (standard edition version 3.0; 1999; Pharsight Corp.). The maximal drug concentration (C_{max}) was derived directly from the experimental data. For estimation of the area under the curve (AUC) from 0 to infinity ($AUC_{0\text{-inf}}$) and from 0 to 96 h (AUC_{0-96}), linear trapezoidal estimation was used for the initial ascending portion of the curve, and logarithmic trapezoidal estimation was used for the descending portion of the curve. For most subjects in the study, the percentage of extrapolation to infinity was $<15\%$, indicating reliable $AUC_{0\text{-inf}}$. Terminal half-life values ($t_{1/2}$) were calculated using the last three to five

non-zero points. Volume of distribution (V_{ss}) was calculated in the same way for total Pt.

In addition to the pharmacokinetic parameters, WinNonlin provides a SE for each parameter for each subject and a percentage coefficient of variation (%CV). These values were examined for each subject, and if the %CV values were >200%, then the parameters for that subject were considered too imprecise and potentially inaccurate and were not reported. Compartmental modeling was tried for the ultrafilterable Pt concentrations, but the %CV values were extremely high, possibly due to the generally low and variable concentrations or to a pharmacokinetic mechanism not adequately described by the available compartmental models. It was concluded that the noncompartmental parameters were the most reliable for ultrafilterable Pt.

The percentage of dose excreted in the urine was calculated from the urinary volumes and concentrations. In addition, the renal clearance (Cl_r) was calculated for each collection interval. Cl_r was calculated using the following formula: $Cl_r = (Fl \times C_{urine})/C_{mean}$, where Fl is the urinary flow rate, C_{urine} is the concentration of total Pt in the urine, and C_{mean} is the mean plasma concentration of total Pt during the collection interval. Flow rate was calculated by dividing the volume of the urine sample by the duration of the collection interval. C_{mean} was calculated by dividing the partial plasma AUC (*i.e.*, AUC_{0-8}, AUC_{8-24}, and AUC_{24-48}) by the duration of the collection interval. Because Cl_r calculated for any given interval is subject to potential error due to incomplete emptying of the bladder and inaccuracies in the estimation of C_{mean}, the values for the three intervals for each subject were averaged to achieve a value for the subject. Cl_r was not calculated for those subjects who did not have three urine collections post-dose. For comparison, plasma clearance (Cl_p) was calculated as dose/$AUC_{0\text{-}inf}$. The pharmacokinetic parameters were reported as mean ± SD.

Table 1 Patient characteristics and tumor response

	No. of patients	% of patients
Total no.	29	
Male/female	17/12	59/41
Median age (range) (yrs)	53 (27–73)	
WHO performance status		
0	6	21
1	16	55
2	7	24
Previous therapy		
Radiotherapy, systemic therapy, and surgery	9	31
Surgery and systemic therapy	18	62
Radiotherapy and systemic therapy	2	7
Prior Pt[a] treatment		
Yes	23	79
No	6	21
Response		
PR	0	
CR	0	
SD	5	17
PD	14	48
NE	10	35
Tumor type		
Colon	5	
Rectal	6	
Non-small cell lung carcinoma	4	
Renal	3	
Ovarian	2	
Melanoma	2	
Gastric	2	
Rectal	1	
Head and neck	1	
Pancreatic	2	
Esophageal	1	

[a] Pt, platinum; PR, partial response; CR, clinical response; SD, stable disease; PD, progressive disease; NE, not evaluable.

RESULTS

Patient Characteristics. A total of 29 patients were included in the trial, and their characteristics are presented in Table 1. The median age of the patients was 53 years (range, 27–73 years), and most patients had a good performance status. As anticipated for a Phase I trial, this population of patients had received extensive prior therapy. All patients had had prior systemic chemotherapy, and in 79% of the patients, this had included a Pt drug-based regimen. The majority of patients had tumors not generally considered to be sensitive to Pt drugs. A total of 62 cycles of AP5280 were administered to the 29 patients. The median number of cycles/patient was 3 [range, 1–7 cycle(s)]. The number of patients treated at each dose level and the number of cycles administered are summarized in Table 2. Four patients were treated at the first dose level. One of these patients was initially planned for dose level 180 mg Pt/m^2 but was included at 90 mg Pt/m^2. Therefore, these data were included at this dose level. Seven patients were treated at dose level 3300 mg Pt/m^2. One patient was not eligible because she suffered from CTC grade 3 hematuria, which was possibly related to AP5280. Because of the patient's preexisting condition (nephrectomy), it was decided that this patient should not be included in the determination of the MTD. This patient was replaced by enrollment of another patient at the same dose level. Nine patients died due to disease progression; the remaining patients went off-study because of objective disease progression or due to adverse events or symptomatic deterioration.

Adverse Events. All patients were evaluable for toxicity. The frequencies per patient of treatment emergent hematological adverse events as a function of dose are presented in Table 3. Overall, hematological toxicity was very mild, and none of the hematological adverse events were clearly dose related. Grade 3 hematological toxicities of any type occurred in only 10 patients (34%), and there were no grade 4 adverse events. The frequencies of treatment emergent nonhematological adverse events as a function of dose are presented in Table 4. Only six patients (21%) suffered a grade 3 adverse event of any type, and there were no grade 4 adverse events. No patient developed neurotoxicity characteristic of the Pt-containing drugs. The most important drug-related adverse events were nausea and vomiting. At the 4500 mg Pt/m^2 dose level, two of the six patients suffered either grade 3 nausea or vomiting on a total of 5 cycles, despite prophylactic treatment with corticosteroids and a 5-HT_3 antagonist. This was considered to be the DLT. The MTD at this schedule was therefore 4500 mg Pt/m^2, and the recommended dose for a Phase II study is 3300 mg Pt/m^2.

Other clinical laboratory abnormalities were a maximal

Table 2 Dose escalation

Dose level (mg Pt/m^2)[a]	90	180	360	720	1440	2200	3300	4500
N	4	1	3	2	3	3	7	6
No. of cycles	9	2	7	4	5	6	13	16

[a] Pt, platinum.

grade 3 creatinine increase (up to 347 μM) at dose level 3300 mg Pt/m^2 and hypomagnesemia, which was observed at grade 3 in one patient and grade 4 in one patient, at the 4500 mg Pt/m^2 dose level.

Pharmacokinetics. Blood samples for the measurement of total and ultrafilterable Pt were obtained from all 29 subjects during the first cycle of treatment, and from 1 of these patients, samples were also obtained on the second cycle.

For both total Pt and ultrafilterable Pt, the lower limit of quantitation was 0.05 μg/ml. Fig. 2 presents graphs of the mean values for both total and ultrafilterable plasma Pt as a function of time after the start of the infusion in patients receiving 2200 mg Pt/m^2. The concentrations of total Pt were generally much higher than the concentrations of ultrafilterable Pt at all doses and time points. The estimated pharmacokinetic parameters are presented in Table 5, A and B. Most subjects had plasma concentrations of total Pt above the lower limit of quantitation for up to 500 h. The decay curve exhibited reasonably well-defined disposition phases. The C_{max} for total Pt occurred at or near the end of the 1-h i.v. infusion of AP5280, and mean C_{max} increased with dose. The terminal half-life of total Pt was influenced by the time period over which the samples were taken. This phenomenon is probably due to the existence of a long terminal half-life for total Pt of approximately 120 ± 25 h, and if sampling is not continued until approximately 500 h, this half-life is poorly defined. Compartmental modeling was not done for total Pt because the bound and ultrafilterable Pt have different pharmacokinetics.

Ultrafilterable Pt concentrations were undetectable in patients receiving doses of AP5280 of 90 and 180 mg Pt/m^2. At the higher dose levels, ultrafilterable Pt concentrations were measurable but highly variable. Maximum measured concentrations ranged from 0.81 to 36.08 μM. The concentration of ultrafilterable Pt increased with time at all dose levels, reaching a peak at an average of 11 ± 5 h after the start of the infusion when all patients and dose levels are considered together. The time at which Cmax was reached (T_{max}) was not related to dose. Unlike the situation for total Pt, the C_{max} of ultrafilterable Pt did increase with increasing dose, but the increase was not exactly dose proportionate due to the limited number of patients and high interpatient variation in C_{max} values. Peak ultrafiltrate Pt concentrations ranged from 0.2% to 1.4% of those for total Pt at the 3300 and 4500 mg Pt/m^2 dose levels.

Noncompartmental analysis of total Pt showed that

Table 3 Likelihood of the relation between observed toxicity and the study drug

Item	Dose level	Grades				Total no. of patients (%)
		1	2	3	4	
Hemoglobin	90	1 (3%)	2 (7%)	0	0	3 (10)
	180	0	0	1 (3%)	0	1 (3)
	360	2 (7%)	1 (3%)	0	0	3 (10)
	720	1 (3%)	1 (3%)	0	0	2 (7)
	1440	2 (7%)	0	1 (3%)	0	3 (10)
	2200	3 (10%)	0	0	0	3 (10)
	3300	2 (7%)	4 (14%)	1 (3%)	0	7 (24)
	4500	1 (3%)	2 (7%)	1 (3%)	0	4 (14)
Platelets	90	1 (3%)	0	0	0	1 (3)
	720	1 (3%)	0	0	0	1 (3)
	1440	2 (7%)	0	0	0	2 (7)
	2200	1 (3%)	0	0	0	1 (3)
	3300	4 (14%)	0	0	0	4 (14)
	4500	1 (3%)	1 (3%)	0	0	2 (7)
WBCs	90	1 (3%)	0	0	0	1 (3)
	720	0	1 (3%)	0	0	1 (3)
	3300	1 (3%)	0	0	0	1 (3)
Neutrophils	720	1 (3%)	0	0	0	1 (3)
	3300	1 (3%)	0	0	0	1 (3)
Lymphocytes	90	1 (3%)	1 (3%)	1 (3%)	0	3 (10)
	180	0	0	1 (3%)	0	1 (3)
	360	1 (3%)	1 (3%)	1 (3%)	0	3 (10)
	720	0	1 (3%)	1 (3%)	0	2 (7)
	1440	0	1 (3%)	1 (3%)	0	2 (7)
	2200	1 (3%)	2 (7%)	0	0	3 (10)
	3300	2 (7%)	2 (7%)	1 (3%)	0	5 (17)
	4500	3 (10%)	2 (7%)	0	0	5 (17)

Table 4 Likelihood of the relation between observed toxicity and the study drug

Toxicity	Dose level	Grades 1	2	3	4	Total no. of patients (%)
Gastrointestinal toxicity						
Nausea	90	4 (14%)	0	0	0	4 (14)
	180	1 (3%)	0	0	0	1 (3)
	360	2 (7%)	0	0	0	2 (7)
	720	1 (3%)	0	0	0	1 (3)
	1400	1 (3%)	0	0	0	1 (3)
	2200	2 (7%)	1 (3%)	0	0	3 (10)
	3300	3 (10%)	3 (10%)	0	0	6 (20)
	4500	3 (10%)	2 (7%)	1 (3%)	0	6 (20)
Vomiting	90	2 (7%)	0	0	0	2 (7)
	180	1 (3%)	0	0	0	1 (3)
	360	0	2 (7%)	0	0	2 (7)
	1400	1 (3%)	2 (7%)	0	0	3 (10)
	2200	1 (3%)	1 (3%)	0	0	2 (7)
	3300	1 (3%)	4 (14%)	0	0	5 (17)
	4500	0	2 (7%)	2 (7%)	0	4 (14)
Constipation	4500	0	1 (3%)	0	0	1 (3)
Dyspepsia	90	1 (3%)	0	0	0	1 (3)
Neurological toxicity						
Muscle contractions	4500	0	1 (3%)	0	0	1 (3)
Convulsions	3300	0	1 (3%)	0	0	1 (3)
Ototoxicity						
Tinnitus	3300	1 (3%)	0	0	0	1 (3)
Other toxicities						
Anorexia	90	1 (3%)	0	0	0	1 (3)
	1440	0	1 (3%)	0	0	1 (3)
	2200	1 (3%)	0	0	0	1 (3)
	3300	0	1 (3%)	0	0	1 (3)
	4500	0	1 (3%)	0	0	1 (3)
Asthenia	2200	1 (3%)	0	0	0	1 (3)
Dysgeusia	360	1 (3%)	0	0	0	1 (3)
	3300	1 (3%)	0	0	0	1 (3)
	4500	0	1 (3%)	0	0	1 (3)
Dehydration	1440	0	1 (3%)	0	0	1 (3)
	3300	0	1 (3%)	0	0	1 (3)
Fatigue	90	2 (7%)	1 (3%)	0	0	3 (10)
	1440	0	0	1 (3%)	0	1 (3)
	2200	1 (3%)	1 (3%)	0	0	2 (7)
	4500	1 (3%)	1 (3%)	1 (3%)	0	3 (10)
Headache	1440	1 (3%)	0	0	0	1 (3)
Pyrexia	1440	1 (3%)	0	0	0	1 (3)
	4500	0	1 (3%)	0	0	1 (3)
Hematuria	3300	0	0	1 (3%)	0	1 (3)
Myalgia	3300	0	1 (3%)	0	0	1 (3)
	4500	2 (7%)	0	0	0	2 (7)
Acute renal Insufficiency	4500	0	1 (3%)	0	0	1 (3)

AUC_{0-96} and AUC_{inf} (Fig. 3; Table 5A) appeared to increase with dose but leveled off between dose level 3300 and 4500 mg Pt/m^2 due to a small dose increment (35%) and high interpatient variation at these dose levels (60–95%). AUC_{inf} for ultrafilterable Pt was <4% of the AUC_{inf} for total Pt in all patients. It is of interest that there was a trend toward increasing plasma clearance and V_{ss} over the dose range from 1400 to 4500 mg Pt/m^2. The V_{ss} for ultrafilterable Pt was calculated. However, no reliable data could be obtained.

Urine Pharmacokinetics. Urine was collected for each of the three scheduled intervals in 24 of the 29 patients. Fig. 4 presents the curves describing cumulative urinary Pt excretion as a function of time. The fraction of the administered dose excreted via the urine did not vary consistently with dose. The mean total cumulative urinary Pt excretion for all dose groups combined was 44 ± 19% within 48 h. Most of the excreted Pt was eliminated within 24 h. Furthermore, based on the shape of the urinary excretion curves (Fig. 4), it appeared that urinary elimination was not completed by 48 h. The mean value for Cl_r for all dose groups combined was 601 ± 270 ml/h, similar to the mean value for Cl_p (644 ± 266 ml/h), indicating that clearance of Pt was entirely or almost entirely renal. For all dose groups combined, the Cl_r:Cl_p ratio was 98 ± 44%. Although the results of this study indicated similar clearances for plasma and renal elimination, there was a moderately large intersubject variability in estimation of both clearances.

Pt-DNA Adduct Formation. Samples were available for measurement of Pt-DNA adduct formation in WBCs from 28

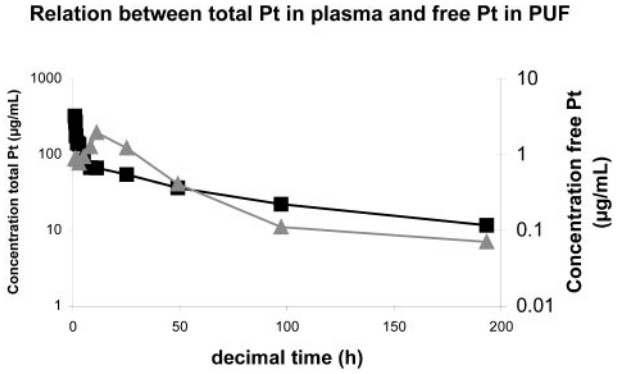

Fig. 2 Plasma concentration-time curve of total and free platinum (Pt) in plasma during course 1 in one representative patient receiving 2200 mg Pt/m^2. ▲, free Pt in plasma; ■, total Pt in plasma.

patients. Adducts were determined in four patients receiving AP5280 at a dose of 90 mg Pt/m^2, one patient receiving AP5280 at a dose of 180 mg Pt/m^2, three patients receiving AP5280 at a dose of 360 mg Pt/m^2, two patients receiving AP5280 at a dose of 720 mg Pt/m^2, two patients receiving AP5280 at a dose of 1440 mg Pt/m^2, three patients receiving AP5280 at a dose of 2200 mg Pt/m^2, six patients receiving AP5280 at a dose of 3300 mg Pt/m^2, and six patients receiving AP5280 at a dose of 4500 mg Pt/m^2 during the first course. Pt-GG concentrations were undetectable in patients receiving doses of AP5280 of 90 and 180 mg Pt/m^2. Pt-GG concentrations ranged from 70 to 1848 amol/μg DNA at the other dose levels. Pt-AG concentrations were detectable only in patients receiving doses of 3300 and 4500 mg Pt/m^2, and they ranged from 36 to 223 amol/μg DNA. Maximal platination of WBC DNA occurred at 24 h after AP5280 administration. Fig. 5 presents a scatter plot of the C_{max} of Pt-GG adducts in WBCs as a function of AUC_{0-24} of total Pt. A scatter plot of C_{max} of Pt-GG adducts in WBCs as a function of $AUC_{0\text{-}inf}$ showed a similar profile (data not shown). The concentrations of Pt-GG adducts in WBC increased with dose and/or AUC, but the relationship appeared to reach a maximum with decreasing increments of additional adduct concentrations at the higher AUC values and doses.

Three patients had a tumor that was easily accessible for a true cut biopsy. The biopsy was performed during the first cycle as close to 24 h after the infusion as possible to investigate the formation of Pt-DNA adducts in the tumor, one each at doses of 360, 3300, and 4500 mg Pt/m^2. Tumor biopsies were obtained from a s.c. lymph node of a patient with metastatic gastric carcinoma and from a patient who had a metastasis around the stoma of the colon, and, in a patient with a carcinoma of the lung, the biopsy was obtained from the lung mass itself at 48 h post-dose. The Pt-GG concentrations were measured in the tumor DNA and in the DNA of WBCs obtained by venipuncture at the same time. The data presented in Table 6 indicate that the tumor DNA adduct concentrations were 1.3–2.0-fold higher than the WBC DNA adduct concentrations at the same time point.

Response. Nineteen patients were evaluable for response. Ten patients were not considered evaluable for response, because they did not receive at least 1 cycle of drug or because they did not have measurable tumor. As shown in Table 1, five patients (17%) had stable disease. The duration of stable disease was measured from the start of the treatment until the

Table 5 Summary of pharmacokinetic parameters for platinum

Data are presented as mean ± SD. SDs were not calculated for $n < 3$.

Summary of noncompartmental pharmacokinetic parameters for total platinum

Dose (mg/m^2)	No. of subjects	T_{max} (h)	C_{max} (μg/ml)	AUC_{0-96} (μg·h/ml)	AUC_{0-inf} (μg·h/ml)	$t_{1/2}$ (h)	Cl_p (ml/h)	V_{ss} (liters)
90	4	1.1 ± 0.1	17.9 ± 6.0	226 ± 72	418 ± 167	134 ± 50	427 ± 157	33.0 ± 7.5
180	1	1.4	28.8	444	593	67	549	22.1
360	3	1.1 ± 0.1	56.7 ± 4.9	751 ± 157	1,153 ± 197	116 ± 39	621 ± 152	36.5 ± 14.2
720	2	0.9	117.1	1,426	2,616	142	456	71.0
1440	3	0.9 ± 0.2	270.8 ± 55.5	3,557 ± 854	6,118 ± 1,822	144 ± 28	488 ± 148	35.9 ± 12.2
2200	3	1.0 ± 0.0	358.3 ± 85.7	4,258 ± 1,061	7,216 ± 1,370	124 ± 40	566 ± 133	42.5 ± 16.8
3300	7	1.3 ± 0.4	439.7 ± 161.4	6,137 ± 2,690	9,604 ± 3,447	111 ± 58	691 ± 242	68.2 ± 41.1
4500	6	1.5 ± 0.4	706.5 ± 185.7	6,432 ± 1,770	10,509 ± 3,048	107 ± 38	821 ± 303	93.5 ± 39.6

Summary of noncompartmental pharmacokinetic parameters for free platinum

Dose (mg/m^2)	No. of subjects	T_{max} (h)	C_{max} (μg/ml)	AUC_{0-96} (μg·h/ml)	AUC_{0-inf} free/ AUC_{0-inf} total	AUC_{0-inf} (μg·h/ml)	$t_{1/2}$ (h)
360[b]	1	6.7	0.14				
720	2	7.7	0.54	14.3	0.6%	15.6	25.6
1440	3	14.8 ± 9.2	1.21 ± 0.47	48.9 ± 19.6	1.0% ± 0.3%	63.0 ± 29.7	44.8 ± 16.0
2200	3	8.2 ± 3.1	1.25 ± 0.66	43.5 ± 16.7	0.7% ± 0.2%	53.2 ± 18.7	44.2 ± 5.7
3300	7	19.9 ± 15.1	2.62 ± 1.19	128.3 ± 107.8	1.9% ± 1.0%	195.1 ± 186.3	68.7 ± 28.3
4500	6	11.0 ± 7.9	3.14 ± 2.14	120.8 ± 64.7	1.8% ± 0.8%	184.3 ± 109.9	86.8 ± 28.1

[a] T_{max}; time at which Cmax is reached; C_{max}, maximal drug concentration; AUC_{0-96}, area under the curve from 0 to 96 h; AUC_{0-inf}, area under the curve from 0 to infinity; $t_{1/2}$, terminal half-life; Cl_p, plasma clearance; V_{ss}, volume of distribution; AUC_{0-inf} free/AUC_{0-inf} total, AUC_{0-inf} of free platinum:AUC_{0-inf} of total platinum.

[b] Only T_{max} and C_{max} values were available for the subject that received 360 mg platinum/m^2.

criteria for progression were met. Two patients (one with non-small cell lung cancer and one with colon cancer) had stable disease for 9 weeks, two other patients (with colon and ovarian cancer) had stable disease for 12 weeks, and one other patient with non-small cell lung cancer was stable for 24 weeks. Fourteen patients (48%) showed disease progression (Table 1). There were no complete or partial responses among this heavily pretreated population of patients (Table 1).

The patient who remained stable for 24 weeks had a bronchoalveolar cell carcinoma and was treated at the 4500 mg Pt/m^2 dose level. She tolerated 8 cycles of treatment with AP5280, and during the course of treatment, she received a total of 56 g of Pt. No serious drug-related toxicity was observed until cycle 8, when she experienced CTC grade 3 vomiting, which was related to the study medication. Tumor stabilization or minimal regression was the best response, but no partial remission was reached.

DISCUSSION

Drug development efforts related to Pt agents in the past decade have focused on identifying analogs with a broader spectrum of antitumor activity and reduced myelosuppression, neurotoxicity, ototoxicity, nephrotoxicity, and nausea and vomiting (7, 8). An alternative approach to improving the therapeutic index of this class of drugs is to target the agent to the tumor. AP5280 was designed to take advantage of two independent mechanisms for attaining selectivity. The first of these is the enhanced permeability and retention effect by which high molecular weight polymers passively accumulate to high levels in tumors (14), and the second is the increased level of expression of proteases capable of cleaving the peptide linker between the polymer and the cytotoxic moiety that is characteristic of many tumors (9). The goal was to develop a drug that would remain in the systemic circulation for a prolonged period of time in an inactive form and only become activated when it encountered an unusually permeable capillary, such as those found in most solid tumors, that permitted entry into the extracellular space. Because of the poor lymphatic drainage of tumors, upon entering the tumor extracellular space the residence time of polymers such as AP5280 is prolonged, and this favors either cleavage of the linker by extracellular tumor proteases or endocytosis and cleavage by lysosomal proteases. The ability of *N*-(2-hydroxypropyl)methacrylamide polymers to passively accumulate to very high levels has been well documented in experimental tumor models (9). Consistent with these concepts, AP5280 was able to deliver 11-fold more Pt to tumor DNA than a maximally tolerated dose of carboplatin, and it exhibited a substantially better therapeutic index than the small molecule Pt drugs.[6]

The results of this Phase I trial indicate that part of the design goals for AP5280 was achieved. Large doses of Pt could be administered with only modest systemic toxicity, indicating that native AP5280 itself and any other forms of Pt resulting from its degradation have very low cytotoxic potency. The desired prolonged half-life for total plasma Pt was attained, and the anticipated dominance of renal clearance was documented. It

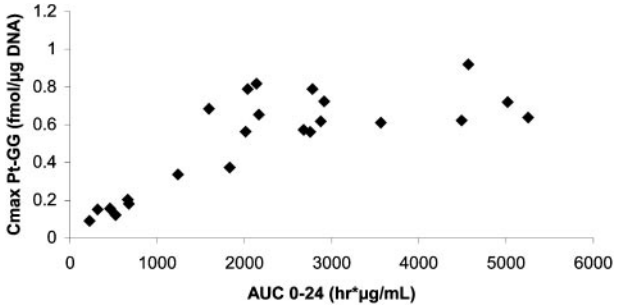

Fig. 5 The relationship between the AUC_{0-24} of total platinum in plasma and maximum concentrations of platinum-GG adducts in WBCs.

Table 6 Pt[a]-GG (amol Pt/μg DNA) concentrations in tumor tissue at 24 h after infusion compared with Pt-GG (amol Pt/μg DNA) concentrations in WBC samples

	WBC Time (h after infusion)				Tumor	
Dose (mg Pt/m^2)	0	1	24	96	24 h postinfusion	48 h postinfusion
360	0	140		156	196	
3300	0	115	920	553	1848	
4500	0	88	724	372		1271

[a] Pt, platinum.

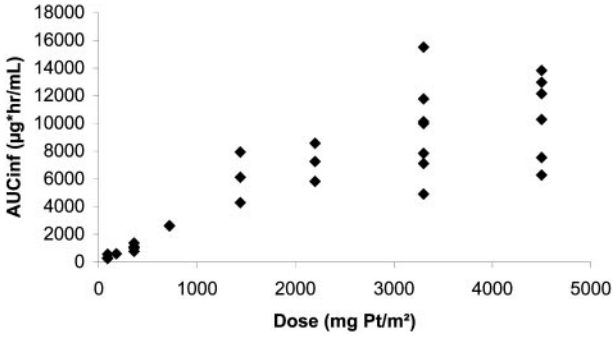

Fig. 3 AUC_{inf} values of total platinum in plasma plotted against the dose of AP5280 (in mg platinum/m^2).

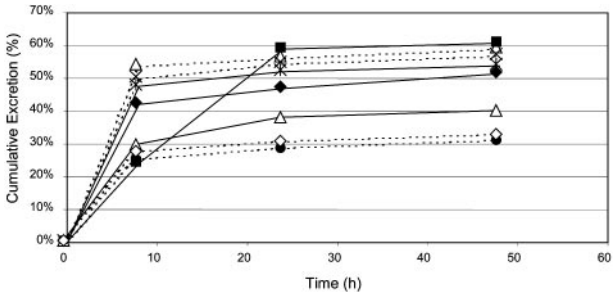

Fig. 4 Mean cumulative urinary excretion of platinum. Pt dose: —◆—, 90 mg/m^2; ···△···, 180 mg/m^2; —■—, 360 mg/m^2; ···×···, 720 mg/m^2; —∗—, 1440 mg/m^2; ···●···, 2220 mg/m^2; —△—, 3330 mg/m^2; ··· ◇ ···, 4500 mg/m^2.

remains to be determined whether AP5280 actually increases delivery of Pt to tumor DNA in man and has a superior therapeutic index relative to low molecular weight Pt drugs in cancer patients.

When AP5280 was administered as a 1-h i.v. infusion once every 3 weeks, the MTD was 4500 mg Pt/m^2. This ranges from 13- to 45-fold higher than the MTD values of 100 mg Pt/m^2 reported for cisplatin, 360 mg Pt/m^2 for carboplatin, and 120 mg Pt/m^2 for oxaliplatin (23–25). The DLT was nausea and vomiting that could not be adequately controlled with prophylactic anti-emetic therapy. Even at the MTD of 4500 mg Pt/m^2, there were few other types of adverse events, and those that occurred were mild. It is noteworthy that at the recommended Phase II dose of 3300 mg Pt/m^2 and even at the MTD of 4500 mg Pt/m^2, there was little evidence of the adverse events that are typically produced by treatment with cisplatin, carboplatin, or oxaliplatin. In particular, the frequency of hematological, neurological, or renal toxicity was low. The fact that AP5280 produced such modest toxicity to other tissues at the dose that produced uncontrollable nausea and vomiting suggests that, relative to its access to other normal tissues, AP5280 has relatively greater access to the chemoreceptor trigger zone of the hypothalamus than the low molecular weight Pt-containing drugs.

After infusion of AP5280, several different forms of Pt-containing compounds are present in plasma, including the Pt in native AP5280, Pt bound to plasma proteins, and low molecular weight forms that have not been characterized. As performed in this study, the measurement of total Pt included the native drug and all other Pt-containing molecules, whereas the measurement of Pt in the ultrafiltrate generated using a M_r 3000 cutoff filter included only molecules containing Pt released from the AP5280. The results of the drug measurements established a number of important points about the pharmacokinetic behavior of AP5280. The kinetics were linear with dose, the terminal half-life was found to be very prolonged relative to that of the low molecular weight Pt drugs, and the clearance was found to be low and almost entirely renal. Total plasma clearance of AP5280 averaged 0.64 liter/h, which is very low compared with a clearance of 20 liters/m^2/h reported for cisplatin (23). The terminal half-life appeared to increase with dose; however, this may be an artifact due to the fact that the concentration of ultrafilterable Pt remained above the lower limit of quantitation for a longer period of time in patients receiving the larger doses. The long terminal half-life is considered advantageous because under circumstances where the rate of uptake of drug from the extracellular fluid into the tumor cell is rate-limiting, continuous delivery of AP5280 over a relatively long period of time may enhance intracellular drug delivery. A mean of only 44% of the administered Pt appeared in the urine in the first 48 h after infusion. Presumably because of the prolonged plasma half-life, some additional Pt was excreted via the renal route over the ensuing days, but it appears that, as for the low molecular weight Pt drugs, a large fraction of the administered Pt remains in the body. The fact that no plasma Pt accumulation was noted on sequential cycles of treatment indicates that this residual Pt must be largely in tissues. Circulating Pt thus may not be pharmacologically active and does not contribute to biological activity, otherwise we would have seen more significant renal toxicities and nonrenal clearance due to tissue binding. Fecal elimination of Pt was not discussed, but it cannot be ruled out. Moreover, elimination of the total dose of Pt does not seem to have been determined.

The plasma concentrations of ultrafilterable Pt were low and highly variable both within and between patients, and thus the pharmacokinetic parameters for ultrafilterable Pt were less accurately estimated than those for total plasma Pt. Nevertheless, the data support several conclusions. The concentration of ultrafilterable Pt was much lower than that of total plasma Pt at all time points. As a result, the AUC for ultrafilterable Pt averaged only 4% of that for total plasma Pt. The low AUC for ultrafilterable Pt relative to total plasma Pt may also be a function of the stability of the intact AP5280 molecule and slow release of low molecular weight Pt species from the polymer. The C_{max} for ultrafilterable Pt occurred substantially later than the C_{max} for total Pt, consistent with the relatively slow release of Pt from the *N*-(2-hydroxypropyl)methacrylamide copolymer. Even though the AUC for ultrafilterable Pt from AP5280 at doses of 720 mg Pt/m^2 or higher (16–184 μg/h/ml) far exceeded that with cisplatin administration at a dose of 80 mg Pt/m^2 [1.1–3.8 μg/h/ml (26–28)], Pt adduct concentrations achieved with AP5280 (70–1848 amol/μg DNA) are much lower than those achieved with cisplatin (0.66–2.05 fmol/μg DNA), indicating that low molecular weight Pt-containing species present in the plasma after injection of AP5280 are largely inactive. This is consistent with the clinical observation that enormous doses of this drug can be given without excessive toxicity. Prior studies using cisplatin have documented that the extent of Pt adduct formation in the DNA of peripheral WBCs is correlated with unbound Pt concentration, indicating that adduct concentrations can be used as a surrogate measure of the amount of free reactive Pt species generated in the plasma compartment (29, 30).

Tumor was available for biopsy and determination of Pt-GG concentrations in DNA from only three of the patients in this trial. These patients received doses of 360, 3300, and 4500 mg Pt/m^2 AP5280. Tumor Pt-GG adduct concentrations (196–1848 amol Pt/μg DNA) were 1.3–2.0-fold higher than those in WBCs harvested at the same time. For comparison, after administration of carboplatin at a dose sufficient to produce an AUC of 4–5 min·mg/ml, Pt-GG concentrations in tumors (0.3–1.0 fmol Pt/μg DNA) were 3–5-fold higher than those in WBCs [0.1–0.2 fmol Pt/μg DNA (31)], and after administration of cisplatin at a dose of 100 mg Pt/m^2, Pt-GG concentrations in tumors were 4-fold higher than those in WBCs (32). However, the number of patients assayed is too small to make any determination regarding whether AP5280 can increase overall Pt delivery to the tumor.

In conclusion, the recommended dose for AP5280, when given as a 1-h i.v. infusion once every 3 weeks is 3300 mg Pt/m^2. The design goal of producing a tumor-targeting polymer platinate with a prolonged plasma half-life but no activation while in the systemic circulation has been achieved. AP5280 displays a markedly different pharmacokinetic behavior as compared with the small molecule Pt compounds. Consequently, its toxicity profile differs from that of cisplatin and carboplatin. Renal toxicity, neurotoxicity, and myelosuppression were minimal for AP5280. Additional clinical trials are now under way to

determine whether this drug can actually increase delivery of the cytotoxic Pt moiety to human tumors.

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