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ORIGINAL ARTICLE

Phase I and pharmacokinetic trial of AP5346, a DACH–platinum–polymer conjugate, administered weekly for three out of every 4 weeks to advanced solid tumor patients

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Abstract
Purpose To determine the maximum tolerated dose (MTD) safety and pharmacokinetics of AP5346, a copolymer-linked 1,2-diaminocyclohexane(DACH)-platinum compound, in advanced solid tumor patients.
Experimental design AP5346 was administered as a 1-hour IV infusion on days 1, 8, 15 of a 28-day cycle. Seven dose levels (DL) were explored: DL1: 40 mg platinum (Pt)/m² (1 patient); DL2: 80 (1); DL3: 160 (3); DL4: 320 (3); DL5: 640 (6); DL6: 850 (6); DL7: 1280 (6) mg Pt/m^2. Dose-limiting toxicity (DLT) included infusion omission and cycle delay >2 weeks.
Results Twenty-six patients received 41 cycles (median 1/patient, range 1–4). No DLT occurred in DL 1–4; 1 DLT in DL5 (RD; renal insufficiency), two in DL6 (MTD; vomiting; fatigue) and 5 in DL7 (neutropenic infection with diarrhea; neutropenia with vomiting; vomiting with fatigue; renal insufficiency; and fatigue). Two deaths occurred due to renal insufficiency (DL5); in both cases patients had disease in or surrounding genitourinary tract whose contribution could not be accurately discerned. Grade 1-2 creatinine abnormalities occurred in seven patients. Nausea/emesis was frequent (92%), reaching grade 3-4 (23%), but controlled by antiemetics. Grade 2-4 allergic reactions occurred in 4 patients. C_{max} and AUC increased linearly with dose for total plasma platinum and ultrafiltrate platinum. Antitumor activity included two partial responses in metastatic melanoma and ovarian cancer, and an additional CA-125 normalization (from 133 IU/l) in a suspected ovarian cancer.
Conclusions AP5346 administered weekly for 3 weeks out of every four is tolerated up to a dose of 640 mg Pt/m² on the first cycle when given with antiemetic prophylaxis. The pharmacokinetics of AP5346 indicates a prolonged half-life, and evidence of antitumor activity was observed at this dose level.

Keywords AP5346 · Phase I trial · Pharmacokinetics · Drug delivery

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Introduction

Platinum-based compounds have been extensively explored in cancer therapy, with cisplatin, and its analogue carboplatin, having become components of

standard treatment regimens for a wide range of cancers. Their well-characterized dose-limiting toxicities, including nephrotoxicity, neurotoxicity, and ototoxicity for cisplatin and myelosuppression for carboplatin, and the frequency of intrinsic or acquired resistance to these fully cross-resistant analogues, places limits on their use [8]. In addition, the analysis of dose-intensification with either cisplatin or carboplatin suggests not only a plateau in their efficacy, as illustrated by response rates reported in testicular and ovarian cancers, but also a marked increase in toxicity with a limited therapeutic index [14, 16]. These limitations have motivated the search for new platinum analogs with different spectra of antitumor activity and reduced toxicity. The last three decades have seen the synthesis and investigation of several thousand platinum derivatives including compounds containing a 1,2-diaminocyclohexane (DACH) carrier ligand. These were recognized as early as 1980 as not being cross-resistant with cisplatin and have received the more attention in recent years. Oxaliplatin, the only DACH platinum to have received marketing approval, has demonstrated antitumor activity in some cell lines with intrinsic or acquired cisplatin/carboplatin resistance [20]. It is also active against cell lines with mismatch repair (MMR) deficiency, which is associated with primary or secondary resistance to cisplatin and carboplatin [1, 26]. The dose-limiting toxicity of oxaliplatin is also distinctive, consisting of a particular form of cumulative acral paraesthesia/dysesthesia, exacerbated by cold, which can become persistent, and frequently results in treatment discontinuation in responding patients.

Efforts to increase the therapeutic index of cytotoxic agents has also proceeded via attempts to improve delivery of drugs to the tumor with one avenue being to couple the cytotoxic agent to a macromolecule in order to take advantage of the 'enhanced permeability and retention' (EPR) effect [11]. The capillaries of growing tumors are frequently hyper-permeable to circulating macromolecules. In combination with the fact that tumors usually have very poorly developed lymphatics, once a macromolecule escapes from vascular circulation into the tumor extracellular fluid it can be trapped and concentrated in the tumor [22]. Concentrations of drugs conjugated to such polymers can reach levels that are 10–100 times higher in tumor tissue than those attained following administration of free drug [5]. In addition, conjugation to the polymer both protects it while in the systemic circulation and renders it inactive until it reaches the tumor to ensure that overall toxicity to non-malignant tissues may be reduced [5].

AP5346 is a rationally designed, macromolecular carrier to which a DACH-platinum is bound to a polymer via a pH-sensitive chelate (Fig. 1). The water-soluble biocompatible polymer backbone is a 90:10 random copolymer of: *N*-(2-hydroxypropyl) methacrylamide (HPMA) and a methacrylamide monomer substituted with a triglycine aminomalonate group providing the primary binding site for the DACH-platinum moiety [19]. The conjugate contains approximately 10% platinum by weight and has a molecular weight of 25 kDa, which was selected to allow glomerular filtration while being large enough to benefit from EPR. While negligible in neutral solutions, release of the DACH-platinum moiety is increased at low pH so that platinum release is favored in environments such as the extracellular space of hypoxic tumors and the intracellular lysosomal compartment [15].

The antitumor activity of AP5346 has been evaluated in several syngeneic murine and human tumor xenograft models [18]. In C57BL/6 mice bearing subcutaneously implanted B16F10 tumors the maximum tolerated dose (~10% weight loss at the nadir) of oxaliplatin, given as a single IP injection, was 5 mg Pt/kg and the maximum tolerated dose of AP5346 was 100 mg Pt/kg. AP5346 produced greater tumor growth inhibition and prolonged growth delay compared to equitoxic doses of oxaliplatin and/or carboplatin in B16 melanoma, Lewis lung, 2008 ovarian and HT-29 colorectal (10, 12). Detailed pharmacokinetic studies in the B16 melanoma model demonstrated that a single dose of AP5346 delivered 16-times more platinum to the tumor, and 14 times more platinum to tumor DNA, than an equitoxic dose of free oxaliplatin (12). The C_{max} was 154 μg Pt/ml and the terminal half-life was 23.3 h. In in vivo preclinical models AP5346 exhibited a toxicity profile typical of platinum compounds, with the dose-limiting toxicity being impairment of renal function and myelosuppression (10). No adverse events outside of those already identified for platinum cytotoxic agents were observed.

We report here the first evaluation of AP5346 in humans in a dose-finding Phase I trial of 1-hour infusions administered weekly for three out of every 4 weeks. This schedule was selected based on the observation that efficacy and drug delivery to the tumor and tumor DNA improved with the more prolonged exposure attainable with weekly dosing of this long-half drug, and prior clinical experience with AP5280, a polymer carrying cisplatin rather than a DACH platinum [17]. The primary objective was to determine the pattern of adverse events and the maximum tolerated dose (MTD) in patients with advanced solid tumors. The secondary objectives were to determine the recommended dose for subsequent Phase II studies, to characterize the pharmacokinetic

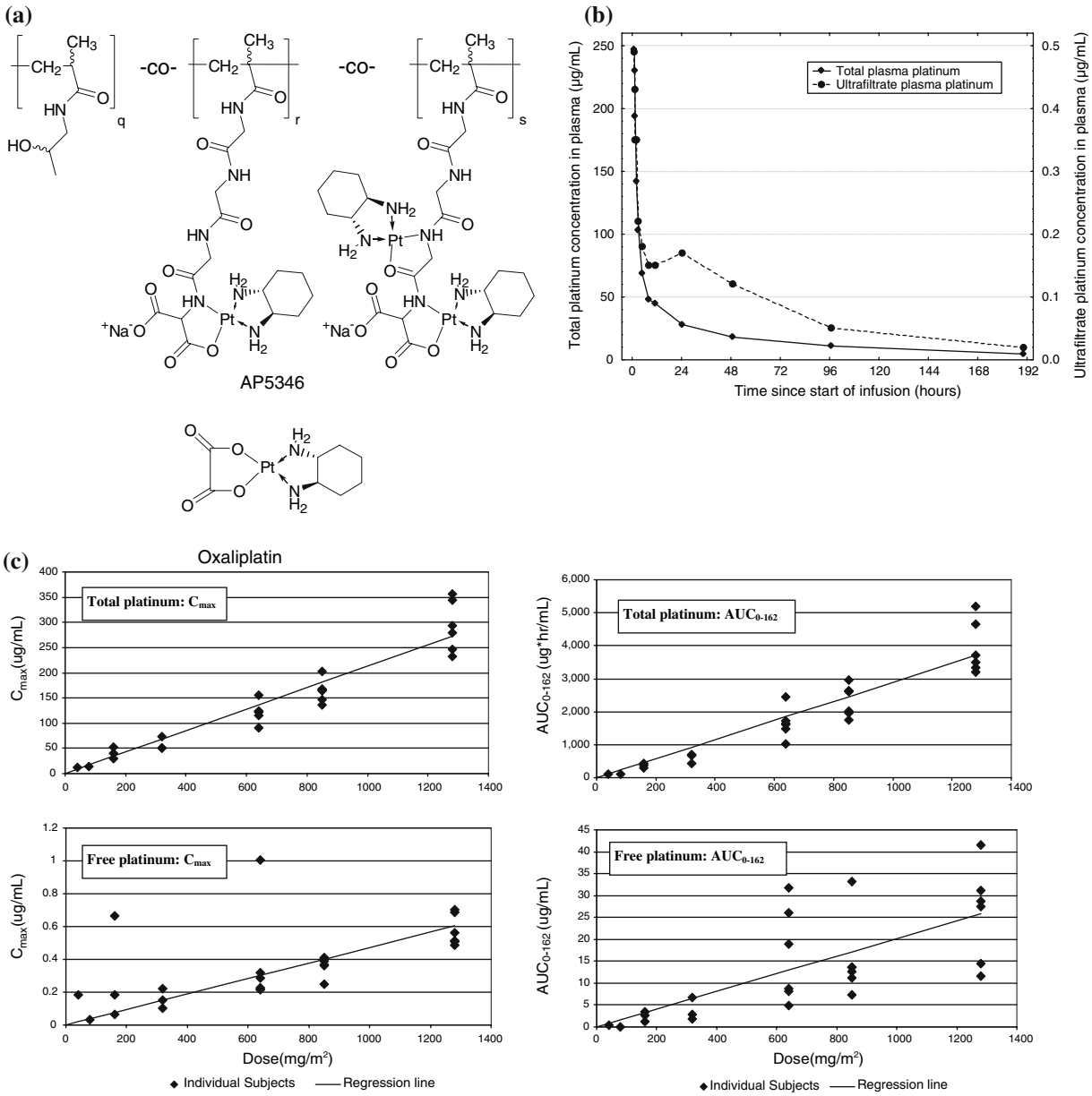

Fig. 1 **a** Chemical structure of oxaliplatin and partial chemical structure of AP5346, a random copolymer conjugated with a DACH-platinum, where Q:R:S is approximately 36–47:2.3:1. **b** Characteristic profile of total and ultrafiltrate platinum concentrations in plasma over the first week of treatment (*dose level 7*) **c** C_{max} and $AUC_{0\text{-}1wk}$ of individual patients for total and ultrafiltrate plasma platinum according to dose level with linear regression lines

profile and to undertake a preliminary assessment of the antitumor activity.

Patients and methods

Patient selection

Patients were to have histologically or cytologically confirmed advanced solid tumors that had failed all therapy of established merit, and meet all the following criteria: age ≥18 years, WHO performance status ≤2, life expectancy ≥3 months, discontinuation of all previous anticancer treatments for at least 4 weeks before first dose of study drug (6 weeks for nitrosoureas, melphalan and mitomycin C), discontinuation of all prior radiotherapy (8 weeks for extensive prior radiotherapy), use of effective contraceptive methods, adequate bone marrow function (absolute neutrophil count $\geq 1.5 \times 10^9$/l, platelet counts $\geq 100 \times 10^9$/l), hepatic

function (serum bilirubin ≤1.5× the upper limit of normal (ULN); aspartate aminotransferase and alanine aminotransferase ≤3× ULN), renal function (serum creatinine and blood urea to be within normal limits and creatinine clearance, calculated according to the Cockroft–Gault formula, >50 ml/min). Patients were ineligible in the event of clinical signs of brain metastases and/or leptomeningeal involvement by tumor, prior nephrectomy, National Cancer Institute—Common Toxicity Criteria (NCI-CTC version 2) grade 2-4 neuropathy or hearing loss ≥ grade 2, or patients with active uncontrolled infection or other serious illness. All patients gave written informed consent and the study protocol was approved by the appropriate ethics committees.

Drug administration and dose escalation

AP5346 was administered in a 1 h IV infusion on days 1, 8, 15 of a 28-day cycle. Seven patients in dose levels 5–7 received hydration and bicarbonate to alkalinize the urine as prophylaxis against potential renal toxicity; this consisted of inpatient IV hydration with 2 l of 4% sodium bicarbonate (over 12 h pre-infusion and over 20–22 h post-infusion) and sodium bicarbonate 1 g PO four times daily between infusions. Antiemetic prophylaxis, when administered, was not standardized and varied between centers and from patient to patient. The day 8 or 15 infusion was to be omitted in the event of grade 3 thrombocytopenia, neutropenia or leukopenia. Cycles could be delayed up to 2 weeks for recovery of toxicity to baseline. Patients were to discontinue treatment in the event of grade 3 neurotoxicity or any grade 4 toxicity, unless there was evidence of clinical benefit, in which case the patient could continue treatment at the next lower dose level.

The starting dose was 40 mg Pt/m^2 per infusion, one-tenth of the MTD in the most sensitive animal species (female mice). AP5346 is approximately 10% Pt by weight; all doses are reported as the amount of Pt injected per meter of estimated body surface area. One patient per dose level (DL) was to be treated and dose escalation was to be undertaken in 100% increments until the occurrence of grade 1 non-hematologic toxicity (excluding alopecia and untreated nausea/vomiting) or grade 2 hematologic toxicity (excluding anemia) using an accelerated titration design [23]. Subsequently, three patients were to be entered at each dose level and dose escalation was to be undertaken in 50% increments until grade 2-4 non-hematologic toxicity or grade 3-4 hematologic toxicity occurred, after which dose escalation was to be by 20–33% increments. Intrapatient dose-escalation was not allowed.

DLT was defined as ≥grade 2 neurotoxicity lasting more than 2 weeks, or grade 3 neurotoxicity of any type, ≥grade 3 other non-hematologic toxicity, grade 4 neutropenia lasting >5 days or complicated by fever or infection, grade 4 thrombocytopenia, inability to receive three weekly consecutive doses of the drug for toxicity reasons which were not dose-limiting, inability to receive a second course of therapy within 2 weeks of day 29 for reasons of persistent toxicity. If a DLT occurred during the first cycle in the initial cohort of 1–3 patients, a total of six patients were to be treated at that dose level. Patients who received fewer than three infusions and did not experience DLT were not evaluable for determination of Maximum Tolerated Dose (MTD). The MTD was defined as the dose resulting in DLT during the first cycle in at least two of six patients. The next lower DL was defined as the dose that would be recommended for phase II trials (RD).

Clinical assessments

Before inclusion in the study and immediately before each treatment cycle, a physical examination, evaluation of WHO performance status and vital signs, neurological exam, complete blood count (CBC), serum biochemistry, calculation of creatinine clearance, urinalysis (including β-microglobulin) and tumor marker assessment were performed. CBC, serum biochemistry and standard urinalysis were also performed weekly. Toxicity was assessed according to NCI-CTC Version 2.0. An audiogram was obtained at baseline and was to be repeated in case of suspected hearing loss. Disease assessment was performed every second cycle. Disease response was evaluated according to RECIST criteria [24].

Pharmacokinetics and pharmacodynamics

Plasma samples for pharmacokinetic assays were obtained prior to each infusion (trough levels), at 15 and 30 min, and at 1, 2, 4, 7, 10, 24, 48, 96 h after the end of the first infusion. Ultrafiltrate platinum was separated using Centricon YM3 filters with a 3 kDa cut-off (Millipore Corp., Billerica, MA). Total and ultrafiltrable platinum plasma concentrations were assayed using a validated flameless atomic absorption spectrometry (FAAS) method, with lower limits of quantification of 0.1 and 0.02 μg/ml, respectively. Pharmacokinetic parameters were calculated from plasma concentration-time data using non-compartmental methods with WinNonlin version 3.1 (Pharsight, Cary, NC). The maximum observed plasma concentration (C_{max}) was determined by visual inspection of the data. The area

under the plasma concentration-time curve from time 0 to the last measurable concentration prior to the day 8 infusion ($AUC_{0\text{-}1wk}$) was calculated using the linear trapezoidal method. The terminal phase elimination rate constant (K_{el}) was estimated from the slope of least square regression analysis of the concentration-time data using the last three or four quantifiable concentrations before the day 8 infusion. The terminal phase half-life ($T_{½}$) was calculated as $0.693/K_{el}$. $AUC_{0\text{-}\infty}$ was computed as $AUC_{0\text{-}t}$ plus the extrapolation from the last time point to infinity using C_t/K_{el}, where C_t is the last quantifiable concentration prior to day 8 infusion. The total body clearance (CL_p) was calculated as $Dose/AUC_{0\text{-}\infty}$ and the apparent volume of distribution (V_z) as $Dose/(AUC_{0\text{-}\infty} \times K_{el})$.

Results

Between April 2003 and December 2004, 26 patients were entered from two different cancer centers and treated at seven dose levels: DL1: 40 mg Pt/m², one patient; DL2: 80 mg Pt/m², one patient; DL3, 160 mg Pt/m², three patients; DL4, 320 mg Pt/m², three patients; DL5, 640 mg Pt/m², six patients; DL6, 850 mg Pt/m², six patients; DL7, 1,280 mg Pt/m², six patients. All patients were eligible. As shown in Table 1, the patient characteristics were comparable at each dose level. The most frequent tumor types entered in the study were melanoma (five patients), breast cancer, ovarian cancer (four patients each) and adenocarcinoma of unknown primary (three patients).

Table 1 Patient characteristics

Number of patients treated	26
Sex	
Female	14
Male	12
Median age (range)	50 (32–74)
Performance status (WHO)	
0	11
1	13
2	2
Primary tumor type	
Melanoma	5
Breast	4
Ovarian	4
Adenocarcinoma	3
Prostate	2
Other[a]	8
Number of prior chemotherapy regimens	1.5 (0–3)
Prior platinum chemotherapy	13

[a] Cervix, esophageal, NSCLC, pancreas, renal, thyroid, Ewing sarcoma, urachus

Treatment

Forty-one cycles and 106 infusions were administered for a median of one cycle per patient (range, 1–4) and three infusions per patient (range, 1–12). Patients in dose levels 2–5 received a median of two cycles. Only two patients received three or four cycles. Treatment was usually administered according to the planned schedule, with a median relative dose intensity of 92% (range, 33–99%). Ten infusions (9%), distributed over all dose levels, were delayed, omitted or interrupted due to treatment toxicity; for allergic reactions following four infusions and creatinine elevations in three. Two patients (9% of patients receiving >1 cycle), both treated at the highest dose level, required dose reductions one for neutropenic infection with diarrhea and one for transiently reduced creatinine clearance. Eleven patients (43%) discontinued treatment due to disease progression, and 9 (35%) due to treatment-related adverse events that included renal toxicity in three cases, allergic reaction and fatigue in two cases each, and neutropenia and nausea/vomiting in one case each.

Dose escalation

Per protocol the MTD was to be defined on the basis of adverse events occurring on the first cycle of AP5346 treatment. As shown in Table 2, no DLT were observed in dose levels 1–4. One patient was entered at each of dose levels 1 and 2 during the accelerated titration phase, and three patients were entered at dose levels 3 and 4 following the observation of grade 1 non-hematological toxicity in DL2. No DLT were observed during the first cycle of the initial cohort of patients at DL5 (640 mg Pt/m²) and escalation proceeded to DL7 (1,280 mg Pt/m²). DL7 was found to be excessively toxic, with two of the first three patients and three of the subsequently entered three patients (total five out

Table 2 Dose-limiting toxicity

	DL 1–4	DL5	DL6	DL7
Number of patients treated	8	6	6	6
Number of evaluable patients	7	6	5	6
Number of patients with DLT in first cycle	–	1	2	5
Type of DLT[a]				
Neutropenia grade 4	–	–	–	2
Renal toxicity grade 2-4	–	1	–	1
Nausea/ vomiting grade 3	–	–	1	2
Diarrhea grade 3	–	–	–	1
Fatigue grade 3	–	–	1	3

[a] A single episode of dose-limiting toxicity could involve more than one toxicity type

of six patients) experiencing a DLT consisting of two cases of grade 4 neutropenia, complicated by diarrhea and infection in one case and by grade 3-4 vomiting, nausea, fatigue, spastic ileus, hypokalemia and hypoalbuminemia (18 g/l) in the other, and one case each of renal insufficiency (with concomitant decreased plasma bicarbonate), grade 3 fatigue and grade 3 nausea, vomiting and fatigue.

An intermediate dose level (DL6, 850 mg Pt/m^2) was then explored; among the five evaluable patients treated at this dose level, two had DLT (grade 3 vomiting; grade 3 fatigue) on the first cycle. To more accurately define the MTD, an additional four patients were treated at DL5 (640 mg Pt/m^2). Of the five evaluable patients treated at DL5, two developed renal insufficiency and subsequently died, one after the first cycle and one after the third cycle, but in both cases kidney function appeared to be impaired by their underlying disease making it difficult to assess the toxicity of AP5346 to the renal injury. The patient who died after the third cycle had no evidence of nephrotoxicity on the first two cycles, nor did the other three patients treated at this dose level. The investigators concluded that DL5 is the current best estimate of a dose appropriate for subsequent trials on the day 1, 8 and 15 schedule in patients receiving IV hydration.

Toxicity

Gastrointestinal toxicity was frequent, with 24 patients (92%) experiencing nausea, vomiting, diarrhea or anorexia, which reached grade 3-4 in six patients (23%). The incidence of emesis was dose related, with grade 2-4 nausea/vomiting reported in 9, 19 and 45% of infusions at dose levels 1-4, 5 and 6-7, respectively. Antiemetic prophylaxis was effective in reducing emesis when administered in an adequate regimen, defined as ≥2 mg granisetron or equivalent with ≥10 mg dexamethasone or equivalent. The rate of grade 2-4 nausea/vomiting in the 30 infusions administered with adequate prophylaxis was 0 and 31% in dose levels 5 and 6-7, respectively (Table 3).

Renal toxicity occurred in nine patients (35%), being characterized mostly by transient grade 1-2 elevations of serum creatinine or decrease of creatinine clearance. Two patients (8%) had grades 3 or 4 creatinine abnormalities that were accompanied by anuria, spastic ileus, hypokalemia and/or oliguria, and resulting in death due to renal insufficiency, considered possibly or probably related to treatment. One of the deaths occurred following the first cycle in a context of progression of a pre-existing bulky renal lesion with renal vein thrombosis; the other occurred following the third cycle of treatment at which time the patient was experiencing

Table 3 Treatment-related adverse events, worst grade per patient

	Dose level									
	1–4		5		6		7		Total	
	Any grade	Grade 3-4	Any grade	Grade 3-4	Any grade	Grade 3-4	Any grade	Grade 3-4	Any grade	Grade 3-4
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N* (%)	*N* (%)
Abdominal pain	2	–	1	–	1	–	–	–	4 (15)	–
Anorexia	2	–	2	1	1	–	1	–	6 (23)	1 (4)
Dehydration	–	–	1	–	–	–	1	–	2 (8)	–
Diarrhea	2	1	2	–	–	–	2	1	6 (23)	2 (8)
Dry mouth	1	–	1	–	–	–	–	–	2 (8)	–
Ileus (spastic)	–	–	1	1	1	–	1	1	3 (12)	2 (8)
Nausea/vomiting	8	–	6	1	4	1	6	3	20 (80)	5 (20)
Weight decreased	–	–	–	–	–	–	2	–	2 (8)	–
Stomatitis	1	–	1	–	1	–	1	–	4 (15)	–
Allergic reaction	2	–	2	2	–	–	–	–	4 (15)	2 (8)
Fatigue	3	–	4	1	1	1	5	3	13 (50)	5 (19)
Pyrexia	1	–	1	1	–	–	–	–	2 (8)	1 (4)
Renal failure	–	–	–	–	–	–	3	–	3 (12)	–
Anxiety	1	–	1	–	–	–	–	–	2 (8)	–
Dizziness	–	–	1	–	–	–	1	–	2 (8)	–
Headache	–	–	2	–	–	–	–	–	2 (8)	–
Sensory neuropathy	2	–	1	–	–	–	–	–	3 (12)	–

progression of para-aortic lymph nodes that was considered a possible concomitant cause of renal insufficiency. The toxicity appeared to be dose-related, with 1 of 8 patients in DL 1–4 experiencing toxicity versus 8 of 18 in DL 5–7. The median day of first occurrence of highest creatinine levels was day 29 (range, 2–124), corresponding to the end of the first cycle. There is evidence that hydration and urine alkalization with IV sodium bicarbonate pre- and post-infusion, employed in seven patients in dose levels 5–7 was effective in restricting the renal abnormalities to grade 1, with no cases of grade 2-4 occurring, versus five cases (45%) in the 11 patients treated in DL 5–7 without IV renal prophylaxis. Neither of the patients with possible treatment-related death received the full renal prophylactic regimen.

The myelosuppressive effect of AP5346 was moderate with no neutropenia observed below DL7, and thrombocytopenia grade 1-2 occurring in only 4 patients (20%) below that dose level (Table 4). Allergic reactions occurred during infusion in four patients (15%) in dose levels 3–5, reaching grade 3-4 in two cases, with one episode of anaphylactic shock and respiratory arrest in a patient with a history of allergy to carboplatin. No ototoxicity was reported, and there were three cases of treatment-emergent grade 1 sensory neuropathy of 1-day duration.

Pharmacokinetics

Pharmacokinetics were assessed in 26 patients in DL 1–7 following the first infusion, with weekly trough samples obtained up to the end of cycle 2 (Table 5). As shown in Fig. 1, total plasma platinum exhibited biphasic elimination, while ultrafiltrate platinum concentrations displayed a secondary peak at 24 h post-infusion. C_{max} and $AUC_{0\text{-}1wk}$ for total plasma platinum increased linearly with dose over the entire range of doses studied for total plasma platinum (Fig. 1). The correlation coefficients for C_{max} and $AUC_{0\text{-}162}$ were 0.903 and 0.870, respectively, indicating excellent correlation of the parameters for total platinum with dose. For the ultrafiltrate platinum the correlation coefficients were 0.346 and 0.511 for C_{max} and $AUC_{0\text{-}162}$, respectively, indicating moderate correlation of the pharmacokinetic parameters with dose.

Only a very small fraction of the total platinum was present free in the plasma, with the ultrafiltrate platinum $AUC_{0\text{-}1wk}$ representing, on average, only 0.6% of the total platinum AUC. Terminal half-life did not appear to vary with dose for total platinum or for ultrafiltrate platinum at doses of $\geq$320 mg/m². Mean terminal half-life was 72.3 ± 16.9 h for total platinum and 56.7 ± 14.7 h for ultrafiltrate platinum at does $\geq$320 mg/m². Only moderate inter-patient variability was observed, with a coefficient of variation generally in the range 10–30% for all parameters. In patients assessed for trough concentrations prior to subsequent infusions, total platinum was detectable in all patients, while ultrafiltrate platinum was only detected at or above 640 mg/m². Trough concentrations 7 days after infusion 2 and after infusion 3 were significantly higher than after the first infusion for total platinum (Wilcoxon $P = 0.002$ and 0.008, respectively) and for ultrafiltrate platinum (dose levels 5–7, $P = 0.018$ and

Table 4 Treatment-emergent hematological and biochemical abnormalities, worst grade per patient

	Dose level									
	1–4		5		6		7		Total	
	Any grade	Grade 3-4	Any grade	Grade 3-4	Any grade	Grade 3-4	Any grade	Grade 3-4	Any grade	Grade 3-4
	N	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N* (%)	*N* (%)
Anemia	4	–	4	1	1	–	4	1	13 (50)	2 (8)
Leukopenia	–	–	–	–	–	–	2	2	2 (8)	2 (8)
Neutropenia	–	–	–	–	–	–	2	2	2 (8)	2 (8)
Thrombocytopenia	1	–	2	–	1	–	4	–	8 (31)	–
Alkaline phosphatase	2	–	4	3	2	–	1	–	9 (35)	3 (12)
ALT	2	–	–	–	2	–	1	–	5 (19)	–
AST	1	–	2	–	2	1	1	–	6 (23)	1 (4)
Bilirubin	1	–	1	–	1	–	–	–	3 (12)	–
Serum creatinine	–	–	3	1	–	–	4	–	7 (27)	1 (4)
Calculated creatinine clearance	1	–	–	2	1	–	5	–	7 (27)	2 (8)
Bicarbonate	1	–	2	–	1	–	2	1	6 (23)	1 (4)
Inorganic phosphorus	–	–	4	3	4	2	3	2	11 (42)	7 (27)
Albumin	2	–	3	1	–	–	5	1	10 (38)	2 (8)

Table 5 Pharmacokinetics of total and ultrafiltrate plasma platinum

	Dose level (mean ± SD)						
	1	2	3	4	5	6	7
Number of patients assessed	1	1	3	3	6	6	6
Total plasma platinum							
C_{max} (μg/ml)	11.7	14	41 ± 12.1	58.1 ± 13.1	122 ± 21	164 ± 23	292 ± 50
$AUC_{0\text{-}1wk}$ (μg h/ml)	108	119	351 ± 45	607 ± 143	1705 ± 463	2460 ± 461	3986 ± 788
$AUC_{0\text{-}\infty}$ (μg h/ml)	128	144	454 ± 57	780 ± 230	2017 ± 485	2767 ± 514	4585 ± 909
$T_{½}$ (h)	71.8	72.6	83.4 ± 25.3	87.7 ± 18.6	74.6 ± 16.8	65.8 ± 16.6	63 ± 10.1
V_z (l)	50.8	98.4	73.8 ± 26.1	91.6 ± 7.4	67.8 ± 27.9	57 ± 19.5	47.2 ± 17.7
Clearance (l/h)	0.49	0.94	0.62 ± 0.11	0.75 ± 0.19	0.64 ± 0.25	0.60 ± 0.16	0.51 ± 0.13
Ultrafiltrate plasma platinum							
C_{max} (ng/ml)	0.19	0.03	0.31 ± 0.32	0.16 ± 0.06	0.38 ± 0.31	0.36 ± 0.06	0.58 ± 0.10
$AUC_{0\text{-}1wk}$ (ng h/ml)	0.39	0.07	2.44 ± 1.22	3.77 ± 2.56	16.9 ± 11.0	16.3 ± 9.3	26.1 ± 11.2
% of $AUC_{0\text{-}1wk}$ for total Pt	0.4	0.1	0.6 ± 0.2	0.6 ± 0.3	0.9 ± 0.5	0.7 ± 0.3	0.7 ± 0.3
$T_{½}$ (h)	6.36	ND	14.5	60.7 ± 33.0	51.8 ± 8.7	57.2 ± 13.9	59 ± 10.9

ND not determinable

0.043, respectively), with median increases >30 and >50%, respectively. Trough concentrations after infusion 3 of cycle 1 showed a tendency to be higher than after infusion 2 ($P = 0.069$ for total platinum and 0.080 for ultrafiltrate). The trough value after infusion 1 of cycle 2 was higher than after infusion 1 of cycle 1 for total platinum ($P = 0.028$). Total platinum was detected in all patients at day 1 of cycle 2, after 2 weeks without an administration, but ultrafiltrable platinum was undetectable in three out of four assessed patients in dose levels 5–7.

Activity

Sixteen patients were evaluable for response to treatment; three patients did not have measurable disease, four patients received fewer than three infusions and discontinued treatment for a reason other than progression, and three patients were not adequately assessed. Evidence of antitumor activity was observed in dose levels 4–7. Two patients obtained a partial response as defined by the RECIST guidelines. One was a woman with ovarian cancer who received first line chemotherapy with carboplatin and paclitaxel and had a 5-year-platinum-free interval and was subsequently treated with carboplatin, paclitaxel, pegylated liposomal doxorubicin, and topotecan. She was treated in dose level 5, and had disappearance of a liver lesion, response of lymph node lesions and CA-125 tumor marker decrease from 83 to 40 IU/l lasting 3.3 months. She discontinued treatment after two cycles due to anaphylactic shock. The second was a man with extensive metastatic melanoma who had received prior therapy with dacarbazine. He was treated in dose level 7 and experienced a 78% decrease of skin, lung and lymph node lesions that lasted 3.6 months. Three patients, one each with cervical cancer, melanoma and esophageal cancer, experienced disease stabilization lasting 1.8^{+}, 2.8 and 3.3 months, respectively. In addition, a patient with adenocarcinoma of unknown primary suspected to be of ovarian origin (peritoneal and retroperitoneal disease, CA-125 133 IU/l) who did not undergo disease assessment due to discontinuation after two infusions, had normalization of CA-125 3 weeks after her last AP5346 infusion, with attained a 5-month clinical and biological response to follow-up treatment with single-agent oxaliplatin.

Discussion

Oxaliplatin is a third generation platinum compound that contains a DACH carrier ligand and has a wider and different spectrum of activity than cisplatin or carboplatin. In particular, oxaliplatin has activity in colorectal carcinoma and has now emerged as a key component of chemotherapy for this disease in combination with 5FU and leucovorin [2, 25]. Oxaliplatin also has activity in various other cancers, notably in ovarian, breast, prostate, gastric and lung cancer [3, 4, 13, 27, 29]. The basis for the differences in the activity spectra of oxaliplatin and earlier platinum compounds is still poorly understood but is probably related to differences in DNA binding, adduct formation, strand breaks, apoptosis and in resistance mechanisms including specific DNA adduct repair mechanisms [21, 28]. The major problem with oxaliplatin is that it produces a cumulative and poorly reversible peripheral sensory

neurotoxicity [9]. When treatment is prolonged, neuropathy often becomes persistent resulting in functional impairment.

AP5346 was designed to enhance the therapeutic index of a DACH-platinum compound both by improving the targeting of the of the drug to the tumor through the EPR effect [11], and by reducing toxicity to normal tissues by keeping the DACH-platinum moiety attached to the polymer until it encountered the low pH environment of the tumor. Dose escalation proceeded via accelerated titration in increments of 100% in cohorts of 1 patient each until toxicity was observed, which was to be followed by a modified Fibonacci dose escalation scheme. However, during execution of the trial a 100% increase in DL was implemented for every dose escalation since DLT was not encountered on cycle 1 at any dose below 1,280 mg Pt/m^2. A total of 26 patients were treated in the seven dose levels explored. The frequency of DLT was dose-related, and the types of DLT experienced by patients were characteristic platinum compound toxicities including nausea and vomiting, diarrhea, fatigue, renal toxicity and neutropenia at the highest dose explored. It is noteworthy that while a maximum tolerated dose of oxaliplatin 135 mg/m^2 oxaliplatin delivers 66 mg Pt/m^2 over a 4 weeks period of time [6], at 620 mg Pt/m^2 AP5346 delivered 1,860 mg Pt/m^2 over the same period.

Two deaths occurred due to renal insufficiency that were considered "possibly" or "probably" treatment-related and seven patients (27%) experienced treatment-emergent, grade 1-2 creatinine elevation or clearance decrease without clinical signs of renal dysfunction. Due to the occurrence of frequent nausea and vomiting, a pre-renal contributory factor is probable. Decrease of creatinine clearance was sometimes associated with other metabolic/renal disturbances, such as hypomagnesemia, hypokalemia, low plasma bicarbonate or proteinuria. Clinical and biological findings suggest proximal tubular damage, possibly related to the release of free platinum in acid urine, due to the inverse relationship between pH and platinum release from the copolymer conjugate. Implementation of renal prophylactic measures, consisting of IV hydration with sodium bicarbonate solution intended to increase renal flux and induce urine alkalization, may have been successful in limiting renal toxicity to grade 1 abnormalities of creatinine or creatinine clearance (0% vs. 45% grade 2-4 renal toxicity in patients with or without prophylaxis in DL 5–7). Notably, the two cases of "possible" or "probable" treatment-related death had not received IV bicarbonate, and both had disease progression in the kidney itself or its vessels that likely contributed to the fatal outcome. However, the ability to reduce renal toxicity with alkalinization remains uncertain.

As observed with other platinum-based therapies, nausea and vomiting were frequent (85%), reaching grade 3-4 in five patients in DL 5–7 (19%). Only one case of severe emesis occurred in patients receiving adequate anti-emetic prophylaxis. Only three patients (12%) reported treatment-related grade 1 sensory neuropathy; the absence of characteristic dose-limiting DACH-platinum neurotoxicity (85–95% with oxaliplatin [12] could be related to the low cumulative exposure of the cohort or decreased delivery of the DACH-platinum to key sites in the peripheral nervous system. No ototoxicity was observed. Hypersensitivity reactions occurred in four patients (15%), an incidence comparable to that reported for carboplatin and oxaliplatin [7, 10], and included one episode of anaphylactic shock. One of the four patients had demonstrated prior carboplatin sensitivity. Hypersensitivity reactions resulted in treatment discontinuation for two patients. Below a dose of 1,280 mg Pt/m^2, AP5346 did not produce significant hematologic toxicity with no neutropenia and only infrequent grade 1-2 thrombocytopenia reported in DL 1–6.

Preliminary evidence of antitumor activity was observed in this Phase I trial with two partial responses that met the RECIST guidelines and one biological marker response. One of the partial responses occurred in a patient with metastatic melanoma (lung, skin and lymph nodes) previously treated with dacarbazine and one in a patient with heavily pre-treated platinum-sensitive advanced ovarian cancer. CA125 normalization was observed after two doses of AP5346 in a patient with adenocarcinoma of unknown primary suspected to be of ovarian origin. Both plasma platinum C_{max} and AUC were linear with dose, and there was only moderate inter-patient variation of pharmacokinetic parameters within dose levels.

In conclusion, AP5346 was tolerated on the initial cycle up to a dose of 640 mg/m^2 with a toxicity profile characterized by frequent grade 1-2 nausea, vomiting, with an absence of grade 3-4 hematotoxicity. Significant increases in serum creatinine in the absence of possible disease-related renal impairment were observed on the first cycle only at very high doses (3,840 mg Pt/m^2 over a 3 weeks period of time); these were only partly reversible. The potential for nephrotoxicity with repeated cycles of therapy cannot be adequately assessed from this trial. Neurotoxicity was not a prominent adverse event although few patients received multiple cycles of treatment. Due to the frequency and severity of nausea and vomiting observed, and the potential impact of dehydration on renal toxicity, strict adherence to

prophylactic, antiemetic and hydration/alkalinization regimens is important, including administration of full dose 5-HT_3 blockers and corticosteroids for emesis and pre- and post-infusion hydration and IV sodium bicarbonate. Adherence to prophylactic regimens is probably an important factor in maintaining tolerability. Exploration of alternative schedules of administration, such as every 2 or 3 weeks, is warranted as are combinations of AP5346 with other chemotherapeutic agents Phase II disease-specific assessment is also warranted, such as in potentially platinum sensitive ovarian cancer, with benchmarking against the clinical activity spectrum of oxaliplatin.

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