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Clinical report

Stability and compatibility of the investigational polymer-conjugated platinum anticancer agent AP 5280 in infusion systems and its hemolytic potential

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AP 5280 is a novel polymer-conjugated platinum anticancer agent currently undergoing phase I clinical trials. It is pharmaceutically formulated as a lyophilized product containing 200 mg platinum per dosage unit. The aim of this study was to determine the reconstitution and dilution fluid of choice, and to investigate the stability and compatibility of AP 5280 in solution under different storage conditions and with several container materials. Furthermore, the hemolytic potential of AP 5280 infusion solution was investigated *in vitro*. AP 5280 slowly released small platinum species in all solutions, although this release was enhanced in normal saline. Accordingly, 5% dextrose in water (D_5W) was selected for reconstitution and dilution of AP 5280. Container material [glass or polyvinylchloride (PVC)] did not influence the stability of AP 5280 in solution. Storage at refrigerated temperature (2–8°C) marginally decreased the release rate of liberated platinum. The infusion solutions are compatible with the PVC infusion system and do not cause hemolysis *in vitro*. In conclusion, AP 5280 lyophilized product should be reconstituted and diluted to infusion concentration with D_5W, and administered within 8 h after preparation to ensure that less than 1.0% of the total platinum concentration is present as liberated platinum. [© 2002 Lippincott Williams & Wilkins.]

Key words: AP 5280, compatibility, hemolysis, infusion devices, stability.

Introduction

AP 5280 (a random copolymer of *N*-2-hydroxypropyl methacrylamide and the methacrylamide of GFLG-ama=$Pt(NH_3)_2$, molecular weight 24 ± 3 kDa, Figure 1) is a novel copolymer-conjugated platinum compound, designed for tumor targeting. In this copolymer, platinum is linked to a *N*-2-hydroxypropyl methacrylamide (HPMA) backbone via a tetrapeptide spacer [glycine–phenylalanine–leucine–glycine (GFLG)] and an amidomalonic acid (ama) chelating agent. Due to the hyperpermeable nature of the neovasculature of tumors in combination with their limited lymphatic and/or capillary drainage, it is expected that AP 5280 will preferentially accumulate at the tumor site.[1–4] Subsequently, platinum is released from the polymer intratumorally by lysosomal thiol-dependent proteinases, enzymes known to be elevated in human tumors.[5] Theoretically, AP 5280 administration will lead to higher intratumoral platinum concentrations and therefore potentially greater efficacy than the currently marketed non-polymer platinates cisplatin, carboplatin and oxaliplatin. Preclinical studies show that AP 5280 has a higher therapeutic index than cisplatin and carboplatin when administered to mice implanted with several different types of tumor.[6]

AP 5280 is pharmaceutically formulated as a lyophilized solid for i.v. infusion containing 200 mg platinum per dosage unit and has recently entered phase I clinical trials.[7] Before commencement of the clinical trials, we investigated the stability of AP 5280 in two commonly used infusion solutions [5% w/v dextrose in water (D_5W) or 0.9% w/v sodium chloride (normal saline)] at various concentrations and storage conditions. Stability was measured as the release of small platinum species ('liberated platinum') from the copolymer carrier into the

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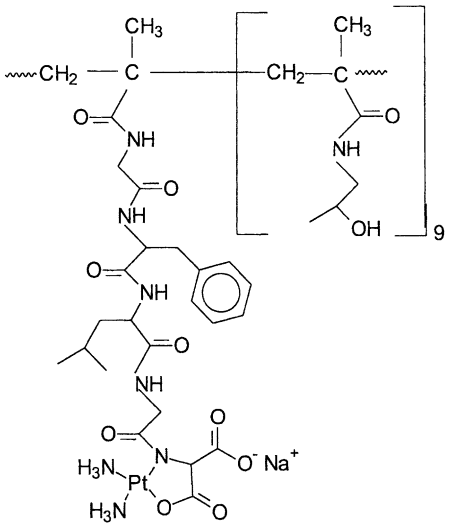

Figure 1. Chemical structure of AP 5280. Molecular weight 24 ± 3 kDa, polydispersity index 1.2–2.3.

infusion solution, a process that could affect both activity and toxicity of the compound *in vivo*. Compatibility with containers composed of glass and polyvinylchloride (PVC) was examined in terms of sorption to container surfaces and release of the plasticizer diethylhexylphthalate (DEHP). Subsequently, infusion simulation experiments were performed and the hemolytic potential of AP 5280 solutions was investigated *in vitro*. This paper describes the stability and compatibility of AP 5280 in solution for clinical application.

Materials and methods

Materials

Access Pharmaceuticals (Dallas, TX) provided AP 5280 drug substance and HPMA homopolymer [poly-HPMA (pHPMA)]. AP 5280 lyophilized product containing 200 mg platinum per dosage unit (2.5 g AP 5280), 5% dextrose in water (D_5W) and normal saline in glass bottles were manufactured in-house (Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands). D_5W in 50- and 500-ml PVC containers (Intraflex) was obtained from Braun Medical ('s Hertogenbosch, The Netherlands). Platinum atomic absorption standard was purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Paraplatin (carboplatin 50 mg/vial) originated from Bristol-Myers Squibb (Woerden, The Netherlands) and Platosin (cisplatin 1 mg/ml) from Pharmachemie (Haarlem, The Netherlands). Fresh, citrated blood and plasma ultrafiltrate were purchased from the local blood bank (Central Laboratory for Blood transfusion, Amsterdam, The Netherlands). Hydrochloric acid 37% was purchased from Merck (Darmstadt, Germany) and methanol from Biosolve (Amsterdam, The Netherlands). All chemicals were of analytical grade and used without further purification. Distilled water was used throughout the experiments.

Methods

Total platinum analysis. Total platinum concentrations were measured using a Perkin Elmer 3100 atomic absorption spectrometer (AAS) (Perkin Elmer, Nieuwerkerk a/d IJssel, The Netherlands). A slit width of 0.7 nm, a wavelength of 266 nm and an air/acetylene flame were employed. Platinum standards (0.0392, 0.03136 and 0.02352 mg/ml) and quality control samples (0.03528, 0.03136 and 0.02352 mg/ml) in 0.4 mg/ml pHPMA in 50/50% (v/v) 0.4% HCl/methanol were used for quantification of total platinum concentrations. Samples analyzed for their total platinum concentration were diluted with 50/50% (v/v) 0.4% HCl/methanol to yield a theoretical total platinum concentration of approximately 0.03 mg/ml.

Liberated platinum analysis. Liberated platinum concentrations were measured using a SpectrA-A 30/40 Zeeman Graphite Furnace AAS [flameless AAS (F-AAS)] (Varian, Techtron, Victoria, Australia), consisting of a spectrometer, a GTA-75 autosampler and a DS-15 data station equipped with the Quality Control Protocol software package (Varian). Absorbances were recorded at 265.9 nm, a slit bandwidth of 0.2 nm and a time constant of 0.05 s. Argon was used to purge the graphite tube. The temperature program of the instrument is shown in Table 1. Carboplatin calibration curves and cisplatin quality control standards were used to quantify the results and validate the analysis.[8]

Before analysis, samples were ultrafiltered through a Centricon YM-3 filter (3 kDa cut-off; Millipore, Etten-Leur, The Netherlands). The platinum concentration in each sample was analyzed in duplicate and the mean value used for further calculations.

Chromatography for DEHP determination. DEHP release from the PVC containers was analyzed using a reversed-phase high-performance liquid

Table 1. Temperature program of the F-AAS instrument

Step no.	Temperature (°C)	Time (s)	Gas flow (l/min)
1	50	1.0	3.0
2	85	5.0	3.0
3	95	30.0	3.0
4	120	20.0	3.0
5	250	30.0	3.0
6	1400	40.0	3.0
7	1400	20.0	3.0
8	1400	2.0	0.0
9	2800	0.7	0.0
10	2800	2.0	0.0
11	2800	4.0	3.0
12	50	13.8	3.0

chromatography method as previously described.[9] Ultrafiltrate samples were injected directly into the system to determine whether any DEHP was present.

^{195}Pt-NMR spectroscopy. ^{195}Pt-NMR spectra were recorded with a Bruker DPX 300 spectrometer with a 5-mm multi-nucleus probe. A variable temperature unit was used to maintain the temperature at 298 K. The ^{195}Pt-NMR spectra were calibrated using K_2PtCl_4 as an external reference at $\delta = -1614$ p.p.m. Samples were measured in solutions containing 5% D_2O. Only AP 5280 reconstituted solutions contained a platinum concentration high enough to perform ^{195}Pt-NMR spectroscopic analysis.

Stability and compatibility. AP 5280 lyophilized product for i.v. infusion containing 200 mg platinum per dosage unit was reconstituted with 14.7 ml D_5W or normal saline in its primary container (30-ml glass type I lyophilization vials; Münnerstädter Glaswarenfabrik, Münnerstadt, Germany). The resulting solutions had a volume of 16 ml and a theoretical platinum content of 12.5 mg/ml, and were stored at either room temperature (20–25°C, ambient light) or refrigerated conditions (2–8°C, dark). Reconstituted solutions were further diluted to yield AP 5280 infusion solutions at concentrations of 0.306, 1.53 and 3.06 mg/ml platinum (3.8, 19.1 and 38.3 mg/ml AP 5280, respectively) in D_5W or normal saline in 50-ml glass containers stored at room temperature. Based upon the initial examinations, D_5W was selected for further stability and compatibility tests. Infusion solutions in 50-ml PVC (Intraflex) containers were prepared and stored at room temperature or refrigerated conditions. All solutions were prepared in triplicate and samples were taken immediately after preparation and after 1, 2, 4, 8, 24, 48, 72 and 96 h of storage, and analyzed for total and liberated platinum concentrations. DEHP concentrations in samples from the solutions stored in Intraflex containers were determined after 96 h storage. Furthermore, immediately after preparation and after 96 h storage, ^{195}Pt-NMR spectra of the reconstituted solutions (12.5 mg/ml platinum) stored at room temperature were recorded. All reconstituted and diluted infusion solutions were visually checked for clarity.

Infusion simulation experiments. AP 5280 infusion simulations were conducted using an infusion system consisting of a 500-ml Intraflex container, PVC tubing regularly used for the infusion of cytotoxic agents (type G52703; IVAC, San Diego, CA) and a needle (Microlance 0.8×40 mm;. Becton Dickinson, Franklin Lakes, NJ). AP 5280 infusion solutions at concentrations of 0.306, 1.53 and 3.06 mg/ml platinum in D_5W were prepared and infusion rates set at 0.35 ml/min for a duration of 24 h. All infusion simulations were performed in triplicate at room temperature. Samples were taken from the needle outlet at 0, 1, 2, 4, 8 and 24 h after preparation, and analyzed for total and liberated platinum content. The 24-h samples were assayed for the presence of DEHP. The total amount of platinum delivered by each infusion system was calculated from the infusion rate and the area under the total platinum concentration–time curves (AUCs): total amount of platinum delivered (mg) = AUC (mg/ml · h) × infusion rate (ml/h).

The AUCs were calculated using the trapezoidal rule. The same calculations were performed to estimate the total amount of liberated platinum delivered by the infusion systems.

Hemolysis. The potential of AP 5280 infusion solutions at a concentration of 3.06 mg/ml platinum in D_5W to cause hemolysis was examined using both the static and dynamic *in vitro* test models as described by Ward *et al.*[10] and Krzyzaniak *et al.*[11–13] The hemolytic potentials of solutions of D_5W, 2.5 mg/ml cisplatin in D_5W and 39.2 mg/ml pHPMA in D_5W were determined for comparison. For the static model, 25, 100 and 250 μl infusion solution was added to 500 μl blood, resulting in formulation:-blood (F:B) ratios of 0.05, 0.2 and 0.5, respectively. The solutions were slowly whirl-mixed for 5 s. For the dynamic model, each solution was infused at rates of 0.3 and 1.2 ml/min using a Model 711 syringe pump (IVAC) into a tube containing blood flowing at a rate of 6 ml/min employing a Model 501 Dz peristaltic pump (Watson Marlow, Rotterdam, The

Netherlands), which resulted in F:B ratios of 0.05 and 0.2, respectively. The contact time with blood was set at 5 s by administering the infusion solution 25 cm from the end of the silicone tubing transporting the blood (1.6 mm diameter; Watson Marlow). For both models, the hemolytic reaction was quenched by addition of 50 ml normal saline to the blood sample. Subsequently, an aliquot of the diluted test solution was centrifuged at 3000 r.p.m. for 10 min.The absorption (A) of the resulting supernatant was measured at 540 nm with a model UV/VIS 918 spectrophotometer (GBC Scientific Equipment, Victoria, Australia).

The baseline degree of hemolysis was measured using normal saline at the same F:B ratios. The 100% hemolysis level was determined by diluting the blood used in both the static and dynamic model with 50 ml distilled water instead of normal saline. As a positive control, a mixture of 40/10/50% (v/v/v) propylene glycol/ethanol/water (PEW) was used.[12] All experiments were run in triplicate. The percentage hemolysis induced by all solutions was calculated as: % Hemolysis$=(A_{\text{test solution}}-A_{\text{normal saline}})/(A_{100\%}-A_{\text{normal saline}}) \times 100\%$.

Results and discussion

Before commencement of phase I clinical trials several pharmaceutical issues of AP 5280 infusion solutions were investigated to ensure the suitability of the solutions to be administered to patients. AP 5280's proposed starting dose in phase I clinical studies was 90 mg platinum/m^2, administered in 500 ml as a 1-h infusion every 3 weeks. We investigated a dose range of 90–900 mg platinum/m^2, corresponding to 153–1530 mg platinum, for a patient with a body surface area of 1.7 m^2.

All currently marketed platinum drug products have specific requirements with respect to the infusion solution employed for reconstitution and dilution; in particular, to the presence of chloride ions. Furthermore, the storage conditions of the platinum infusion solutions may influence the stability. For instance, cisplatin (Platinol) is only chemically stable in solutions containing at least 0.2% NaCl; in solutions with a lower chloride concentration, one or both of cisplatin's chloride ions are displaced by water, forming the toxic mono- and diaqua species. Furthermore, when stored at refrigerated temperatures, formation of a precipitate occurs which is difficult to redissolve, necessitating storage of cisplatin solutions at 15–25°C.[14–16] Carboplatin (Paraplatin) solutions in normal saline, on the other hand, degrade more rapidly than solutions in D_5W, which are stable for at least 24 h at room temperature.[14–16] Contact of oxaliplatin (Eloxatin) with normal saline results in chemical modifications and formation of a precipitate, requiring the use of D_5W for reconstitution and dilution.[17,18] Carboplatin and oxaliplatin solutions can be stored at either room temperature or refrigerated conditions.[14,18]

For AP 5280, initially small-scale stability and compatibility studies were performed to determine the optimal infusion solution, container and storage condition. Subsequently, an infusion simulation was carried out employing administration parameters intended for use in the clinical setting.

Release of liberated platinum

A 3-kDa cut-off value was used to define 'small platinum species' and thus the liberated platinum content. Figures 2 and 3 depict the percentage platinum released from the polymer with time (expressed as the liberated platinum concentration relative to the total platinum concentration) for AP 5280 solutions in D_5W and normal saline, respectively, when stored at room temperature in glass containers. The total platinum concentration in all solutions remains constant with time and is in agreement with the theoretical total platinum concentration. This indicates that there is no platinum loss due to, for example, sorption to container walls.

The data shown in Figures 2 and 3 indicate that a low concentration of small platinum species is present in all investigated AP 5280 solutions. In D_5W, the level of liberated platinum shows an initial burst, probably due to release of loosely bound platinum resulting from the manufacturing process and the presence of small polymer species that successfully pass through the pores of the filter membrane. After 8–24 h a plateau of approximately 1.5% liberated platinum is reached, which is independent of the AP 5280 concentration. However, in normal saline this release process occurs continuously and the liberated platinum concentration increases to 3–4% after 96 h at room temperature (Figure 3). These results indicate that release of small platinum species from AP 5280 in solution is enhanced by the presence of sodium chloride or one of its components, most likely chloride ions. In normal saline solutions, the release process is

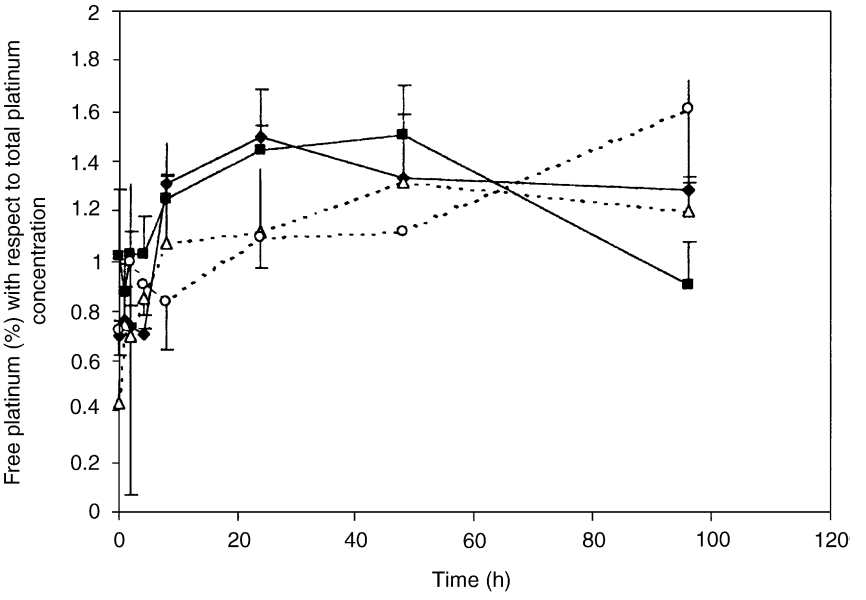

Figure 2. Release of liberated platinum from AP 5280 in D_5W solutions when stored in glass containers at room temperature. (Diamonds) AP 5280 after reconstitution (12.5 mg/ml Pt), (squares) AP 5280 high-concentration infusion solution (3.06 mg/ml Pt), (triangles) AP 5280 medium-concentration infusion solution (1.53 mg/ml Pt) and (circles) AP 5280 low-concentration infusion solution (0.306 mg/ml Pt).

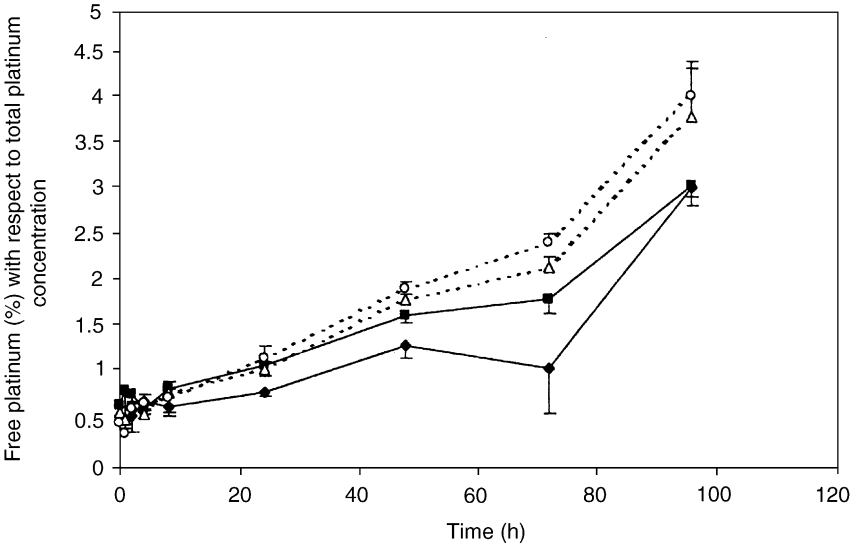

Figure 3. Release of liberated platinum from AP 5280 in normal saline solutions when stored in glass containers at room temperature. (Diamonds) AP 5280 after reconstitution (12.5 mg/ml Pt), (squares) AP 5280 high-concentration infusion solution (3.06 mg/ml Pt), (triangles) AP 5280 medium-concentration infusion solution (1.53 mg/ml Pt) and (circles) AP 5280 low-concentration infusion solution (0.306 mg/ml Pt).

concentration dependent, with the lowest AP 5280 concentration releasing, relatively, the most liberated platinum. This is most likely due to the relative abundance of chloride ions with respect to AP 5280 at low concentrations.

D_5W is more suitable as reconstitution and dilution fluid for AP 5280 than normal saline. Therefore, further investigations into the stability and compatibility of AP 5280 were conducted using D_5W.

Stability and compatibility

Figure 4 shows the percentage liberated platinum with respect to total platinum concentration of AP 5280 in D_5W solutions stored in glass containers at refrigerated conditions. Again, total platinum concentrations remain unchanged, while the liberated platinum concentration reaches a plateau after 8 h storage, which is slightly lower (0.2–0.5%) than observed for the solutions in D_5W stored at room temperature (1.5%, see Figure 2).

Table 2 shows the total and liberated platinum concentrations of AP 5280 infusion solutions in D_5W stored in Intraflex PVC containers at both room temperature and refrigerated conditions at selected time points. For all solutions a plateau in liberated platinum release is reached after 8–24 h storage, which appears concentration and marginally storage condition dependent. The highest concentration AP 5280 infusion solution at room temperature shows a maximum liberated platinum concentration of about 1.5% and the lowest concentration AP 5280 infusion solution at refrigerated condition of about 0.8%. Percentages liberated platinum found in Intraflex containers are comparable to the percentages found in AP 5280 solutions stored in glass containers.

Total platinum for all investigated AP 5280 solutions is stable in time and approximately equal to the theoretical total platinum concentrations, indicating that no sorption to container walls takes place during storage. Any deviation from the theoretical total platinum concentrations is a result of the preparation of the solutions and analytical variation. No precipitate formation was observed in any of the AP 5280 solutions. The lack of visual detection of precipitation is confirmed by the stable total platinum concentrations in time. AP 5280 is very soluble in water, and hence not likely to precipitate.

A drawback for the use of PVC administration sets is the possible extraction of plasticizers ('leaching') by the solubilized formulation. Leaching of DEHP, for instance, has been described for infusion solutions containing surfactants.[19–21] AP 5280 infusion solutions are free from such additives. Nevertheless, as teratogenic and hepatotoxic effects have been ascribed to DEHP,[22,23] it was deemed important to check for any leaching of DEHP due to AP 5280 infusion solutions. No DEHP could be detected in any of the samples (detection limit: 0.5 μg DEHP/ml) and clearly AP 5280 infusion solutions do not cause significant leaching of DEHP from the PVC (Intraflex) containers.

Figure 5 depicts the ^{195}Pt-NMR spectrum of the reconstituted solution in D_5W at room temperature after 96 h of storage. The spectra of the same solution immediately after preparation and of the AP 5280 reconstituted solutions in normal saline immediately after preparation and after 96 h storage were identical. These results indicate that platinum-binding characteristics do not change for at least 96 h. It should be noted, however, that the sensitivity of the method is inadequate to detect a small percentage

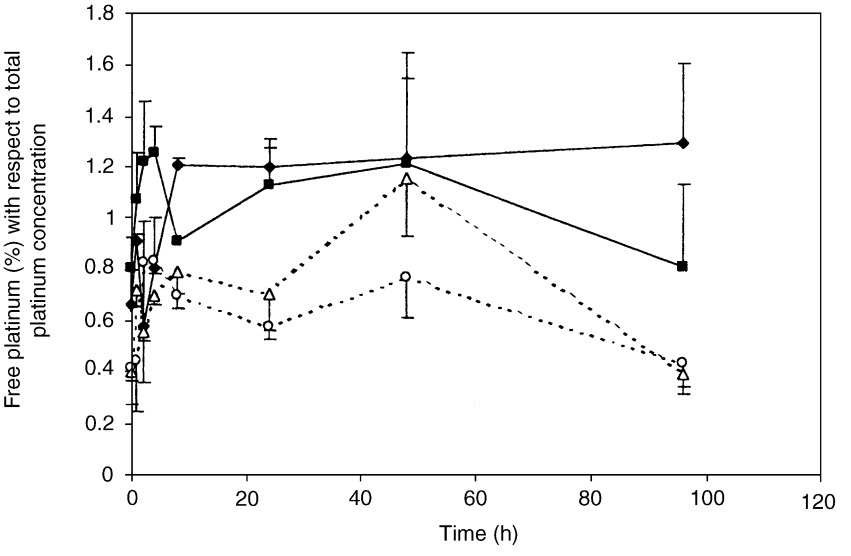

Figure 4. Release of liberated platinum from AP 5280 in D_5W solutions when stored in glass containers at refrigerated conditions. (Diamonds) AP 5280 after reconstitution (12.5 mg/ml Pt), (squares) AP 5280 high-concentration infusion solution (3.06 mg/ml Pt), (triangles) AP 5280 medium-concentration infusion solution (1.53 mg/ml Pt) and (circles) AP 5280 low-concentration infusion solution (0.306 mg/ml Pt).

Table 2. Total and liberated platinum concentrations (mg/ml) ± SD of AP 5280 infusion solutions in D_5W in 50-ml PVC containers stored at room temperature or refrigerated conditions

Storage condition	Infusion solution concentration	Total platinum concentration (mg/ml) ± SD				Liberated platinum concentration (mg/ml) ± SD (percentage of total platinum concentration)			
		0 h	8 h	24 h	96 h	0 h	8 h	24 h	96 h
Room temperature	low	0.33 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.39 ± 0.01	0.0018 ± 0.0003 (0.55%)	0.0032 ± 0.0014 (0.89%)	0.0037 ± 0.0006 (1.03%)	0.0038 ± 0.0007 (0.97%)
	medium	1.43 ± 0.02	1.64 ± 0.06	1.64 ± 0.02	1.57 ± 0	0.0080 ± 0.0014 (0.56%)	0.0158 ± 0.0042 (0.96%)	0.0191 ± 0.0053 (1.16%)	0.0186 ± 0.0072 (1.18%)
	high	2.69 ± 0.09	2.92 ± 0.04	3.00 ± 0.07	2.96 ± 0.04	0.0182 ± 0.0055 (0.68%)	0.0288 ± 0.0019 (0.99%)	0.0404 ± 0.0095 (1.35%)	0.0437 ± 0.0052 (1.48%)
Refrigerated conditions	low	0.33 ± 0.01	0.34 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.0022 ± 0.0007 (0.67%)	0.0020 ± 0.0005 (0.59%)	0.0026 ± 0.0010 (0.74%)	0.0027 ± 0.0012 (0.77%)
	medium	1.49 ± 0.02	1.58 ± 0.02	1.57 ± 0.06	1.58 ± 0.02	0.0077 ± 0.0004 (0.52%)	0.0131 ± 0.0058 (0.83%)	0.0149 ± 0.0005 (0.95%)	0.0143 ± 0.0052 (0.91%)
	high	2.69 ± 0.15	2.94 ± 0.31	2.90 ± 0.18	2.86 ± 0.18	0.0163 ± 0.0017 (0.61%)	0.0258 ± 0.0059 (0.88%)	0.0306 ± 00122 (1.06%)	0.0287 ± 0.0022 (1.00%)

High = 3.06 mg/ml Pt, medium = 1.53 mg/ml Pt, low = 0.306 mg/ml Pt.

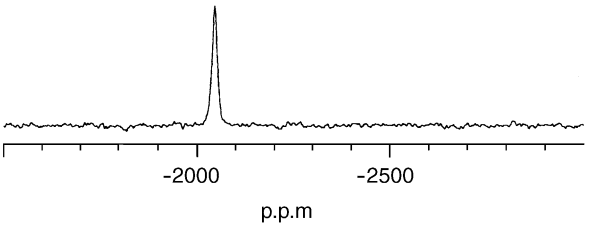

Figure 5. ^{195}Pt-NMR spectrum of AP 5280 after reconstitution with D_5W after 96 h storage at room temperature.

release of liberated platinum. This is illustrated by the fact that the solution in normal saline after 96 h storage shows the same ^{195}Pt-NMR spectrum as the solution in D_5W, while its concentration of small platinum species is 4%. The presence of liberated platinum in a concentration as high as 4% of the total platinum concentration is not detected by ^{195}Pt-NMR spectroscopy.

The initial experiments show that AP 5280 solutions in D_5W are chemically stable for at least 96 h. As no difference was observed between the stability of AP 5280 in D_5W solutions stored in glass or PVC containers, it was decided to employ Intraflex containers for AP 5280 infusions in the clinic. This choice was made because of the smaller chance of breakage, and thus exposure of both hospital staff and patients to cytotoxic agents, and convenience of handling.

Infusion simulation experiments

In order to set the final administration parameters, infusion simulation experiments were performed employing 500-ml Intraflex containers containing AP 5280 in D_5W, a PVC infusion line (1.5 m. length) and a needle. An infusion of 24 h was employed to evaluate the liberated platinum release profile in the infusion system.

Table 3 shows the total and liberated platinum concentrations in time and the total amounts of platinum and liberated platinum delivered after 24 h by each infusion system. Again, all three concentrations showed a stable total platinum concentration in time. The total amount of platinum delivered was within 90–110% of the theoretical amount for all concentrations tested. Deviations from 100% total platinum delivery can be attributed to the preparation of the infusion solutions and analytical variation.

Based on its proposed mode of action, the integrity of AP 5280 upon administration is of great importance. Stability of AP 5280 in solution has been

Table 3. Total and liberated platinum concentrations (mg/ml) ± SD of AP 5280 infusion solutions in D_5W in 500 ml PVC containers for the infusion simulation study

Time (h)	Total platinum concentration (mg/ml Pt) ± SD			Liberated platinum concentration (mg/ml Pt) ± SD (percentage of total platinum concentration)		
	High	Medium	Low	High	Medium	Low
0	2.97 ± 0.11	1.52 ± 0.02	0.35 ± 0.03	0.0104 ± 0.0053 (0.35%)	0.0077 ± 0.0017 (0.51%)	0.0021 ± 0.0002 (0.60%)
1	2.97 ± 0.04	1.52 ± 0.04	0.35 ± 0.03	0.0108 ± 0.0007 (0.36%)	0.0071 ± 0.0001 (0.47%)	0.0013 ± 0.0003 (0.37%)
2	2.89 ± 0.08	1.48 ± 0.02	0.35 ± 0.02	0.0195 ± 0.0012 (0.67%)	0.0094 ± 0.0036 (0.64%)	0.0015 ± 0.0004 (0.43%)
4	2.82 ± 0.08	1.52 ± 0.04	0.34 ± 0.02	0.0140 ± 0.0024 (0.50%)	0.0087 ± 0.0028 (0.57%)	0.0016 ± 0.0001 (0.47%)
8	2.75 ± 0.04	1.45 ± 0.02	0.32 ± 0.03	0.0162 ± 0.0037 (0.59%)	0.0080 ± 0.0021 (0.55%)	0.0019 ± 0.0006 (0.59%)
24	2.83 ± 0.07	1.41 ± 0.08	0.34 ± 0.03	0.0130 ± 0.0028 (0.50%)	0.0099 ± 0.0049 (0.70%)	0.0029 ± 0.0009 (0.85%)
Total amount of (liberated) platinum delivered	1413.6 mg (92.4%)[a]	741.6 mg (96.9%)[a]	168.8 mg (110.7%)[a]	7.36 mg (0.52%)[b]	4.38 mg (0.59%)[b]	1.08 mg (0.64%)[b]

High = 3.06 mg/ml Pt, medium = 1.53 mg/ml Pt, low = 0.306 mg/ml Pt.
[a]Relative amount of platinum administered with respect to the theoretical total platinum dose.
[b]Relative amount of liberated platinum administered with respect to total platinum dose.

evaluated by the extent and rate of platinum release ('liberated platinum') from the polymer. Using the ultrafiltration method to separate bound from liberated platinum, all platinum species smaller than 3 kDa are gathered in the ultrafiltrate. At this moment, identities of the platinum species in the ultrafiltrate are unknown, as are their pharmacological effects. Therefore, it is felt that it is important to keep the levels of liberated platinum as low as possible in AP 5280 infusion solutions. In the 24-h infusion simulation experiment, approximately 0.6% of the total platinum dose was delivered as liberated platinum, regardless of the infusion concentration. For the moment, the specification for the liberated platinum concentration has been set at 1.0% of the total platinum concentration. In order to keep within safe margins of this specification, AP 5280 should be reconstituted, diluted and administered within 8 h.

No DEHP was detected in any of the 24-h samples.

Hemolysis

Hemolysis can cause a wide range of undesirable medical conditions, such as jaundice, kernicterus, hemoglobinuria, nephrosis and acute renal failure. Death can occur when hemolysis becomes severe. Every effort must therefore be made to minimize the occurrence of hemolysis and an evaluation of the ability of a formulation to induce this condition is therefore an important component of the development of an i.v. formulation.[24]

AP 5280 infusion solutions are iso-osmotic and of neutral pH, and are therefore not expected to cause large disruptions in erythrocyte integrity. To date, not many polymers have been found to cause hemolysis. In fact, some polymers such as poyvinylpyrrolidone, dextran and hydroxyethylstarch act in an antihemolytic manner.[25] Other polymers such as poly(amidoamines)[26] and polyimides[27] cause little to no hemolysis. However, some solid-phase poly (methyl methacrylate) formulations were shown to cause hemolysis.[28] As AP 5280 is related to poly (methyl methacrylate), it was felt important to test it for its hemolytic potential.

AP 5280 will be administered at an infusion rate of 8.3 ml/min. The venous blood flow is approximately 40 ml/min (an estimate for the broad range of blood flows in the circulatory system[12]), leading to a F:B ratio of 0.2. To evaluate the effect of a varying infusion rate, F:B ratios of 0.05 and 0.5 were also investigated. Instead of a contact time of 1 s between the test solution and blood, as described by

Krzyzaniak *et al.* to be physiologically realistic for an i.v. bolus injection,[11–13] a longer contact time of 5 s was employed to mimic the continuous exposure of blood to the administered agent during prolonged i.v. infusions.[9]

To differentiate between possible hemolytic effects caused by platinum and those caused by polymer, solutions of 2.5 mg/ml cisplatin (the maximum solubility of cisplatin) and 39.2 mg/ml pHPMA (corresponding to a platinum concentration of 3.06 mg/ml in AP 5280) in D_5W were tested.

No hemolysis was detected for any of the solutions tested in either the static or dynamic model, except for the positive control, which showed increasing degrees of hemolysis with increasing F:B ratios; up to 88% hemolysis for the F:B ratio of 0.5 in the static model (data not shown). Therefore, infusion of AP 5280 solutions is not expected to cause any hemolysis upon i.v. administration.

Conclusions

AP 5280 lyophilized product for i.v. infusion was subjected to a series of *in vitro* tests to evaluate its suitability for i.v. administration and for its potential to cause hemolysis. AP 5280 in solution slowly releases liberated platinum, a process enhanced by the presence of chloride ions. Therefore, AP 5280 lyophilized product should be reconstituted and diluted with D_5W. Container material (glass or PVC) does not affect the stability of AP 5280 in solution. Storage at refrigerated conditions slows down the liberated platinum release in AP 5280 solutions. In the infusion simulation experiments, the total amount of liberated platinum delivered was low and no DEHP leaching was observed. Finally, no hemolysis was shown to occur upon static and dynamic hemolysis tests. In conclusion, AP 5280 should be reconstituted and diluted using D_5W and either glass or PVC containers can be employed for administration of AP 5280 infusion solutions, although for practical reasons PVC containers are preferred. Administration should take place within 8 h after preparation of the infusion solutions to ensure that less than 1.0% of the total platinum concentration is present as liberated platinum.

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