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Short communication

Suitability of cisplatin solutions for 14-day continuous infusion by ambulatory pump*

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Summary. The stability of cisplatin (DDP) solutions (1 and 1.6 mg/ml in saline-mannitol) in plastic infusion bags was studied for up to 14 days at 25° C, 37° C and 60° C. Small changes in the solution were observed, but no evidence of any decomposition product was seen. Some precipitation of DDP was seen in the 1.6-mg/ml solution at the lower temperatures. Fluid loss from the bags was significant at the higher temperatures.

Introduction

The delivery of cytotoxic drugs by continuous i. v. infusion has a number of potential advantages over i. v. bolus administration. First, the maintenance of serum drug levels over a prolonged period should enable a larger number of tumour cells to be exposed to cytotoxic agents during the vulnerable phases of the cell cycle [1]. Such considerations are particularly relevant for drugs such as cisplatin (DDP), which exhibits a $t_{1/2}$ value of between only 25 and 49 min [3, 6] as compared with a cell-cycle period of up to 88 h in some cancers [2]. Secondly transport of the drug into the tumour cell may depend not only on the concentration of drug in the extracellular space but also on the duration of exposure, which is inevitably prolonged during continuous infusion [7]. Finally, toxicity related to peak drug levels after bolus administration seems likely to be reduced by a delivery technique that results in low serum concentrations. This certainly appears to be the case for doxorubicin, for which the incidence of nausea and vomiting is markedly reduced during continuous infusions [4, 5]. However, doxorubicin-induced stomatitis is probably more severe after continuous infusion [4], an observation that draws attention to the possibility of increased cytotoxicity following prolonged low-dose drug exposure.

DDP is known to be stable in saline solution and is now commercially available in solution (David Bull Laboratories, Warwick, UK). The present study determined the stability of DDP solutions contained in Pharmacia Deltec medication cassettes (100 ml; St. Paul, Minn., USA) for a period of 14 days.

Materials and methods

DDP solutions were used at two concentrations (1 and 1.6 mg/ml). The lower concentration used was the commercially available Cisplatin Injection formulation (100-ml vials containing 100 mg DDP, 900 mg NaCl and 100 mg mannitol; David Bull Laboratories, Warwick, UK). The higher concentration was prepared using vials of DDP that were reconstituted with water to give a final concentration of 1.6 mg/ml (50-mg vials containing sodium chloride and mannitol; Farmitalia Carlo Erba Ltd., St. Albans, UK). This higher concentration necessitated gentle warming to ensure complete dilution. The solutions were placed in Deltec bags (Pharmacia) according to the manufacturer's instructions, and the bags were either placed in ovens set at 60° C or 37° C or were kept at room temperature (24° C ± 2° C). All samples were protected from light by aluminium foil.

Analysis. The purity of DDP was determined by high-performance liquid chromatography (HPLC) using a Waters 600 MS solvent delivery system along with a Waters 484MS UV/VIS detection system (220 nm). Two separate chromatographic separations were performed. One separation procedure involved the application of DDP onto a Techopak 10 phenyl semi-micro column (15 cm × 2 mm; HPLC Technology, Macclesfield, UK) using an isocratic solvent system [H_2O (95%) and methanol (5%)] run at a flow rate of 1 ml/min. The internal standard used was barbital, which gave baseline resolution when it was injected as a DDP-barbital mixture (Fig. 1). The retention times for DDP and the internal standard were 50 and 215 s, respectively. A second internal standard (1-phenylalanine) was also used; the solvent system was isocratic (100% water, 0.4 ml/min), and the retention times were 122 s (DDP) and 209 s (phenylalanine). HPLC analysis was also performed using an Asahipak ODP column (Asahi Chemical Company) along with an isocratic solvent system [H_2O (90%), methanol (10%), 1.25 ml/min] and 1-phenylalanine as the internal standard. The retention times were 169 s (DDP) and 242 s (phenylalanine). Mass spectrometry was carried

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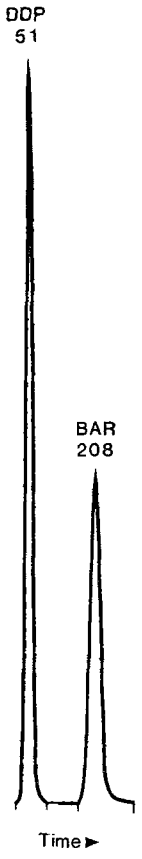

Fig. 1. Separation of DDP and barbital on the Techopak column

out on a VG Trio 2 instrument using fast-atom-bombardment ionisation and a thioglycerol matrix.

Sample preparation. On each day of analysis, the bags containing the drug were weighed, drug was removed with a syringe, and the bags were reweighed and replaced in the appropriate environment. The DDP solution was spiked with the internal standard (final concentrations, 0.62 mg/ml phenylalanine or 0.14 mg/ml barbital). The concentration of internal standard was chosen such that approximately equivalent drug peak areas would be obtained. The more concentrated DDP solutions (1.6 mg/ml) were diluted with saline (×2) immediately prior to the addition of internal standard and injection into the HPLC system. Injections was either 50 or 25 µl DDP solution, and drug estimations were performed in triplicate. The concentration of DDP (peak area) was calculated relative to the internal-standard peak area. The internal standard solutions were stored frozen in 1-ml aliquots (prepared from the same solution), and a fresh vial was used each day. The experiments were performed using the two HPLC analytical methods on alternate days.

Drug hydrolysis. A further test of the ability of the two analytical systems to detect any breakdown was carried out by studying the hydrolysis of DDP in water. Pure drug was dissolved in water (1 mg/ml), and HPLC analysis was performed using the two analytical methods described above at various times for up to 5 days. Control experiments using DDP in the formulation matrix (NaCl, mannitol) were conducted simultaneously.

Statistical analysis. Statistical analysis was performed using the Minitab statistical package (Minitab Inc., State College of Pennsylvania, USA) on a minicomputer (Microvax).

Results

The infusion-pump reservoirs showed loss of weight over the 14 days of the experiment. As the bags showed no sign

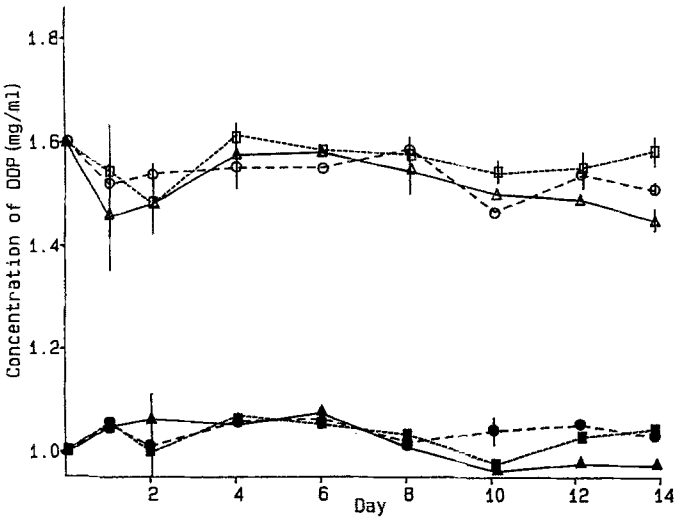

Fig. 2. Stability of DDP over 14 days. The DDP concentration has been arithmetically corrected for fluid loss. The *open symbols* represent the stability of DDP at a concentration of 1.6 mg/ml (□– – –□, $T = 60°C$; ○– – –○, $T = 37°C$; △——△, $T = 25°C$). The *closed symbols* represent the stability of DDP at a concentration of 1 mg/ml (▲——▲, $T = 60°C$; ●– – –● $T = 37°C$; ■– – –■ $T = 25°C$). Bars represent standard errors

of any leakage, this loss was attributed to evaporation. The weight loss was greater at the highest temperature (60° C) and smallest at the lowest (25° C) and remained constant at each temperature over the duration of the experiment ($T = 25°C$, weight loss = 0.06 ± 0.01 g/day; $T = 37°C$, weight loss = 0.19 ± 0.04 g/day; $T = 60°C$, weight loss = 0.59 ± 0.12 g/day). The cumulative fluid loss became significant at higher temperatures [$T = 25°C$, 0.74 g (0.74%) over 14 days; $T = 37°C$, 2.69 g over 14 days; $T = 60°C$, 8.21 g over 14 days]. The concentrations of platinum were corrected for this evaporation.

The effect of time and temperature on DDP in the infusion bags is illustrated in Fig. 2, and the data analysis is shown in Table 1. It can be seen that there was little change in the infusion bags containing the lower (1 mg/ml) concentration of DDP. The slope of the concentration-time plots did not differ significantly from zero at this drug concentration over the 14 days at any of the three temperatures examined. A significant change in DDP concentration was seen at the higher (1.6 mg/ml) concentration, especially at the lowest temperature investigated (25° C). A rapid drop in DDP concentration (~15%) occurred on day 1 and was associated with the appearance of small yellow crystals of DDP; these were not seen at the lower DDP concentration. The mass spectra of the solutions were identical at the start of the experiment and after 14 days, with a strong molecular ion being detected at m/e = 298, corresponding to DDP. No evidence was found of new ions corresponding to hydrolysis products that might have formed during the study. Similarly, no evidence of any new peaks corresponding to breakdown products was found using the Asahipak analytical system.

The ability of the analytical methods to detect changes in the DDP were tested by drug hydrolysis in water. This resulted in a rapid decrease in the level of the DDP peak when the drug was dissolved in water. No similar change

Table 1. Analysis of the stability of DDP in infusion bags

Analytical system	Temperature	DDP concentration (mg/ml)	% Change in DDP concentration per day (±50)	*P* value	Calculated % change in DDP concentration on day 14	Observed % change in mean DDP concentration on day 14
Techopak (Barbital)	25°C	1	+0.07(0.14)	0.624	<1	1
	37°C	1	+0.02(0.11)	0.850	<1	1
	60°C	1	–0.07(0.23)	0.766	<1	–5
	25°C	1.6	–0.25(0.38)	0.517	–3.5	–10
	37°C	1.6	–0.4 (0.21)	0.073	–5.6	–7
	60°C	1.6	+0.64(0.33)	0.066	+8.9	–2
Techopak (Phenylalanine)	25°C	1	–0.16(0.16)	0.322	–2.2	–1
	37°C	1	–0.06(0.12)	0.632	<1	–4
	60°C	1	–0.15(0.08)	0.061	–2.1	–11
	25°C	1.6	–1.58(0.13)	<0.001	–22	–14
	37°C	1.6	+0.2 (0.21)	0.405	+2.8	–5
	60°C	1.6	–0.04(0.2)	0.811	<1	–5

The change in the DDP: barbital ratio was calculated by linear regression-analysis of variance. The standard deviations and the probability that the gradient would be different from zero are shown

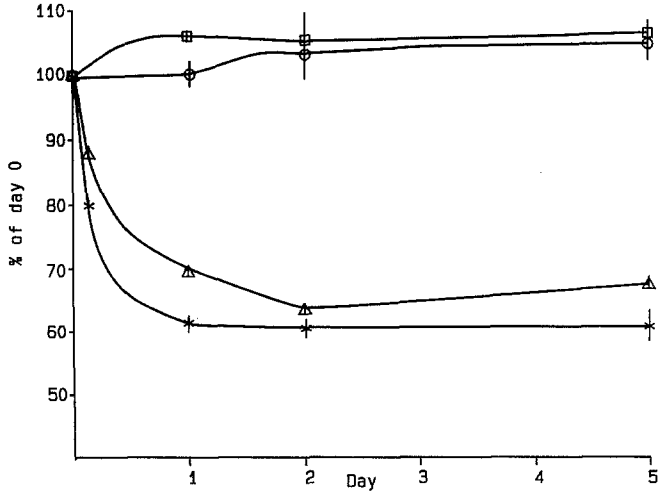

Fig. 3. Decomposition of DDP in water. The graph shows the decrease in the DDP: internal standard ratio (expressed as a percentage of the ratio on day zero). □, ○, DDP (1 mg/ml) dissolved in saline/mannitol at 25°C; △, X, DDP (1 mg/ml) dissolved in water at 25°C. Bars represent standard errors. □,X, Use of phenylalanine as the internal standard; ○, △, use of barbital as the internal standard

was seen when saline was used as the solute. The Techopak analysis systems revealed a decrease in the peak corresponding to DDP (Fig. 3). However, under the chromatography conditions used with this column, no product peak was seen. The Asahipak column disclosed both a decrease in the DDP peak and an increase in a new peak that presumably represented a hydrolysis product. The retention time for the new peak (150 s) was too close to that of DDP (169 s) for the achievement of baseline resolution on the chromatogram. No attempt was made to characterise this hydrolysis product further.

The bags were examined daily for any changes caused by the drug or solvent. No damage to the bags was observed. Those containing the higher concentration of DDP (1.6 mg/ml) at the lower temperatures (25° and 37°C) did show the formation of a few extremely small, yellow crystals whose colour was similar to that of DDP. Despite the small size and low number of crystals in this precipitate, the latter might have been responsible for the apparent decrease in the DDP: internal standard ratio that was observed at the higher drug concentrations and the lowest temperature (25°C).

Discussion

The slow infusion of drugs to treat cancer may offer significant advantages in the treatment of a number of diseases. The use of these systems relies on two factors: (1) the stability of the drug throughout the infusion, and (2) the lack of an interaction between the drug and solvent and the reservoir.

DDP is known to be stable at ambient temperature in glass bottles. This stability is evidenced by the use of a DDP solution in a drug-formulation system [1 mg/ml DDP in mannitol (1 mg/ml) and saline (9 mg/ml; David Bull Laboratories, Warwick, UK)]. It is therefore not surprising that the HPLC assays showed no great change in the drug during the 14 days of this study. However, the bags exhibited a reasonably high fluid loss, especially at the higher temperatures investigated. At 25°C, the loss was relatively low (<1%), but it became more pronounced at the higher temperatures examined (<3% at 37°C and >8% at 60°C). The precipitation of small crystals at the higher concentration of DDP (1.6 mg/ml) may have reduced the concentration of drug in the reservoirs at the lower temperatures studied. No such precipitation was observed in the 1-mg/ml samples. The dark-coloured plastic reservoirs were not exposed to direct light except during the removal of samples. This mimics the clinical situation in which the reservoir is placed inside a coloured plastic container, which is generally then covered with cloth.

In conclusion, little change in the DDP concentration was seen at the lower (1 mg/ml) concentration tested. No evidence of any decomposition product was seen, although the analytical methods used have been shown to be capable

of detecting the hydrolysis of DDP in water. The failure to detect any hydrolysis products, the lack of temperature effects, an the known stability of DDP in saline/mannitol are indicative that the changes observed during the present study did result from drug destruction. Some change in the concentration of DDP in the 1.6-mg/ml solution were seen; this was considered to be attributable to precipitation of the drug in this solution, especially at lower temperatures. Any use of the higher concentration of drug in continuous-infusion regimens must therefore be approached with caution.

A potential drawback of this infusion system may be the fluid loss (presumably due to evaporation) that occurred during the experiment. This could have led to an increase in the concentration of DDP, which was small (<1% of the total over 14 days) at 25° C but increased at the higher temperatures tested (<3% at 37° C and 8.2% at 60° C).

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