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The suitability of carboplatin solutions for 14-day continuous infusion by ambulatory pump: an HPLC–dynamic FAB study

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Abstract: The stability of aqueous carboplatin solutions over 14 days has been studied at 37 and 60°C. High-performance liquid chromatography and dynamic FAB mass spectrometry studies have shown that carboplatin solutions were stable at 37°C but degraded at 60°C. Fluid loss through evaporation was significant at the higher temperature.

Keywords: *Carboplatin; dynamic FAB mass spectrometry; high-performance liquid chromatography; stability.*

Introduction

The delivery of cytotoxic drugs by continuous intravenous (i.v.) infusion has a number of potential advantages over i.v. bolus administration. Firstly, the maintenance of serum drug levels over a prolonged period should allow a larger number of tumour cells to be exposed to cytotoxic agents during vulnerable phases of the cell cycle. Secondly, transport of the drug into the tumour cell may depend not only on the concentration of drug in the extracellular space but on the duration of exposure which is inevitably prolonged during continuous infusion [1]. Finally, toxicity related to peak drug levels after bolus administration seems likely to be reduced by a technique associated with low serum concentrations and this certainly appears to be the case for doxorubicin where the incidence of nausea and vomiting is markedly reduced [2, 3]. However, doxorubicin-induced stomatitis is probably worse after continuous infusion [2], an observation which draws attention to the possibility of increased cytotoxicity following prolonged low dose drug exposure.

There are also economic advantages of administering drugs by continuous infusion: the patient only needs to attend a clinic once to receive the regimen, the daily routine of the patient is undisturbed and the patient can resume a normal lifestyle.

Cisplatin has already been shown to be suitable for continuous infusion [4]. Two studies [5, 6] have previously investigated the stability of the second generation platinum drug, carboplatin for periods of up to *14* days. However, neither study was directly comparable to a proposed clinical trial involving carboplatin infusion over a 14-day period in patients using ambulatory pumps. This study ascertained the stability of carboplatin solutions contained in Pharmacia Deltec medication cassettes (100 ml; St Paul, MN, USA) over a period of 14 days at 37°C, and at 60°C where any degradation would be accelerated.

Materials and Methods

Carboplatin solutions were used at two concentrations (6.0 and 10.0 mg ml^{-1}). The higher concentration used was the commercially available carboplatin (Bristol–Myers). The lower concentration solution was prepared by diluting the commercially available material with a 5% solution of dextrose in water. Fifty millilitres of each solution were placed in the Deltec infusion bags and any air in the bags was expelled. The bags containing 6.0 mg ml^{-1} carboplatin were maintained at 60 and 37°C. A

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Heraeus B2025 oven was used for the higher temperature. This has a temperature reproducibility of ±1°C at 60°C. The 37°C sample was stored in a cell culture hot room where temperature variations are minimal (±1°C). No attempt was made to control humidity. The bag containing 10.0 mg ml^{-1} carboplatin was placed in an oven set at 37°C. A single bag was set up for each concentration–temperature combination. The samples were protected from light by aluminium foil, as this mimics the clinical situation where the dark plastic reservoirs are in holsters and often hidden under clothing.

Table 1
Intra-day and inter-day precision and accuracy of carboplatin concentrations

Prepared conc. (mg ml^{-1})	Measured conc. (mg ml^{-1})	Relative SD (%)	Bias (%)
intra-day			
6	6.06	3.2	1
10	9.77	2.4	2.3
inter-day			
6	5.85	4.0	2.5
10	9.99	4.1	0.1

$$\text{Bias} = \frac{\text{measured conc.} - \text{prepared conc.}}{\text{prepared conc.}} \times 100.$$

Analysis

The purity of the carboplatin solutions was determined using high-performance liquid chromatography (HPLC) on a Waters 600 MS solvent delivery system along with a Waters 484MS UV–vis detection system (220 nm). The HPLC detection procedure involved the application of carboplatin onto a Techopak 10 phenyl semi-micro column (150 × 2 mm; HPLC Technology, Macclesfield, UK) using an isocratic solvent system (water–methanol, 97.5:2.5, v/v) run at a flow rate of 1.0 ml min^{-1}. The internal reference used was barbital (5,5-diethyl-2,4,6-1*H*,3*H*,5*H*-pyrimidinetrione), which gave baseline resolution from carboplatin when injected into the HPLC system as a carboplatin–barbital mixture. The retention times for carboplatin were 0.91 ± 0.01 min and 2.52 ± 0.07 min, respectively.

The carboplatin concentration was calculated relative to a standard carboplatin solution (10 mg ml^{-1}) spiked with barbital. The analyte/internal standard ratio curve was linear over the concentration range studied (1–10 mg ml^{-1}, Peak ratio = 0.014 + 0.048 [carboplatin], $r^2 = 0.99$). The inter- and intra-day variations are shown in Table 1.

Mass spectrometry

Static mass spectrometry was carried out on a VG Trio 2 instrument using fast atom bombardment ionization (FAB). The bombardment gas used was argon with the atom gun operating at 1 mA at 8.5 kV and the carboplatin solution was mixed with thioglycerol before insertion into the mass spectrometer. Dynamic FAB mass spectrometry was performed with the same instrument. The HPLC system employed the above column using an isocratic solvent system (water–methanol–glycerol, 97.5:2.5:0.5, v/v/v) run at flow rate of 0.5 ml min^{-1}. Following elution from the column, the eluent passed via the light detector through a flow splitter which diverted 2–5 μl min^{-1} to the mass spectrometer. A flow rate which resulted in a pressure of 2×10^{-5} mbar in the analyser was found to be optimal. Argon was the bombardment gas with the atom gun operating at 1.6 mA at 6.4 kV.

Sample preparation

On each day of analysis, the Deltec bags containing the carboplatin solutions were weighed, the drug removed with a syringe, and the bags reweighed and replaced in their appropriate environments. No air was allowed to enter the bag during sample removal. The flexible nature of the Deltec bag meant that the liquid–bag area of contact did not change during the experiment. The drug solution was checked under a microscope for microbial contamination. The removed 6.0 mg ml^{-1} carboplatin solutions were spiked with the internal standard (final concentration 1.0 mg ml^{-1} barbital). Drug solutions were filtered (Acrodisc 0.45 μm, Gelman Sciences, Ann Arbor, MI, USA) and the filters examined for precipitate formation. The higher concentration solution was diluted to a concentration of 6 mg ml^{-1} with 5% dextrose solution in water and spiked with the internal standard (final concentration 0.6 mg ml^{-1} barbital). The concentration of internal standard was chosen so that approximately equivalent drug peak areas would be obtained. Injection volumes were 5 μl carboplatin/internal standard solution and drug estimations were carried out in triplicate. The concentration of carboplatin (peak area) was calculated relative to the

internal standard. The barbital solution was stored frozen at −20°C in 1 ml aliquots (prepared from the same solution), and a fresh aliquot was used on each day.

Drug degradation

A test of the ability of the HPLC system to detect any drug breakdown was carried out by studying the acidic hydrolysis of carboplatin in 2 M hydrochloric acid. The carboplatin solution (1 ml; 10 mg ml^{-1}) was treated with 2 M hydrochloric acid at 25°C and the mixture analysed by HPLC as described above. Control experiments were performed with the formulated carboplatin solution.

Results

The Deltec infusion pump reservoirs showed a loss of weight over the 14 days of the experiment. The bags showed no sign of leakage, so this loss of weight was attributed to evaporation. The weight loss was greater at the higher temperature (60°C) and remained approximately constant at each temperature over the 14-day period [T = 60°C, weight loss = 1.26 ± 0.33 g day^{-1}, T = 37°C (6.0 mg ml^{-1}), weight loss = 0.26 ± 0.07 g day^{-1}; T = 37°C (10.0 mg ml^{-1}), weight loss = 0.26 ± 0.06 g day^{-1}]. The 14-day cumulative weight loss was more notable at the higher temperature [T = 37°C (6.0 mg ml^{-1}), weight loss = 3.75 g; T = 37°C (10.0 mg ml^{-1}), weight loss = 3.59 g; T = 60°C, weight loss = 17.69 g]. The concentrations of the carboplatin solutions were corrected for this weight loss. No evidence of plasticisers was seen during this study.

The effect of concentration, time and temperature of the carboplatin solutions is depicted in Fig. 1. These data have been corrected for fluid loss. It can be seen that at 37°C there is no statistically significant alteration in the concentration of the carboplatin solution during the 14-day experiment for either of the concentrations tested [P = 0.219 (6 mg ml^{-1}), P = 0.267 (10 mg ml^{-1}) linear regression/analysis of variance]. However, at 60°C a significant loss of carboplatin concentration with time ($P < 0.001$, linear regression/analysis of variance) was apparent. After 14 days at 60°C the carboplatin had fallen to a concentration of 3.1 mg ml^{-1} from an initial concentration of 6.0 mg ml^{-1}. This decrease in the carboplatin peak was accompanied by the appearance of a breakdown product on the HPLC chromatogram with a retention time of 0.77 min. This is accompanied by the formation of small black crystals. The loss in concentration can be attributed to the degradation of the drug in solution at this higher temperature. This suggested that the carboplatin solution was breaking down when subjected to a temperature of 60°C. The nature of the breakdown product is unknown.

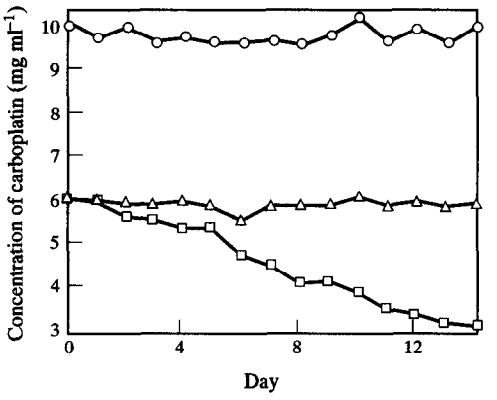

Figure 1
Stability of carboplatin over 14 days. The concentrations of carboplatin have been corrected for fluid loss. ○——○, 10.0 mg ml^{-1}, 37°C; Δ——Δ, 6.0 mg ml^{-1}, 37°C; □——□, 6.0 mg ml^{-1}, 60°C. Error bars represent standard deviations of the mean from triplicate samples.

No microbial contamination was noted at either concentration or temperature.

The HPLC analysis of the carboplatin solution treated with 2 M hydrochloric acid indicated rapid degradation. After 1 h at 25°C no carboplatin could be detected and a peak with a retention time of 0.77 min appeared. Baseline resolution was not achieved, however two peaks were readily distinguishable (Resolution factor = 0.82). This peak has a similar retention time to that observed following incubation of the carboplatin solution at 60°C. No similar change was noted in the control solution of carboplatin.

The static mass spectra of the carboplatin solutions gave a molecular ion of 371 (M + H). The mass spectra were indistinguishable for both concentrations and both temperatures at day 0 and day 14 of the experiment. No other major ions could be detected in the spectrum of the higher temperature carboplatin solution. The dynamic FAB experiments were carried out on both concentrations of carboplatin kept at 37°C following the stability study. This dynamic FAB mass spectrometry confirmed that the major peak was carboplatin (Fig. 2).

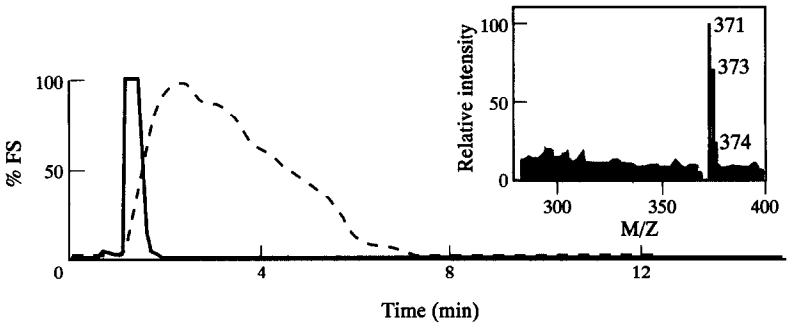

Figure 2
HPLC–dynamic FAB mass spectrometry of carboplatin. The solid trace is the HPLC of the carboplatin solution (10.0 mg ml^{-1}) at 220 nm. The broken trace is the corresponding ion current (m/z = 371). The inset is the mass spectrum derived from the ion current trace. FS means full scale.

Discussion

The use of cytotoxic chemotherapy has had a major impact on the management of a number of human cancers. However, the drugs currently available are not completely specific for tumour cells and this can result in damage to normal tissues. Successful therapy must, therefore, depend on the drug attaining a sufficiently high concentration in the plasma to exert its anti-tumour effects but without reaching levels likely to seriously or irreversibly damage normal tissues. The route and method of delivery can radically alter drug pharmacokinetics. The use of a continuous infusion regimen is believed to offer advantages over bolus injection. This route may avoid the transiently high levels of drug seen after bolus injection, which have been implicated in the toxicity of these agents. Similarly, infusions may be used to increase the duration of exposure of the tumour to drug.

This study shows carboplatin to be stable at 37°C at concentrations of 6 and 10 mg ml^{-1}. These concentrations will allow a clinical study on the effects of carboplatin given as a 14-day infusion by ambulatory pump to be carried out. This may allow chemotherapy to be given on an outpatient basis, even for the platinum drugs which are known to have potentially serious toxic side effects.

The stability of carboplatin has been the subject of a number of studies [5–8]. We have shown carboplatin to be stable at 37°C in aqueous solution at concentrations of 6 and 10 mg ml^{-1} for periods up to 14 days in Deltec infusion reservoirs. This result is in agreement with the work of Northcott *et al.* [5] who demonstrated carboplatin stability at 37°C over a similar timescale but under different storage conditions and concentration range. Two studies, however, have indicated that carboplatin is degraded under similar conditions. Sewell *et al.* [6] showed a carboplatin loss of 3.1% over 24 h (37°C), and Allsopp [8] a 5% loss in water in 52.7 h at 25°C. Obviously degradation at these rates would lead to a significant loss of carboplatin over 14 days. However, our study, in agreement with the work of Northcott [5], shows no degradation of carboplatin. This is of clinical importance, as degradation of carboplatin could lead to either loss of anti-cancer activity, or increased toxicity, as toxic cis-platinum-like species have been proposed as products following degradation of carboplatin [8].

Increased temperature (T = 60°C) does lead to degradation. However, the loss of carboplatin is considerably slower than that reported by Allsopp [8] at 59°C (k_{obs} (pseudo first-order rate constant) = 2×10^{-3} h^{-1}, compared to $k_{obs} = 11.24 \times 10^{-3}$ h^{-1}). The final breakdown products are unknown but hydrolysis probably proceeds by the mechanisms proposed by Allsopp [8]. Care must be taken that the formulation does not contain chloride or other ions which have been shown to increase degradation of carboplatin [8].

In conclusion carboplatin has been shown to be stable for 14 days at 37°C in Deltec infusion reservoirs both at 10 mg ml^{-1} (the commercial preparation) or diluted to 6 mg ml^{-1} with 5% dextrose.

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