Investigation on the Decomposition of Carboplatin in Infusion Solutions

I. Validation of HPLC conditions for the quantification of the decomposition and the 1,1-cyclobutanedicarboxylic acid release from carboplatin

Beate Schnurr and Ronald Gust*

Institut für Pharmazie der Freien Universität Berlin, Königin Luise Str. 2 + 4, D-14195 Berlin, Germany

Abstract. A high performance liquid chromatographic (HPLC) method to study the stability of carboplatin (cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II)) in commercial products was developed and validated according to the recommendations of the International Harmonization Conference [1]. For analytical applications a Nucleosil-100-5 C18 column and an isocratic system of CH3OH/(0.001 N H2SO4/0.02 M Na2SO4) 5/95 (V/V) or 10/90 (V/V) at a flow rate of 0.6 ml/min with UV spectroscopic detection at λ = 220 nm was used. It allows a quantification of carboplatin and 1,1-cyclobutanedicarboxylic acid (CBDCA) with limits of 1.03×10⁻⁴ mg/ml (=2.8×10⁻⁴ mol/l carboplatin) and 2.8×10⁻⁵ mg/ml (=1.9×10⁻² mol/l CBDCA), respectively. Investigations on a carboplatin infusion solution (Ribocarbo3K-L) showed that besides carboplatin and CBDCA hydrophilic as well as hydrophobic decomposition products are formed during the time of storage. A comparative study using conditions according to the European Pharmacopoeia 1997 (Nucleosil-120-7 NH2-column, acetonitril/water 90/10 (V/V), isocratic conditions at a flow rate of 1.98 ml/min), indicated in the same solution carboplatin in a purity of 100%. Admixture of CBDCA (0.75 mg/ml) could not be detected in the chromatogram, so this investigation gives rise to the assumption that the conditions of the European Pharmacopoeia 1997 are not suitable for carboplatin purity analyses.

Key words: Carboplatin; CBDCA; HPLC; stability.

Cis-diamminedichloroplutunum(II) (cisplatin, Scheme 1) is a widely used metal complex in the therapy of malignant tumors [2]. However, its therapeutical application is limited due to its toxic side effects and its low water solubility. These drawbacks could be overcome by exchange of the Cl⁻ leaving groups by the diianion of 1,1-cyclobutanedicarboxylic acid (CBDCA) (→carboplatin, see Scheme 1) [3, 4, 5].

In carboplatin CBDCA is chelate bound and undergoes a dynamic conversion in aqueous solution [6]. Due to this interconversion of the 6-membered chelate ring the cyclobutane moiety is shielding the central platinum atom from the attack of nucleophiles [7]. As a consequence the stability of carboplatin is more than 100 times higher than that of cisplatin (rate constants for hydrolysis reactions in aqueous solutions:

Scheme 1 Structural formulae of carboplatin, cisplatin and CBDCA.

*To whom correspondence should be addressed
Scheme 2 Hydrolysis of carboplatin in aqueous solution.

\[ k_{37°C}(\text{cisplatin}) = 4.16 \times 10^{-5} \text{ s}^{-1}; \quad k_{37°C}(\text{carboplatin}) = 4.67 \times 10^{-7} \text{ s}^{-1} \] [8, 9].

The slow but still important hydrolysis of carboplatin takes place in a two-step consecutive reaction [6, 10]:

1. Opening of the 6-membered chelate ring by attack of a water molecule
2. Release of CBDCA

Both steps - illustrated in generalized form in Scheme 2 - are acid catalysed, so at neutral conditions (pH = 7.0–7.4) degradation by hydrolysis takes place in small amounts only. The high water solubility of carboplatin (18.6 mg/ml) [5] and its low hydrolysis rate make it possible to produce ready-to-use infusion solutions facilitating handling and administration enormously.

For clinical use, the long-term stability of such market products and the range of degradation products is of high interest. Despite several publications about investigations on carboplatin stability by means of HPLC [8, 9, 11–14] only a few are detecting CBDCA as well [15, 16] but have not been tested for their suitability to investigate long-term stored ready-to-use infusion solutions. For this reason chromatographic conditions were developed to quantif the carboplatin and the CBDCA release as parameter for the formation of highly toxic aqua- or diaquaplatinum(II) complexes in such infusion solutions. The development and validation of an adequate HPLC system is described in this paper.

Experimental

Drugs and Materials

Market products and gold standards were gifts from Ribosepharm, Munich, Germany, (Ribocarbo®-L 450 mg) and Lachema, Brno, Czech Republic (Cycloplatin® 450, Carboplatin and CBDCA gold standards). The calibration curves were confirmed with carboplatin, batch no. 46H1004, obtained from Sigma, Munich, Germany and CBDCA (99% free acid), batch no. 01196, from Lancaster, Eastgate, England. Acetonitril, methanol, Na_2SO_4, H_2SO_4 were obtained from Merck, Darmstadt, Germany. Deionized water from a MilliQ filter system (>18 MΩ) was used during all experiments.

Calibration Standards

The quantification of carboplatin and CBDCA was performed by use of calibration standard curves. For the carboplatin calibration standards approximately 1 mg/ml lyophilized carboplatin was dissolved in water and diluted to the working range of 0.075–0.11 mg/ml. For the CBDCA calibration standards an aqueous stock solution of approximately 10 mg/10 ml CBDCA (>99% free acid) was prepared and the pH value was adjusted with 2.5% NH_3 to 5.5–6.5, the pH range of the ready-to-use infusion solutions. Subsequently, the solution was diluted to the working range of 0.02–0.85 mg/ml.

HPLC Apparatus

The HPLC analyses were performed with a Kontron high pressure mixing gradient system (pump 422; autosampler 465; UV detector 430A) and the Kromasystem 2000 software package. A 250 × 4 mm Nucleosil-100-5 C18 column (Macherey & Nagel, Düren, Germany) with a Nucleosil-120-5 C18 30 × 4 mm precolumn (Macherey & Nagel, Düren, Germany) was used for chromatography.

Chromatographic Conditions

After being loaded onto the column, the sample (40 μl) was eluted at room temperature at a flow rate of 0.6 ml/min with an isocratic system consisting of a mixture of CH_3OH and an aqueous solution of 0.001N H_2SO_4 and 0.02M Na_2SO_4 (carboplatin: 5/95 (V/V); CBDCA and side products: 10/90 (V/V)). Substances were detected at λ = 220 nm, the peak area was used for quantitation. While CBDCA and decomposition products were determined directly from the commercial products, a dilution of 1:100 was necessary for the quantification of carboplatin. To assess the suitability of this HPLC assay for the analysis of carboplatin market products the standard conditions of the European Pharmacopoeia 1997 [17] were adjusted and used for chromatography additionally (Nucleosil 120-7 μM NH_2-column (250 × 4 mm) (Macherey & Nagel, Düren, Germany), injection volume: 40 μl, mobile phase of acetonitril/water 90/10 (V/V) under isocratic conditions at 1.98 ml/min, UV-detection at λ = 230 nm).

Results and Discussion

Carboplatin is a widely used well tolerable second generation platinum anticancer drug. The low toxicity is thought to be due to its low reactivity caused by the chelate bound CBDCA. The rate of hydration is very low and the major reaction path of carboplatin is via direct attack by nucleophiles rather than via a hydration step. This allows the preparation of ready-to-use