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Investigations on the Stability of Carboplatin Infusion Solutions

Ronald Gust* and **Beate Schnurr**

Institut für Pharmazie I, Freie Universität Berlin, D-14195 Berlin, Germany

Summary. The stability of the carboplatin market drug products Ribocarbo® and Ribocarbo-L® in 0.9% sodium chloride or 5% glucose infusion solution was investigated by HPLC analysis using a Nucleosil-120-5- C18 column, an eluent consisting of methanol and an aqueous solution of H_2SO_4 (0.001 *N* with Na_2SO_4 (0.02 *M*); 10:90 or 5:95 (v/v)), and UV detection.

At room temperature, carboplatin is stable in 0.9% NaCl solution for 1 h only. During the following 168 h, a 10% degradation to cisplatin and the intermediate diamminechloro[O^1-1-carboxylato-1-carboxycyclobutane]platinum(II) takes place. This reaction is independent of the carboplatin concentration used.

Under identical conditions in 5% glucose solution the carboplatin concentrations decrease during 72 h by about 2–3% and by 5–6% after 168 h of storage. At 4°C, a 10 mg/ml solution is stable during the experiment, whereas from a 1 mg/ml carboplatin infusion, 2% of the drug are lost after day 7 and about 3% after day 28. Degradation products were unequivocally identified. The presence of highly toxic carboplatin hydrolysis products, however, could be excluded.

Keywords. Carboplatin; Stability; 0.9% Sodium chloride; 5% Glucose.

Untersuchungen zur Stabilität von Carboplatin-Infusionslösungen

Zusammenfassung. Die Stabilität der Carboplatin-Fertigarzneimittel Ribocarbo® und Ribocarbo-L® in 0.9% NaCl- und 5% Glucose-Infusionslösungen wurde mittels HPLC untersucht (Nucleosil-120-5 C18-Säule, Methanol/verdünnte H_2SO_4 (0.001 *N* mit Na_2SO_4-Zusatz (0.02 *M*) 10:90 oder 5:95 (v/v), UV-Detektion).

Bei Raumtemperatur ist die Wirkstoffkonzentration in 0.9% NaCl-Lösung nur 1 h lang konstant. Im Lauf von 168 h erfolgt ein 10%-iger Abbau zu Cisplatin und Diamminchloro[O^1-1-carboxylato-1-carboxycyclobutan]platin(II). Dieser Carboplatinabbau ist unabhängig von der verwendeten Wirkstoffkonzentration.

Auch in 5%-iger Glukoselösung wird innerhalb von 72 h ein 2–3%-iger Carboplatinverlust beobachtet, der nach 7 Tagen 5–6% beträgt. Bei 4°C ist die Carboplatinstabilität wesentlich höher. Eine Infusionslösung mit 10 mg/ml Wirkstoff ist während des gesamten Beobachtungszeitraums stabil, während bei Verwendung einer Wirkstoffkonzentration von 1 mg/ml ein Carboplatinabbau von *ca.* 2% nach 7 Tagen und *ca.* 3% nach 28 Tagen zu beobachten ist.

Abbauprodukte wurden eindeutig nachgewiesen; der Abbau zu hochtoxischen Carboplatin-Hydrolyseprodukten konnte jedoch ausgeschlossen werden.

* Corresponding author

Introduction

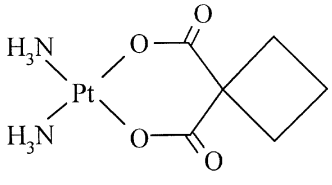
Cisplatin (diamminedichloroplatin(II); see Scheme 1) is one of the therapeutically most important chemotherapeutics in tumor therapy [1, 2]. However, its therapeutical index is limited due to its poor water solubility and strong toxic side effects [1]. The latter result from the high reactivity of the complex against bionucleophiles and the formation of the active aquated metabolites $(NH_3)_2PtCl(OH_2)^+$ and $(NH_3)_2Pt(OH_2)_2^{2+}$ which are also subject to irreversible binding to S-containing bionucleophiles, especially to albumin, the major protein in plasma [3, 4]. This process leads to a decrease of the free drug level and therefore to a deterioration of its antitumor potency (compare also Ref. [5]).

For the development of platinum(II) complexes with better pharmacokinetic properties, the anion of cyclobutane-1,1-dicarboxylic acid (*CBDC*) proved to be a useful leaving group. Carboplatin, the *CBDC* derivative of cisplatin (Scheme 1) is highly stable and yields markedly enhanced free drug levels *in vivo* [6]. Another remarkable feature is its markedly higher water solubility (approx. 18.6 mg/ml [7]).

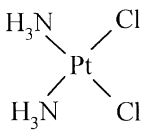
The high inertness of carboplatin against nucleophilic attack results from the six-membered chelate ring of the Pt(malonato) moiety. Analogously to cisplatin, the geometry around the platinum atom of carboplatin is square planar [8, 9, 10], but the puckering malonato chelate ring undergoes a dynamical conversion in solution [11]. According to this, the cyclobutane ring gets close to the platinum central atom and thereby hinders the attack of nucleophiles.

In aqueous solution, the decomposition of carboplatin is solely determined by its hydrolysis. Although H_2O does not represent a strong nucleophile, first a chelate ring opening (1st hydrolysis step) and finally a liberation of the *CBDC* group (2nd hydrolysis step) can be observed (see Scheme 2). The reaction rate of this consecutive substitution is entropically controlled by the chelate effect of the *CBDC* leaving group. The rate constant for the hydrolysis of carboplatin is distinctly lower than that of cisplatin (at room temperature: carboplatin, $k_s = 8.14 \times 10^{-8} s^{-1}$ [12]; cisplatin, $k_s = 6.32 \times 10^{-5} s^{-1}$ [13]) and depends on the temperature as well as on the *pH* value. In the presence of other nucleophiles, *e.g.* chloride, iodide, or molecules containing S- and O-centers, a considerably more rapid degradation of the drug could be observed. The available data, however, are very contradictory regarding these reactions. This is especially true for stability data of carboplatin in infusion solutions [14–16] containing sodium chloride or glucose.

To prepare infusions, solid market drug products or aqueous injection solutions of carboplatin (Ribocarbo-L®, Carboplat®, Cycloplatin®) are diluted with 0.9% NaCl solution or 5% glucose solution to the desired final concentration. In this



Carboplatin



Cisplatin

Scheme 1. Structural formulae of carboplatin and cisplatin

study, we investigated the stability and the decomposition kinetics of carboplatin (Ribocarbo® and Ribocarbo-L®) in these infusion vehicles.

Results and Discussion

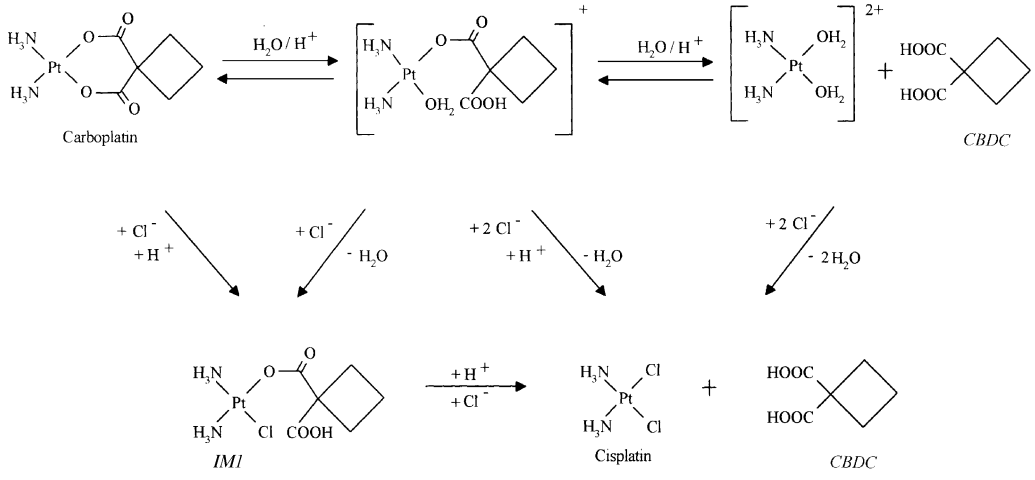
Stability of Ribocarbo-L® in 0.9% NaCl solution at room temperature

Ribocarbo-L® 450 was dissolved in physiological NaCl solution (0.9%) to final concentrations of 0.5, 1.0, 5.0, and 10.0 mg/ml. These solutions were stored in brown flasks at room temperature, and the carboplatin concentration was determined during the experiment (168 h) by use of HPLC techniques. The peak areas in the chromatograms were compared with those of an external carboplatin standard. Each measurement was repeated twice. The results are listed in Table 1.

The analytical conditions (column: Nucleosil-120-5 C18; eluent: methanol/aqueous solution of H_2SO_4 (0.001 *N* with Na_2SO_4 (0.02 *M*) 10:90 (v/v); UV detection at 229 nm) allowed the identifications of carboplatin, the degradation product cisplatin, and the intermediate diamminechloro[O^1-1-carboxylato-1-carboxycyclobutane]platinum(II) (*IM1*, Scheme 2).

Table 1. Stability of carboplatin in 0.9% NaCl solution

Concentration of carboplatin (mg/ml)	Content of carboplatin (%) at various times of storage						
	0 h	0.5 h	1 h	6 h	24 h	48 h	168 h
0.5	100	100.3±0.6	99.3±0.2	97.5±0.7	95.8±1.0	94.8±1.0	90.1±0.6
1.0	100	100.5±0.7	99.5±0.3	97.5±0.8	95.7±0.3	94.3±0.5	90.4±0.7
5.0	100	100.0±0.2	99.2±0.5	97.4±0.2	95.9±0.8	94.5±0.7	90.1±0.6
10	100	99.5±0.7	99.8±0.1	97.3±0.2	95.3±0.7	94.0±0.5	89.9±0.7



Scheme 2. Hydrolysis of carboplatin in aqueous solution and reaction with Cl^-

As listed in Table 1, the stability of carboplatin was independent of the applied carboplatin concentration. During 1 h of storage the content of carboplatin remained nearly constant. After 6 h, about 2.5% of carboplatin reacted with Cl^- to cisplatin. Both cisplatin and the intermediate diamminechloro[O^1-1-carboxylato-1-carboxycyclobutane]platinum(II) could be identified in the chromatograms. Further degradation products, *e.g.* hydrolysis products of cisplatin or carboplatin, were not formed. It is of interest to note that the HPLC system used indicated a higher hydrophobicity of the intermediate *IM1* as compared to cisplatin and carboplatin, expressed in a higher retention time ($t_R = 10.21$ min; carboplatin: $t_R = 5.80$ min, cisplatin: $t_R = 4.06$ min).

During the experiment (168 h time of storage), the peak area of carboplatin decreased to 90% of the starting area. After 6 h, an amount of 2% of cisplatin could already be detected in the chromatograms. This means that using this solution for infusions administers an inacceptable amount of cisplatin to the patients which could induce toxic side effects.

Stability of Ribocarbo-L® in 5% glucose solution

To investigate the compatibility of carboplatin (10 mg/ml and 1 mg/ml) with glucose, we dissolved Ribocarbo® and Ribocarbo-L® in 5% aqueous glucose solution using the *Baxter* adapter system. Peaks resulting from the glucose solution were identified by eluting a carboplatin free reference solution. To minimize the UV absorption of glucose for the detection, a wavelength of 316 nm was used. In the chromatogram (eluent: methanol/aqueous solution of H_2SO_4 (0.001 *N* with Na_2SO_4 (0.02 *M*); 5:95 (v/v)), only one relatively broad peak was detected with a retention time of $t_R = 42.10$ min, well separated from that of carboplatin ($t_R = 8.88$ min; see Fig. 1).

Directly after dissolution of carboplatin (day 0) the infusion solutions were analyzed as to the quantity of carboplatin and possible impurities. From the beginning the glucose and carboplatin peaks were accompanied by small amounts (< 0.5%) of nonidentified impurities ($t_R = 6.37$ and 7.34 min) which did not change their areas during the experiment.

Dependent on the conditions of storage, the peak area of carboplatin decreased. At room temperature, a degradation of the drug was found at a concentration of 1 mg/ml as well as of 10 mg/ml. At the end of the experiment, in both cases over 10% of carboplatin were lost (Table 2).

The breakdown of carboplatin was accompanied by the formation of a side product (*SP1*), detected in the chromatograms at $t_R = 11.02$ min (Fig. 1). An HPLC analysis of Ribocarbo® as well as Ribocarbo-L® indicated that *SP1* did not originate from the market drug product. It appeared for the first time in the chromatograms after 3 d in small amounts. During 28 d of storage its peak area increased up to 10–15% compared to the initial carboplatin peak area. In the case of the 1mg/ml carboplatin solution, 9% of *SP1* were formed (Fig. 1). This correlates very well with the loss of carboplatin; therefore we assume that *SP1* represents a reaction product of carboplatin and glucose.

The stability of carboplatin increased upon storing the infusion bags at 4°C. As listed in Table 2, the stability of carboplatin depends on the concentration used.

Table 2. Stability of carboplatin in glucose solution 5%

Concentration (mg/ml)	Temperature of storage (°C)	Time of storage (d)	Content of carboplatin[a] (mg/ml)	(%)
1	4	0	1.03	100.0
		3	1.02	98.8
		7	1.01	98.0
		28	1.01	97.4
10	4	0	9.17	100.0
		3	9.16	99.9
		7	9.12	99.5
		28	9.12	99.4
1	23	0	1.02	100.0
		3	0.99	97.2
		7	0.97	94.9
		28	0.91	89.7
10	23	0	9.22	100.0
		3	8.99	97.6
		7	8.69	94.3
		28	7.84	85.1

[a] Values within ±0.8%

The infusion containing 10 mg/ml of carboplatin exhibited no impurities or degradation products, whereas in the infusion with 1 mg/ml of carboplatin 2.5% of *SP1* were detected after 28 d of storage (Fig. 1A).

To prove the existence of hydrolysis products we added KI (10 mg) to the infusions. Iodide represents a strong nucleophilic agent for platinum complexes and displaces H_2O immediately from its coordination site [5]. The compounds to be considered, diammine[O^1-1-carboxylato-1-carboxycyclobutane]iodoplatinum(II) and diamminediiodoplatinum(II), were not detected. Furthermore, the peak area of *SP1* remained nearly unchanged, thus excluding a hydrolysis product as a possible structure for *SP1*.

Our results agree very well with those of *Cheung et al.* [14] who found a 5% degradation of carboplatin in physiological sodium chloride solution (0.9%) during the storage of 24 h. Therefore, we do not recommend its use. Although the conversion of 5% of carboplatin to cisplatin seems to be small, it is of clinical importance because of the five times greater toxicity of cisplatin.

A suitable injection solution for carboplatin is represented by 5% glucose. *Benaji et al.* [15] have described a constant concentration of 2.4 mg/ml in PVC bags over a storage period of 9 d at room temperature. *Amador et al.* [17] stored their infusions for 21 d at room temperature and lost 3–5% of carboplatin, independent of its initial concentration. Contrarily to these studies, we found a stability at room temperature for 3 d; only during this period less than 3% of carboplatin are lost.

The HPLC system used allowed the detection of breakdown products in the infusions. In physiological sodium chloride solution (0.9%), cisplatin and

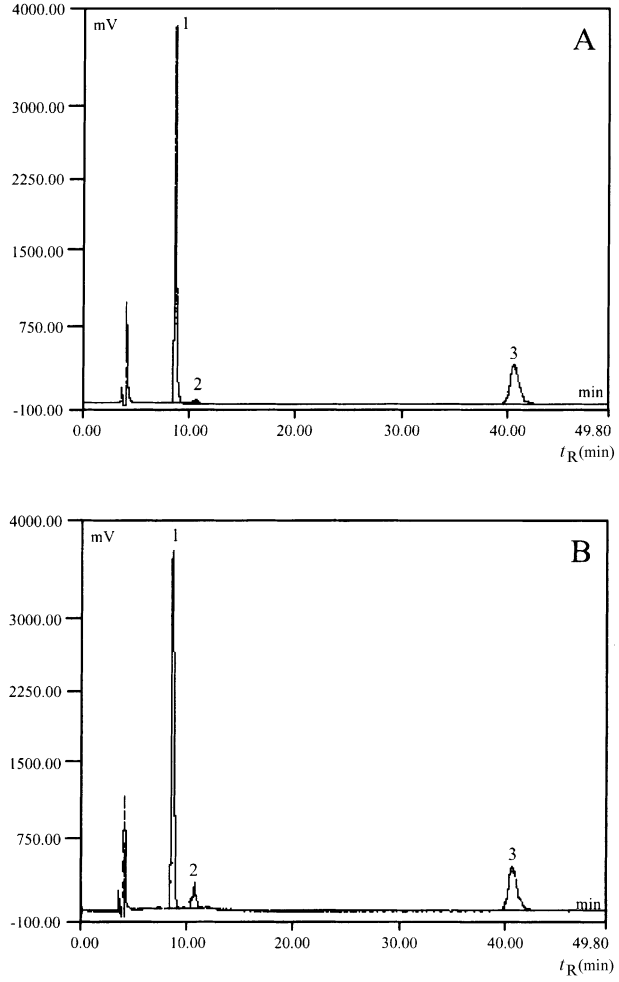


Fig. 1. Chromatograms of 5% glucose infusions containing 1 mg/ml carboplatin after a storage of 28 d at 4°C (A) and room temperature (B); peak 1: carboplatin; peak 2: side product SP1; peak 3: glucose

diamminechloro[O^1-1-carboxylato-1-carboxycyclobutane]platinum(II) were identified. In glucose solution, a reaction product (*SP1*) was detected. Its amount corresponds to the degradation of carboplatin, suggesting a reaction of carboplatin with glucose. Since there is no information about the toxicity of this compound, carboplatin infusions with 5% glucose as vehicle should be stored at 4°C. In this case the solution is stable for 28 d.

Conclusions

Considering the results of our study, we recommend a reconstitution of Ribocarbo® and Ribocarbo-L® in 5% glucose solution and a storage at 4°C. Only these conditions guarantee a high stability of carboplatin. Storing the infusion bags at room temperature (20–25°C) results in a sufficient stability of only 3 days. After

this time a nonacceptable, nonidentified reaction product of carboplatin and glucose will be formed.

Experimental

Drugs and materials

Cisplatin was obtained from Aldrich (Steinheim, Germany), carboplatin from Sigma (Munich, Germany). Ribocarbo 200®, Ribocarbo 50®, and Ribocarbo-L 450® market drug products were a gift of *Ribosepharm* (Munich, Germany). The minibag/mix adaptor system as well as the 5% glucose solutions were purchased from Baxter Co. (Munich, Germany).

HPLC analyses of the product mixtures

The HPLC analyses were performed with a Kontron high pressure mixing gradient system (Kontron HPLC pump 430; Kontron HPLC autosampler 460; Kontron HPLC UV detector 430). A 0.4×25 cm Nucleosil-120-5 C18 column (Macherey-Nagel, Düren, Germany) with a 0.4×3.0 cm precolumn was used for chromatography. After being loaded onto the column, the sample (50 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 H_2SO_4 (0.001 *N* with Na_2SO_4 (0.02 *M*) 10:90 or 5:95 (v/v)). For the detection of the drugs, a wavelength of $\lambda = 229$ or 316 nm was used. Of each sample at least 3 analyses were made. Integration of the peak areas was achieved using the Kontron 450 MT data system. The carboplatin concentration was determined by comparing the respective peak areas with that of an external carboplatin standard.

Stability of Ribocarbo-L® in 0.9% NaCl solution at room temperature

Ribocarbo-L® 450 was added to physiological sodium chloride solution to final carboplatin concentrations of 0.5 , 1.0, 5, and 10 mg/ml. The solutions were stored in brown glass vials. During the experiment, the carboplatin content was determined after 0.5, 1, 6, 24, 48, and 168 h by use of HPLC (CH_3OH/H_2SO_4 (0.001 *N* with Na_2SO_4 (0.02 *M*), 10:90 (v/v)). For these purposes, 1 ml of solution was taken from the bag after intensive shaking *via* a 1 ml injection needle and filled into a silanolized 1.7 ml vial. 70 µl of this sample was loaded onto the column for analyzing.

Beside the carboplatin peak ($t_R = 5.80$ min), peaks of diamminechloro[O^1-1-carboxylato-1-carboxycyclobutane]platinum(II) ($t_R = 10.21$ min) and cisplatin ($t_R = 4.06$ min) could be registered. For assignment purposes carboplatin and cisplatin standard reference substances were used.

Stability of Ribocarbo® in 5% glucose solution at 4°C and room temperature

To prepare the final infusions, the minibag/mix adapter system according to *Baxter* was used. The vehicle was filled into the carboplatin bottles using the adapter system. After this, the carboplatin was dissolved by thorough shaking. The bottle reached a high pressure point through pumping motions which returned the solution into the bag. Threefold repetition of the procedure led to a quantitative transfer of the carboplatin into the infusion bag. By employing this closed system, a contamination-free dissolution of the cytostaticum was guaranteed.

The final concentration of 10 mg/ml was obtained by dissolution of the contents of two bottles Ribocarbo 200® and Ribocarbo 50® each. This corresponds to a total of 500 mg carboplatin in 50 ml 5% glucose.

To prepare the 1 mg/ml solutions, 5 ml were removed from the prefilled glucose infusion bag and replaced by 5 ml of Ribocarbo-L® 450 infusion solution containing 50 mg of carboplatin.

The reconstituted solutions were stored at room temperature (23°C) and at refrigerator temperature (4°C), respectively, for 28 d. Carboplatin contents were determined by HPLC on days 0, 3, 7 and 28 as described above but using an eluent of CH_3OH/H_2SO_4 (0.001 *N* with Na_2SO_4 (0.02 *M*), 5:95 (v/v)) and a wavelength of 316 nm. Under these conditions, the carboplatin peak appeared at $t_R = 8.88$ min. Beside this, a peak at $t_R = 42.10$ min resulting from the glucose solution was identified in the chromatograms.

Acknowledgements

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