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Stability of cisplatin and its monohydrated complex in blood, plasma and ultrafiltrate — implications for quantitative analysis*

ANITA ANDERSSON† and HANS EHRSSON

Karolinska Pharmacy, Box 160, S-171 76 Stockholm, Sweden

Abstract: The stability of cisplatin and its monohydrated complex has been studied in blood, plasma and plasma ultrafiltrate at 37°C (pH 7.4). Intact cisplatin and the monohydrated complex were determined by liquid chromatography with post-column derivatization. The half lives for cisplatin and the monohydrated complex were 1.43 ± 0.03 h (SEM) and 0.36 ± 0.03 h (SEM), respectively, in blood and 0.88 ± 0.05 h (SEM) and 0.26 ± 0.02 h (SEM), respectively, in plasma. The compounds were unstable at −25°C ($t_{½}$ for cisplatin was 52 ± 5 h (SEM) and for the monohydrated compound 26 ± 2 h (SEM)), but at −70°C both compounds were stable for at least 3 weeks. The monohydrated complex was found to be formed to a small extent when cisplatin was added to plasma (37°C, pH 7.4). A sampling procedure using centripetal ultrafiltration of whole blood was evaluated and found applicable if the samples were stored at 0°C and ultracentrifuged within 1 h.

Keywords: Cis-*diamminedichloroplatinum(II);* cis-*diammineaquachloroplatinum(II)-ion; cisplatin; sample handling; stability.*

Introduction

Cisplatin is one of the most important anticancer drugs for the treatment of solid tumours. When cisplatin is dissolved in aqueous solution, the chloride ions are displaced by water molecules [1] (see Scheme 1 for definitions used throughout the text). It is generally assumed that cisplatin is present as intact drug in plasma, due to the high chloride concentration [2]. Inside the cell the chloride concentration is considerably lower favouring the formation of monoaqua (Scheme 1) which is believed to be the ultimate cytotoxic agent reacting with DNA [3–5].

A wide variety of analytical techniques has been presented for the quantitative determination of cisplatin (for a recent review see [6]), while techniques for the determination of the monohydrated complex are more sparse [7].

The stability of cisplatin in plasma and plasma ultrafiltrate (UF) has been investigated in several studies at 37°C. However, it is difficult to draw any firm conclusions about the fate of the intact drug since many of these studies are based on techniques with low selectivity. Moreover, studies where intact cisplatin have been measured are complicated by the fact that the pH has not been stabilized.

Cisplatin was found to be stable for at least 72 h during storage conditions in plasma UF at −10°C [8]. Yang and Drewinko [9] studied the cytotoxic efficacy of cisplatin stored at +4, −20 and −70°C in saline for 3 weeks and found that a significant degree of efficacy was lost regardless of temperature. Cisplatin was unstable in urine at −11°C, but there was no significant degradation at −60°C after 48 h [10].

In the present study the stability of both cisplatin and its monohydrated complex has been studied using an analytical technique based on liquid chromatography with post-column derivatization [7]. The stability has been studied in whole blood, plasma and UF at 37°C (pH 7.4). The stability during storage conditions at −25 and −70°C in plasma and UF and in blood at 0°C has also been evaluated. To investigate the possible formation of the monohydrated compound, cisplatin was added to

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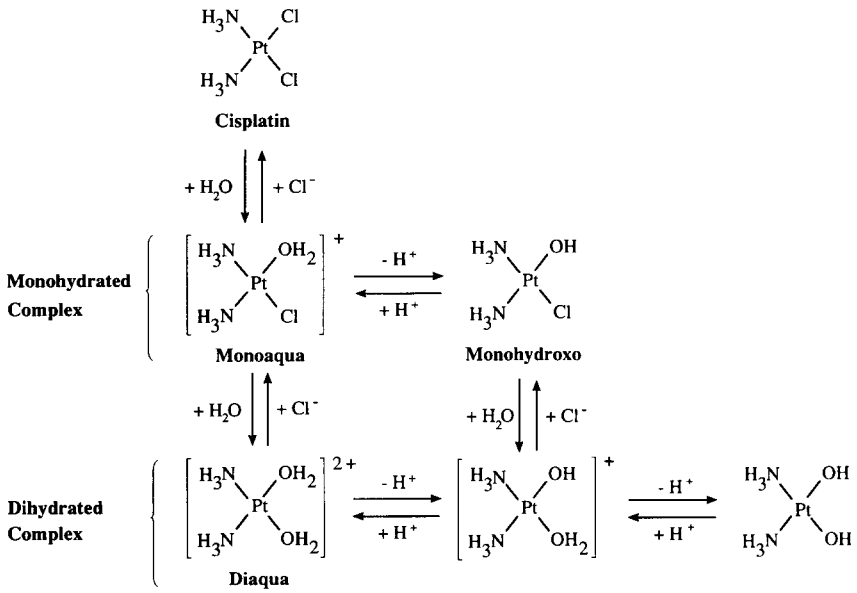
†Author to whom correspondence should be addressed.



Scheme 1
Equilibria of cisplatin in aqueous solution.

plasma whereafter the monohydrated complex was monitored. Also the reverse, formation of cisplatin after adding monohydrated compound was followed.

Experimental

Chemicals

Cisplatin was purchased from Ventron, Karlsruhe, Germany. The monohydrated complex was isolated from a hydrolysis equilibrium obtained by dissolving cisplatin in distilled water (4 mM) and allowing the solution to stand overnight at room temperature. The monohydrated complex was isolated by LC on a Hypercarb S column (100 × 4.6 mm i.d., Shandon Scientific Ltd, Runcorn, UK) using an aqueous mobile phase (manuscript in preparation). Analytical grade methanol was from Merck (Darmstadt, Germany) and 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) was obtained from the Sigma Chemical Company (St Louis, MO, USA).

Apparatus

The instrumentation and conditions for the quantitative determination of the monohydrated compound and cisplatin are described elsewhere [7]. Briefly, a strong anionic exchange column (Nucleosil 5 SB) was used when cisplatin was studied and a strong cationic exchange column (Nucleosil 5 SA) was used for the monohydrated complex. In both cases the mobile phase consisted of 0.125 M succinic acid adjusted to pH 5.2 with sodium hydroxide and methanol (2:3, v/v). The post-column reaction was performed with a packed bed reactor using sodium diethyldithiocarbamate as derivatization reagent. The UV absorbance was measured at 344 nm.

The pH was measured using an Orion model SA 520 pH meter equipped with a 810300 Semimicro Ross combination pH electrode (Orion Research Inc., Cambridge, MA, USA).

Stability studies

Cisplatin or the monohydrated complex were mixed with blood, plasma or ultrafiltrate to a final concentration of 50 μM.

Blood. Fresh blood was allowed to equilibrate to 37°C in a cell incubator (5% CO_2 and 85% humidity) before it was mixed with cisplatin or the monohydrated compound. Teflon tubes were used to aspirate the samples to maintain the environment in the incubator. The samples were chilled and immediately ultrafiltrated centripetally using a 10 000 MW cutoff filter (Centrisart®, Sartorius AG, Göttingen, Germany) for 15 min at 4000*g* at 4°C. The pH was measured at the end of the experiment.

Plasma. Fresh plasma was buffered with 0.01 M HEPES to pH 7.4 (<10% v/v). The plasma was placed in a thermostatted bath at 37°C and allowed to equilibrate. Cisplatin or the monohydrated compound was added and samples taken were immediately ultrafiltrated using the same conditions as above. The plasma was allowed to cool to room temperature prior to the pH measurement.

Ultrafiltrate. The pH of the ultrafiltrate was adjusted to 7.4 with 0.05 M HEPES. The vial was thermostatted at 37°C, cisplatin or the monohydrated complex was added and samples were taken and directly injected into the LC system. The degradation was followed for 20 h. In one experiment 0.1 M NaCl was added to the ultrafiltrate and further divided into two aliquots, where the pH was lowered to 5.5 with perchloric acid in one fraction while the other was left untreated. Cisplatin was added to a concentration of 200 μM and the stability was followed at 37°C.

Low temperature studies. Fresh plasma or ultrafiltrate was spiked with cisplatin or the monohydrated compound. The samples were individually frozen in plastic vials (CMA/Microdialysis AB, Stockholm, Sweden) at −25°C and −70°C. The ultrafiltrate (50 μl) was thawed and directly analysed. Plasma samples (0.5 ml) were thawed and ultrafiltrated for 10 min as described in the study of blood. The stability in blood at 0°C was studied without control of CO_2 concentration.

Statistical treatment of data

All data are given as mean ± SEM. The evaluation of the constants was performed with nonlinear regression analysis (GraphPad Prism™ ver. 1.00, GraphPad Software Inc., San Diego, CA, USA). The number of exponents in the equations expressing the degradation processes was established by the *F*-ratio test [11].

Results

The experimental data and curves fitted to a monoexponential (blood and plasma) and a biexponential decay (UF) for cisplatin and the monohydrated compound at 37°C (pH 7.4) are shown in Fig. 1 and the half lives are given in Table 1. The rate of degradation of the monohydrated compound was about four times higher than that of cisplatin in blood, plasma and UF. Cisplatin was more stable in blood as compared to plasma, while there was no significant difference for the monohydrated complex.

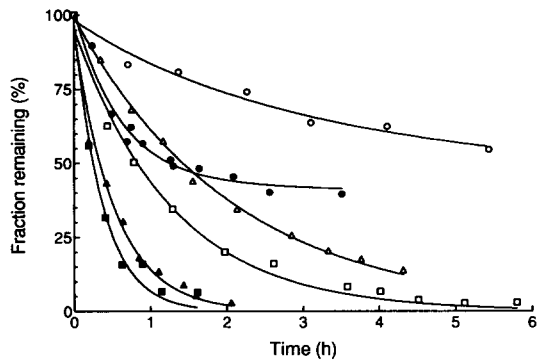


Figure 1
Degradation of cisplatin and the monohydrated complex at pH 7.4 (37°C) in various human media. Key: Cisplatin in ultrafiltrate (○), plasma (□), blood (△) and the monohydrated complex in ultrafiltrate (●), plasma (■), blood (▲).

Table 1
Half lives (h) of cisplatin and the monohydrated complex at 37°C (pH 7.4)

Medium	Cisplatin (mean ± SEM)	Monohydrated complex (mean ± SEM)
Blood	1.43 ± 0.03	0.36 ± 0.03
Plasma	0.88 ± 0.05	0.26 ± 0.02
Ultrafiltrate	α = 1.7 ± 0.4*	α = 0.42 ± 0.05*
	β = 22 ± 3	β = 51 ± 13

*Biexponential decay.

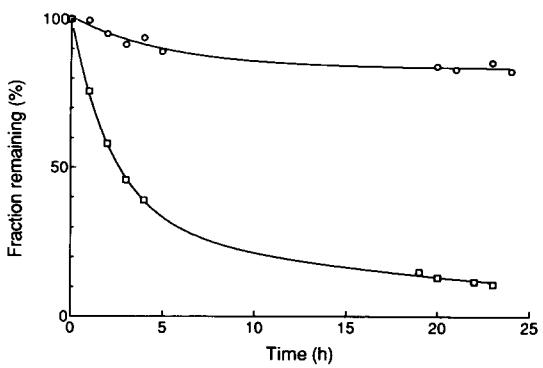


Figure 2
Degradation of cisplatin in ultrafiltrate at 37°C with added NaCl (0.1 M) at pH 5.5 (○) and pH 8.3 (□).

The stability of cisplatin in ultrafiltrate with added NaCl (0.1 M) at alkaline (without buffer) and acidic pH (5.5) is given in Fig. 2. There was a significant drift of pH in the sample without pH control from 8.3 to 8.9 after 24 h. The half life was 1.6 ± 0.2 h for the α-

phase and 15 ± 3 h for the β-phase. The half life for the degradation at the lower pH could not be calculated with sufficient accuracy since cisplatin was only degraded to a small extent.

The formation of the monohydrated complex was studied in plasma at 37°C after addition of cisplatin (Fig. 3). The maximum yield (10%) was reached after about 1 h. About 30% of the added monohydrated complex was also converted to cisplatin (Fig. 3).

The stability of cisplatin and the monohydrated complex at low temperatures is given in Table 2. The stability of cisplatin and the monohydrated complex was remarkably low in plasma at −25°C. The time for 90% of the initial concentration was remaining ($t_{0.90}$) was calculated to 8 h for cisplatin and 4 h for the monohydrated compound. In blood (0°C) cisplatin was stable at least 4 h, while $t_{0.90}$ for the monohydrated compound was 1.3 h. No degradation was observed in plasma at −70°C during 3 weeks.

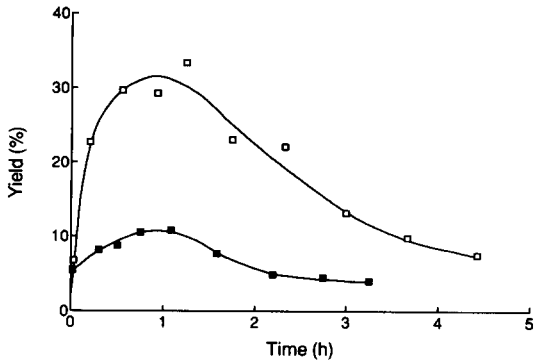


Figure 3
In vitro formation of cisplatin (□) after addition of the monohydrated complex and formation of the monohydrated complex (■) after addition of cisplatin in plasma at 37°C (pH 7.4).

Discussion

The stability of cisplatin is well documented in pharmaceutical preparations with regard to chloride ion concentration [12–14], pH [15], light exposure [12, 14, 15] and container material [15–19]. Protected from light the drug is very stable in physiological sodium chloride at low pH (~4).

Studies on the stability in biological media during storage are scarce for cisplatin and lacking for the monohydrated complex. Data for the stability of cisplatin in blood [20], plasma [20–22] and ultrafiltrate [21, 22] at 37°C have previously been presented (see Table 3). There is a divergence in the half lives for cisplatin in plasma in this study compared to

Table 2
Stability at low temperature of cisplatin and the monohydrated complex in human media

Medium	Temperature (°C)	Cisplatin ($t_{½}$, mean ± SEM)	Monohydrated complex ($t_{½}$, mean ± SEM)
Blood	0	Stable at least 4 h	8.8 ± 0.2 h
Plasma	−25	52 ± 5 h	26 ± 2 h
Ultrafiltrate	−25	31 ± 4 days	4.4 ± 0.7 days
Plasma	−70	Stable at least 21 days	Stable at least 21 days
Ultrafiltrate	−70	Stable at least 13 days	Stable at least 13 days

Table 3
Stability of cisplatin at 37°C in various human media

Medium	Method of determination	$t_{½}$ (h)	Reference
Plasma	Total platinum ETA–AAS*	2.77	21
Plasma	Cisplatin LC–ED†	1.54	21
Plasma	Cisplatin LC–ETA–AAS*	1.5	22
Plasma	'Free' platinum LC–UV‡	1.55	20
Plasma	Cisplatin LC–UV§	0.88	This study
Blood	'Free' platinum LC–UV‡	0.93	20
Blood	Cisplatin LC–UV§	1.43	This study
Ultrafiltrate	Cisplatin LC–ETA–AAS*	2.3	22
Ultrafiltrate	Cisplatin LC–UV§	$t_{½(\alpha)}$ = 1.7 $t_{½(\beta)}$ = 22	This study

* Electrothermal atomization–atomic absorption spectroscopy.
† Electrochemical detection.
‡ Pre-column derivatization.
§ Post-column derivatization.

previous studies, which probably is due to differences in pH. There was no pH control in the previous studies and in fact the pH could be as high as 9 in plasma and plasma ultrafiltrate when no buffer is added. The sulphydryl groups (SH) of albumin are involved in the degradation [22, 23–25]. At alkaline pH the SH-groups react with L-cysteine or L-cystine forming mixed disulphides [26, 27] with lower nucleophilic character [28]. The albumin, molecule also shows pH induced conformation changes [29, 30], but the implications for the stability of cisplatin in this respect is unknown. In this study pH was regulated with HEPES buffer in plasma and in UF. It has previously been shown that HEPES does not to react with the monohydrated complex [31]. Occasionally there was a small drift towards higher pH during the experiment (<0.2 pH-units), which was considered to be acceptable.

The studies in blood were carried out in an atmosphere of 5% CO_2 and the pH was stable throughout the experiment. The half life for cisplatin was 1.43 h (37°C) and it is interesting to note that cisplatin was more stable in blood than in plasma ($t_{½}$ = 0.88 h), indicating a higher stability when the drug is distributed to the red blood cells. Although, for the monohydrated compound there was no significant difference in the stability between plasma and blood (Table 1). Preliminary studies at our laboratory indicate that the partition of cisplatin to erythrocytes is approximately unity (unpublished observations). Since the process of distribution most probably involves passive diffusion it can be assumed that the distribution of the monohydrated complex is in the same order of magnitude. Inside the red blood cell the compounds are protected from albumin and other plasma proteins. On the other hand glutathione (GSH), another major reactant, is present in significantly higher concentration inside erythrocytes than in plasma (millimolar vs micromolar range [32, 33]). At pH 7.4 the reaction between cisplatin and GSH is an order of magnitude slower compared to the monohydrated compound and GSH (unpublished data). The difference in reaction rate with GSH might explain why a difference in stability is found between blood and plasma for cisplatin but not for the monohydrated compound.

The data for the monohydrated complex in UF was fitted to a biphasic decay, as judged by the *F*-ratio test. Depending on either a very slow degradation or that the monohydrated compound goes towards an equilibrium concentration, the calculation of the β-phase is uncertain.

The effect of added sodium chloride (0.2 and 0.1 M) on the stability of cisplatin in UF has previously been studied [21, 22] and it was concluded that the rate of degradation was the same as without added NaCl. The probable reason for this is the high pH obtained with no buffer added to the ultrafiltrate, i.e. the formed monoaqua (Scheme 1) is deprotonated and becomes considerably less reactive [31, 34]. As seen in Fig. 2 the degradation of cisplatin in ultrafiltrate with 0.1 M NaCl added is pH dependent. However, it is not recommended to acidify the ultrafiltrate to stabilize the samples if the individual platinum species are to be determined since the equilibria (Scheme 1) are disturbed.

Storage of plasma or ultrafiltrate samples containing cisplatin and the monohydrated complex at −25°C is only possible for short periods of time (Table 2). The process of degradation is unclear, but most probably a reaction with albumin is involved even at low temperature, since the rate of degradation is considerably slower in UF. At −70°C (Table 2) the compounds were stable in both plasma and UF during the period studied (21 and 13 days, respectively).

The possibility of using ultrafiltration of whole blood without a previous separation of the plasma fraction was studied at 0°C. A likely time span for sampling a patient receiving a 1 h infusion is *ca* 3 h [35]. During this time cisplatin is stable and if the samples are ultracentrifuged within one hour the degradation of the monohydrated compound will be less than 10% ($t_{0.90}$ = 1.3 h).

The pharmacokinetics of the monohydrated complex is presently being studied and it is therefore essential to evaluate its conversion to cisplatin *in vitro*. Figure 3 shows that 30% is converted to cisplatin after one hour at 37°C. Thus, this exchange reaction is easy to avoid by rapidly freezing the sample. Figure 3 also shows that small amounts of the monohydrated compound are formed in plasma at 37°C. The stock solution of cisplatin contains small amounts of the monohydrated complex when it is prepared in physiological sodium chloride [36], which is seen as a *y*-intercept. It is of interest to note that the monohydrated complex is formed in plasma despite its high

chloride ion concentration, since the general consensus is that the monohydrated compound is formed intracellularly.

In conclusion, to accurately determine cisplatin and the monohydrated complex, blood samples should be cooled to 0°C. At this temperature cisplatin is stable for at least 4 h and the monohydrated compound for about 1 h. Both cisplatin and the monohydrated complex are rapidly degraded in plasma at −25°C. Plasma and ultrafiltrate samples should be stored at −70°C, where both compounds are stable for at least 3 weeks.

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