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THE REACTIVITY OF CISPLATIN IN PLASMA. IMPLICATIONS FOR SAMPLE HANDLING IN PHARMACOKINETIC STUDIES

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SUMMARY

Cisplatin reacts with blood and plasma at 37°C with a half-life of about 2 h. The effects of adding chloride and freezing of samples on the stability of cisplatin in plasma samples have been studied. It was found that addition of up to 0.2 M chloride does not noticeably decrease the rate of loss of cisplatin in biological fluids. However, rapid freezing of the samples and storage at ≤ -10°C results in good stability of cisplatin for limited periods. The implications of these findings to sample handling in pharmacokinetic studies are discussed.

INTRODUCTION

Cis-dichlorodiamine-plantinum(II) (cisplatin; Formula I) is an important coordination complex used in the treatment of a variety of cancers (Einhorn and Williams, 1979; Wittes et al., 1977; Briscoe et al., 1978). The chemistry of cisplatin in aqueous media has been well studied (Reishus and Martin, 1961; Lee and Martin, 1976). In recent reports, the stabilizing effect of added chloride ion on aqueous solutions containing cisplatin for intravenous use has been demonstrated (Hincal et al., 1979; Greene et al., 1979). In the light of those findings it might appear logical to expect that addition of chloride ion to samples of cisplatin in blood, plasma or other biological media would prevent or at least reduce the rate of degradation of the drug during the course of pharmacokinetic studies. Recent work in this laboratory has shown that this is not the case.

The purpose of this work was to determine the effects of factors such as added chlo-

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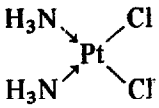
ride ion concentration, temperature and time of storage on the levels of intact cisplatin found in biological samples.



Formula I: cisplatin

MATERIALS AND METHODS

Unless otherwise stated all chemicals used were analytical grade reagents. Cisplatin was obtained from the National Cancer Institute (Bethesda, Md.) as the bulk drug and was used as supplied. Plasma ultrafiltrate was obtained by passing recovered human plasma through Amicon CF25 ultrafiltration cones which retain essentially all species possessing a molecular weight greater than 25,000.

Cisplatin was separated from plasma components and any degradation products by use of a modification of the high pressure liquid chromatographic method previously described (Chang et al., 1978). A Waters Associates Model 6000A Pump, model U6K Universal Injector and Model 450 variable wavelength absorbance detector operated at 300 nm, were used. Separations were accomplished on a Whatman Partisil-10 SAX (strong anion exchange) column. The mobile phase consisted of 20% (v/v) acetic acid/sodium acetate buffer (pH 3.8, 0.1 M) and 80% methanol, with a flow-rate of 2 ml/min. Cisplatin eluted with a retention volume of 6.6 ml. The injection volumes were 50 μl. Quantitation of cisplatin was achieved either by peak height measurements (which were previously shown (Hincal et al., 1979) to be linearly related to cisplatin concentration) or by atomic absorption spectrophotometry (Change et al., 1978). Due to the rapid loss of cisplatin in plasma and related fluids, replicate analyses were not attempted.

Kinetics of loss of cisplatin in various biological media were determined by dissolving accurately weighed amounts of drug in the desired medium that had previously been equilibrated at 37°C. The resulting solutions were maintained at 37°C by using a constant temperature water bath. Aliquots of the solution were withdrawn as needed and immediately subjected to HPLC analysis.

The first-order rate constant for the aquation of cisplatin was determined from the cisplatin remaining vs time data as described previously (Hincal et al., 1979). The apparent first-order rate constant for loss of cisplatin in ultrafiltrate and plasma solutions were obtained directly from the slopes of semi-logarithmic plots of intact cisplatin remaining vs time.

A short time (10 h) low temperature stability study was conducted in a similar fashion to the kinetic studies but the cisplatin solution was divided into 5 fractions after preparation and each fraction was placed in a separate polypropylene vial and immediately frozen in a dry-ice–acetone bath. The frozen samples were subsequently stored in a freezer (−10°C). At the indicated times, a sample was taken from the freezer and allowed to thaw at room temperature and at 50-μl aliquot was used for HPLC analysis. Portions of the sample remaining after injection of the aliquot into the HPLC were discarded.

A separate 72 h low temperature study was also carried out. Whole blood (100 ml) was

spiked with an aqueous solution (250 μl) containing 1.6 mg of cisplatin and 5.8 mg of sodium chloride per ml. This yielded a solution initially containing 4 μg of cisplatin per ml of blood.

At various times, over a 2-h period, aliquots (15 ml) of the blood were removed and centrifuged at ~750 X*g* for 5 min. The plasma (~5 ml) was removed and ultrafiltered at ~500 X*g* for 15 min. A portion of the ultrafiltrate obtained was immediately analyzed for cisplatin, using HPLC and atomic absorption spectrophotometry according to the method of Chang et al. (1978). The remainder of the ultrafiltrate was divided into two parts which were flash-frozen and stored at −10°C. At 24 h a sample was thawed and analyzed for cisplatin. The third sample was similarly treated at 72 h. The results are shown in Table 3.

RESULTS

The data obtained from kinetic studies of the loss of cisplatin in various biological media are presented in Table 1. Semi-log plots of cisplatin remaining as a function of time in all of the biological media were linear for more than 4 half-lives indicating loss of cisplatin occurred by apparent first-order processes. For comparative purposes, the first-order rate constants for the loss of cisplatin through aquation in simple aqueous solutions is included in Table 1. As reported previously (Hincal et al., 1979) the data for apparent rate of loss of cisplatin in water was not simply a first-order process and required some mathematical manipulation in order to obtain the first-order constant.

It is apparent from the data in Table 1 that the rate of loss of cisplatin in plasma ultrafiltrate (with and without added sodium chloride) are characterized by similar values for their half-lives. Furthermore, their apparent first-order rate constants are essentially identical to the value of the corresponding rate constant (k_1 in Eqn. 1) for the aquation of cisplatin in water. Removal of the proteins from plasma (by ultrafiltration) results in ~30% enhancement in stability of cisplatin. Perhaps the most striking result in Table 1 is that the addition of 0.2 M sodium chloride to plasma ultrafiltrate had no effect on the stability of cisplatin in that medium.

TABLE 1

KINETIC DATA FOR THE DISAPPEARANCE OF CISPLATIN IN VARIOUS MEDIA AT 37°C

Reaction media	$(Cisplatin)_0$ (M)	k_{obs} (h^{-1})	Observed half-life (h)
Water	5.1×10^{-4}	0.32 [a]	2.2 [b]
Plasma ultrafiltrate [c,d]	$8.1-8.6 \times 10^{-4}$	0.32	2.2
Plasma ultrafiltrate [c] with 0.2 M added NaCl	8.2×10^{-4}	0.32	2.2
Plasma	8.3×10^{-4}	0.46	1.5

[a] This value (k_1 in Eqn. 1) pertains to the aquation reaction and was obtained by methods described previously (Hincal et al., 1979).

[b] This value (see Eqn. 1) was calculated from the value of k_1 (Hincal et al., 1979).

[c] 25,000 molecular weight exclusion limit.

[d] Replicate determinations in this concentration range resulted in the same observed half-life.

TABLE 2

HEIGHTS OF HPLC PEAKS CORRESPONDING TO CISPLATIN INJECTION OF 50 μl ALIQUOTS OF A SOLUTION OF CISPLATIN IN PLASMA ULTRAFILTRATE (25,000 MOLECULAR WEIGHT CUTOFF) STORED AT −10°C FOR VARIOUS TIMES. INITIAL CISPLATIN CONCENTRATION = 9.5×10^{-4} M

Time (h)	Peak height (cm) [a]
0.07	20.0
2.18	19.1
4.87	18.4
7.45	18.7
10.60	18.8

[a] The mean value and standard deviation for all of the values = 19.0 ± 0.6.

Tables 2 and 3 present the results of two separate studies on the stability of cisplatin in plasma ultrafiltrate which was frozen in a dry-ice–acetone bath and stored at −10°C for various periods of time after which samples were thawed and analyzed for cisplatin. Although there is some scatter in the values representing remaining cisplatin, there appears to be no appreciable loss of cisplatin in either study which clearly indicates the stabilization afforded by freezing and storage at the lower temperature. It should be noted that the data in Table 3 represent cisplatin concentrations which approximate those encountered clinically.

DISCUSSION

The loss of cisplatin in water is known (Reishus and Martin, 1961; Lee and Martin, 1976) to occur by nucleophilic displacement of chloride by water (aquation) to form cis-

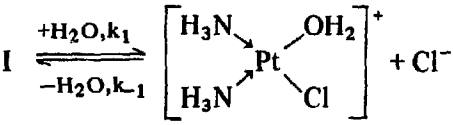
TABLE 3

COMPARISON OF CISPLATIN REMAINING IN PLASMA ULTRAFILTRATE BEFORE AND AFTER FLASH-FREEZING AND STORAGE AT −10°C FOR 24 AND 72 h

Sample	Cisplatin concentration (μg/ml) in samples after freezing [a]		
	0 h	24 h	72 h
A	2.27	2.26	2.02
B	1.37	–	1.38
C	1.05	1.00	1.02
D	0.86	0.83	0.74
E	0.53	0.50	0.52

[a] The 0 h samples were assayed immediately without freezing.

dichloro-monoaquodiamine-platinum(II), (II, Eqn. 1).



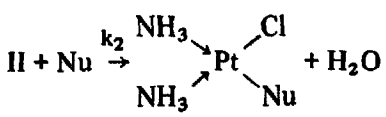
(1)

Formula II

In accord with Eqn. 1, it has been clearly demonstrated (Hincal et al., 1979; Greene et al., 1979) that in aqueous solutions, the overall apparent rate and extent of cisplatin disappearance is dependent upon cisplatin concentration. In aqueous solutions initially containing either 500 or 50 μg cisplatin/ml, it was found at 24 h that intact cisplatin remaining was ~30% and ~20%, respectively. When sodium chloride was added (at 0.15 mol/l), only about 2% of the cisplatin was lost over the 24-h period. This decrease in the extent of loss of cisplatin produced by chloride ion may be understood by considering the equilibrium shown in Eqn. 1 from which it is clear that the rate of the forward reaction (involving k_1) is unaffected by chloride ion. However, the reverse reaction (involving k_{-1}) exhibits a first-order dependence on the concentration of the chloride ion. Consequently, according to the law of mass action, addition of chloride shifts the equilibrium to the left, resulting in an increase in the fraction of intact cisplatin at equilibrium.

The data in Table 1 show that the rate constant for loss of cisplatin in aqueous media (k_1, Eqn. 1) is identical to values of the observed first-order rate constants for the loss of cisplatin in ultrafiltrate. However, unlike simple aqueous systems, addition of up to 0.2 M chloride ion * (as sodium chloride) had no effect on the rate of loss of cisplatin in ultrafiltrate (Table 1). Furthermore, the reaction of cisplatin in plasma ultrafiltrate results in total loss of the drug via an apparently irreversible reaction as demonstrated previously (Repta and Long, 1980).

Based on the data presently available, either of two pathways could account for the observed rate of loss of cisplatin in ultrafiltrate and for the lack of chloride ion effect on that rate. The first involves the reaction shown in Eqn. 1 as the rate-determining step in the loss of cisplatin. The aquated form produced (II) subsequently reacts rapidly (relative to the reaction shown in Eqn. 1) with strong nucleophiles (Nu) as shown in Eqn. 2.



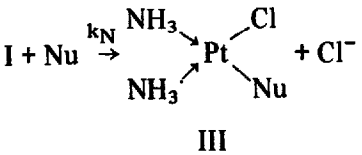
(2)

It can be shown (Repta and Long, 1980; Long, 1980) that if the reaction in Eqn. 2 is much faster than the reaction in Eqn. 1, the presence of added chloride ion would have insignificant effects on the observed rates.

A second mechanism involves direct attack of nucleophilic species on cisplatin as

* Use of higher chloride ion concentrations was attempted, but precipitate formation in the plasma sample was observed in those cases.

shown in Eqn. 3.



III (3)

According to either of the proposed kinetic schemes, there is a common product (III) and no reversibility between III and I which would be directly chloride-dependent as in the simple aquation reaction. Consequently, it is not surprising that the addition of chloride ion does not noticeably affect the rate of loss of cisplatin in biological media such as plasma ultrafiltrate.

As can be seen from the data in Table II, it is possible to substantially decrease the rate of loss of cisplatin by employing low temperatures. When samples of ultrafiltrate-containing cisplatin (at initial concentrations of 285 μg/ml) were flash frozen and stored at −10°C for a period of up to nearly 11 h, then thawed and analyzed for cisplatin by measurement of HPLC peak heights (Hincal et al., 1979), no substantial differences or trends in the height of the peaks corresponding to cisplatin were observed as a function of time over the 10-h period. In fact all values were within the range of the mean ± two standard deviations and represented a total range of values which varied by less than 10%.

The effects of flash-freezing and storage at −10°C of frozen samples containing low levels of cisplatin in ultrafiltrate were examined in a separate study. The objectives of the study were to determine whether or not the results in Table 2 also held for concentrations of cisplatin similar to those which might be encountered in clinical use of the drug, and to determine if samples could be stored in frozen state for periods of up to 72 h. In this study human blood at 37°C was spiked with cisplatin. At various times over a 2-h period aliquots were removed. The plasma was separated and ultrafiltered, and the ultrafiltrate was divided into 3 fractions. Fraction one was analyzed for cisplatin (Chang et al., 1978) immediately while the remaining two fractions were flash-frozen and stored at −10°C for either 24 or 72 h. At each time one of the fractions was thawed and analyzed. As is obvious from Table 3, there is little if any change in cisplatin concentrations over the 72-h period indicating that flash-freezing and storage of −10°C essentially avoids any loss of cisplatin in the ultrafiltrate even at these low levels of drug.

As observed in Table 1, the rate of loss of cisplatin in plasma is greater than the rate of loss from plasma ultrafiltrate. The mechanism for this loss is currently a topic of study in these laboratories. While the exact mechanism is not known, the enhanced rate in plasma may indicate that the loss of cisplatin occurs as a result of direct reaction of cisplatin with nucleophilic sites on protein sites in addition to reaction with low molecular weight nucleophiles present in the plasma ultrafiltrate. In the light of the enhanced rate of loss of cisplatin in the presence of plasma proteins, ultrafiltration of plasma samples appears to be desirable prior to storage and analysis.

Further studies aimed at a better understanding of the mechanisms of reaction of cisplatin with components of human blood and the effect of temperature on the rate of reaction of cisplatin in blood and plasma samples are continuing.

CONCLUSIONS

As a result of these studies, it is apparent that the rate of cisplatin loss in blood or plasma-derived media is not decreased by addition of as much as 0.2 M chloride. After evaluating the results of this work, it is clear that plasma and plasma ultrafiltrate are very reactive media for cisplatin and conclusions drawn from studies conducted in aqueous media cannot be readily applied to biological media.

The data and results accumulated in this study suggest that appropriate sample handling in biological studies of cisplatin disposition should at least include: (1) rapid centrifugation of blood samples and removal of erythrocytes; (2) ultrafiltration of plasma to remove proteins; and (3) immediate analysis *or* flash freezing and storage of the ultrafiltrate at $\leqslant -10^{\circ}C$ (for up to 72 h) when immediate sample analysis is impractical or undesirable. While this 3-step procedure may appear lengthy, a volume of ultrafiltrate sufficient for HPLC analysis may be obtained within 10 min. The use of this protocol would ensure that cisplatin remains intact for pharmacokinetic studies in blood-derived samples for up to 72 h and permits analysis of samples at a later more convenient time.

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