J. Pharm. Pharmacol. 1987, 39: 691–697
Received December 30, 1986

Influence of time and chloride ions on the interaction of cisplatin with human albumin in-vitro

R. MOMBURG, M. BOURDEAUX*, M. SARRAZIN, M. CHAUVET AND C. BRIAND

Laboratory of Pharmaceutical Physics, Faculty of Pharmacy, 27 Bd Jean Moulin, 13385–Marseille Cedex 5, France

The interaction of *cis*-dichlorodiammineplatinum (II) (cisplatin) with human serum albumin (HSA), dissolved in phosphate buffer with or without sodium chloride (0·1 M) has been examined at pH 7·4 and $\mu = 0{\cdot}154$. Equal volumes of cisplatin and HSA solutions were incubated at 37 °C for various times and filterable platinum concentrations versus time measured by flameless atomic absorption spectrophotometry. Binding kinetics differed depending on the buffer solutions used and on the time elapsing between cisplatin dissolution and outset of incubation with HSA. Experimental data were fitted to a theoretical equation used to calculate the number of nucleophilic sites per HSA molecule. Titrations of the HSA sulphydryl group content before and after incubation with a cisplatin solution were made, from which it was shown that the lone SH-group of the HSA macromolecule is involved in cisplatin binding. We also studied HSA's sensitivity towards denaturing agents when it was complexed with cisplatin. This sensitivity was decreased upon cisplatin binding. Also, the binding capacities of HSA and the HSA-Pt(II) complex to both tryptophan and warfarin were compared to determine the possible influence of cisplatin upon the binding to HSA of other drugs; this influence was negligible.

cis-Diamminedichloroplatinum(II) (cisplatin), an antitumour drug first described by Rosenberg et al (1965), is clinically active for the treatment of various solid tumours which are refractory to other forms of chemotherapy (Rosenberg et al 1969; Wolpert-De Fillipes 1980). The intravenous route is usually chosen, although intraperitoneal administration (Wolpert-De Fillipes 1980; Casper et al 1983; Pretorius et al 1983) or arterial infusion (Campbell et al 1983a; Patt et al 1980; Mavligit et al 1981) has more recently been used.

The physicochemical properties of this Pt(II) complex have presented particular problems: cisplatin is susceptible to nucleophilic attack which provokes a substitution reaction involving a five coordinate transition state with trigonal bipyramidal geometry (Howe-Grant & Lippard 1980). Therefore many buffer salt systems contain potential ligands, such as phosphate or chloride ions. The addition of such ions to cisplatin solutions modifies the chemical nature of the Pt(II) complex. This modification is concentration-dependent.

Moreover, biological systems have many donor atoms and binding sites for Pt(II) (Howe-Grant & Lippard 1980). In particular, cisplatin or its aquation products bind to DNA which is considered to be the biological target, and thus inhibit replication (Riley & Sternson 1984). As with nucleic acids, proteins have Pt(II) binding sites (Howe-Grant & Lippard 1980). The extent of binding to these sites depends on the composition of the medium and the time allowed for incubation.

Our purpose was to study the in-vitro interaction of various cisplatin solutions with human serum albumin (HSA), the major plasma protein, because cisplatin is usually administered intravenously and as a result reacts with that protein. This reaction occurs by an essentially irreversible mechanism (Long & Repta 1981). This binding decreases the concentration of the free Pt(II) species which is responsible for antitumour activity (Patton et al 1978; Gormley et al 1979). Many reports have dealt with cisplatin binding to human plasma and plasma fractions (Gullo et al 1980; Repta & Long 1980; Campbell et al 1983b); in particular, Repta & Long (1980) have examined the influence of chloride on the rate of loss of intact cisplatin in 5% HSA phosphate buffer solutions, at constant ionic strength. They have concluded that the loss of intact cisplatin may involve reaction with the intact molecule as well as with the aquation products, but no model has successfully described this.

Therefore, we have studied the kinetics of the binding to HSA of cisplatin in various solutions. Actually, cisplatin in solution can be transformed into other low molecular weight species, which are likely also to react with HSA. The total kinetics of all these species were studied. Moreover, a mathemat-

* Correspondence.

ical model was fitted to determine adequately the number of HSA nucleophilic sites reacting with low molecular weight Pt(II) derivatives, the concentration of which changed according to buffers used and drug storage times.

It has been suggested that protein sulphydryl groups would be expected to be the most reactive nucleophilic agent towards cisplatin (Basolo & Pearson 1976). Therefore the sulphydryl content of HSA solutions incubated for 48 h with cisplatin was determined.

Lastly, HSA modifications due to Pt(II) binding were examined by spectrofluorometric measurements and denaturation experiments. Moreoever, the ability of HSA, when complexed with Pt(II), to bind tryptophan and warfarin was tested.

Thus our aim was not only to study the kinetics of the binding to HSA of cisplatin in various solutions, but also to identify the possible resultant modification of the HSA macromolecule.

MATERIALS AND METHODS

Materials

All the chemicals used were analytical grade reagents. *cis*-Dichlorodiammineplatinum(II) (cisplatin) was supplied by R. Bellon, France, human serum albumin (HSA) dried and purified was purchased from Koch Light Laboratories Ltd, UK, sodium warfarin was from Merrel Dow, France, [α-^{14}C]warfarin and [G-^{3}H]L-tryptophan were from Amersham International, L-tryptophan was from Sigma Chemicals, St Louis, USA, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) was from Fluka A. G., Buchs S. G.; urea R.P. was from Prolabo, France and guanidine-HCl ultra pure was from Schwarz/Mann, New York.

Kinetics studies

Cisplatin was always dissolved at a concentration of about 5×10^{-4} M in a phosphate buffer solution prepared from NaH_2PO_4, H_2O, at pH 7·4 and $\mu = 0{\cdot}154$. Some experiments were performed in $2{\cdot}4 \times 10^{-2}$ M phosphate buffer with NaCl added (0·1 M), others were performed without NaCl in $6{\cdot}8 \times 10^{-2}$ M phosphate buffer. These solutions were stored at 4 °C until use 24 h and 8 days later. Also HSA was dissolved at the same concentration in these two media. Equal volumes of HSA solutions and corresponding cisplatin solutions were mixed and incubated at 37 °C. Aliquots were taken at various times and quickly ultrafiltered through a YMT ultrafiltration membrane of the Amicon Micropartition System with a membrane cut off value of 25 000 daltons. Ultrafiltrate platinum determinations were made by flameless atomic absorption spectrophotometry on a Philips Pye Unicam SP9 apparatus equipped with an autoinjector. The platinum line was monitored at 265·9 nm. The lamp current was maintained at 10 mA. A four-stage heating programme was used: a 20 s dry stage at 115 °C, a 15 s ash stage at 950 °C, a 3 s atomizer stage at 2800 °C and a 3 s clean stage at 2950 °C. The heating platform was cooled with water and the carbon rod was purged with nitrogen (3 L min^{-1}). Pyrocoated tubes were used. The injection volume was 10 μL of ultrafiltrate appropriately diluted. The atomic absorption response was linearly related to cisplatin concentration in the 80 to 240 ng mL^{-1} range. Measurements performed on cisplatin standard solutions either in distilled water or in each phosphate buffer led to identical results; that was the proof of the absence of any matrix effect. Samples were injected in triplicate and absorbances compared with standard solution injected after every third test solution. The coefficients of variation were less than 5%.

Mathematical fitting

The free Pt concentrations, Y, measured by flameless absorption spectrophotometry were the global concentrations of all low molecular weight Pt(II) derivatives not bound to HSA. Each component species, the concentration of which was Y_i, was taken as reacting at a rate constant k_i with HSA nucleophilic sites according to a second order kinetics:

$$dY_i/dt = -\Sigma_i k_i N Y_i$$

where N is the nucleophilic site concentration changing with respect to incubation time, N_0 is initial concentration.

A 1/1 stoichiometry was assumed and all the nucleophilic sites taken as available for binding of the various low molecular weight Pt(II) species. So: $N = N_0 - Y_0 + Y$ where Y_0 is the initial free Pt concentration. Then the rate of free Pt loss is:

$$dY/dt = -\Sigma_i k_i (N_0 - Y_0 + Y)\, Y_i \qquad (1)$$

Nothing is known about Y_i. But it was assumed to remain proportional to Y ($Y_i = \alpha_i Y$) which implies that the equilibrium between all low molecular weight Pt(II) species does not change during HSA binding. Although this hypothesis was not based on any experimental indications, it is the only way to resolve equation (1), furthermore, experimental results are then fitted satisfactorily.

So,

$$dY/dt = -\Sigma_i k_i (N_0 - Y_0 + Y)\, \alpha_i Y \qquad (2)$$

The resolution of this differential equation is:

$$1/Y = \{N_0/[(N_0 - Y_0)Y_0]\}\ e^{(N_0-Y_0)t\Sigma_i k_i\alpha_i} - [1/(N_0 - Y_0)]$$

The experimental results were fitted to this equation by a non-linear least squares computation.

Recording of the cisplatin UV spectrum

Aqueous 5×10^{-4} M solutions of cisplatin (pH = 7·4 and μ = 0·154) were prepared either in a $2{\cdot}4 \times 10^{-2}$ M phosphate buffer containing 0·1 M sodium chloride or in a $6{\cdot}8 \times 10^{-2}$ M phosphate buffer without sodium chloride. Spectra were recorded from 220 nm to 340 nm by a UVIKON 820 spectrophotometer. Their change versus time was checked over 8 days.

Sulphydryl titration

Since DTNB reacts with aliphatic thiols compounds to produce coloured compounds (εm = 13 600 M^{-1} cm^{-1} at λ = 412 nm) (Ellman 1959), it can be used to measure thiol concentrations. SH titration was performed on HSA solution and HSA-Pt(II) complex solution incubated for 48 h. The free platinum content was then so low that its interference with DTNB was negligible, as verified on a test sample. The measurements were carried out with a Beckman ACTA III spectrophotometer.

Spectrofluorometric and denaturation measurements

Fluorescence measurements were performed on an SFM 25 KONTRON apparatus equipped with thermostated cell holder. Excitation and emission slit widths were both 5 nm. All experiments were at 25 °C at the same final HSA concentration ($2{\cdot}5 \times 10^{-5}$ M) in phosphate buffer containing 0·1 M sodium chloride. The HSA-Pt(II) complexes were obtained by incubation of HSA solution at 5×10^{-4} M with equal quantities of cisplatin solutions at 5×10^{-4} M during 48 h at 37 °C and then ten fold diluted. Urea was used at a 8 M concentration and guanidine HCl at a 6 M concentration.

Drug binding to HSA-Pt(III) complexes

Equilibrium dialysis experiments were performed with a Dianorm equilibrium dialyser using cells of 400 µL total volume. Hydrated cellulose membranes (Diachema, type 10·14, molecular weight cut off of 50 000 daltons) were prepared and used as recommended by the manufacturer. Each drug was dissolved in a $2{\cdot}4 \times 10^{-4}$ M phosphate buffer containing 0·1 M sodium chloride (pH = 7·4, μ = 0·154) at concentration varying in the range 5×10^{-5}–5×10^{-7} M.

An alkaline aqueous solution (15 µL) of [α-^{14}C]warfarin ($5{\cdot}4 \times 10^{-4}$ M) was added to 3 mL of the non-labelled warfarin solution used for dialysis experiments. The specific activity of [α-^{14}C]warfarin was 46 mCi $mmol^{-1}$. In the case of tryptophan, 1 µL of a ethanol–water (1 : 1) solution of tritiated tryptophan ($7{\cdot}8 \times 10^{-6}$ M) was added to 3 mL of the non-labelled tryptophan solution. The specific activity of [G-^{3}H]L-tryptophan was 6·4 Ci $mmol^{-1}$. The ligand solution was added to the buffer compartment at the start of dialysis. The initial radioactivity added into the cell as 3·3 nCi for tryptophan and 24·8 nCi for warfarin. HSA was used at about 5×10^{-6} M at 37 °C. Equilibrium was achieved within 2½ h for the warfarin solutions and within 3 h for the tryptophan solutions. Concentrations of labelled warfarin or labelled tryptophan in the two dialysis cell compartments were determined by measuring the radioactivity of the beta emitters with a LS 100 C Beckman apparatus. The association constants and numbers of binding sites of the HSA molecule were calculated by computer analysis for each drug using Scatchard's plots (Scatchard 1949). Dialysis experiments were also performed on 5×10^{-6} M HSA-Pt(II) complex solutions. This complex was obtained by incubating HSA and cisplatin solutions in phosphate buffer for 48 h at 37 °C; then the concentration of free platinum species was verified as negligible and thus free platinum species did not interfere with warfarin or tryptophan binding to HSA.

RESULTS AND DISCUSSION

Kinetic studies

The measured concentrations of free Pt, i.e. of low molecular weight derivatives not bound to HSA, are plotted versus HSA incubation time in Fig. 1. Experimental conditions varied.

Experiment 1 (Fig. 1A) was carried out in phosphate buffer ($2{\cdot}4 \times 10^{-2}$ M) with sodium chloride (0·1 M) and the results were similar whatever the time elapsed between drug solution preparation and start of HSA incubation.

Experiments 2 and 3 were carried out in phosphate buffer ($6{\cdot}8 \times 10^{-2}$ M) without sodium chloride. Cisplatin solutions were prepared 24 h (Fig. 1B) and 8 days (Fig. 1C) before the start of HSA incubation. The kinetics obtained were obviously different. Thus buffer composition influenced the kinetics of binding to HSA. In experiment 1, Pt(II) species in solution containing sodium chloride did not change over time, as shown by UV spectrophotometry (Fig. 2). However, the spectrophotometric method is not sensitive enough to ascertain that no hydrolysis

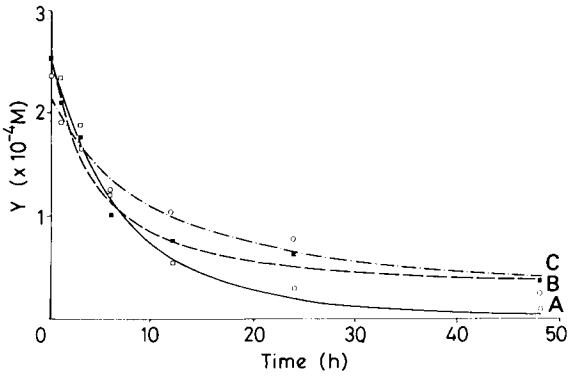

FIG. 1. Kinetics at 37 °C of filterable Pt(II) species (Y) in phosphate buffer. A: containing 0·1 M of sodium chloride; theoretical curve ——, experimental data □. B: without sodium chloride (24h); theoretical curve – – –, experimental data ■. C: Without sodium chloride (8 days); theoretical data –·–·–, experimental data ○.

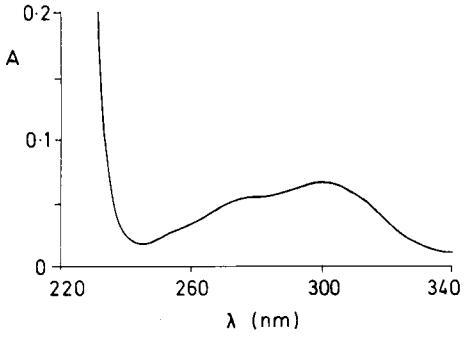

FIG. 2. Cisplatin UV spectrum in phosphate buffer with 0·1 M sodium chloride at the time of cisplatin dissolution, 24 h and 8 days after cisplatin dissolution.

would take place (Lee & Martin 1976) and that phosphate ions would not react as nucleophilic agents towards cisplatin, two reactions which have been proved by NMR experiments (Boze et al 1984; Sarrazin et al 1986). Nevertheless our results are in agreement with previous studies (Hincal et al 1979; Petering et al 1984) proving that cisplatin exhibited good stability in the presence of 0·1 M sodium chloride. Intact cisplatin would seem to be the predominant derivative in these conditions.

Results plotted in Fig. 1B and 1C, when no sodium chloride was added to buffer, were different. It seems reasonable to suppose that cisplatin, during storage time, continues to undergo at a very slow rate some hydrolysis or nucleophilic attack by phosphate ions even 24 h after drug solution preparation, as shown by spectrophotometric measurements (Fig. 3). So the kinetics studied were dependent on the low

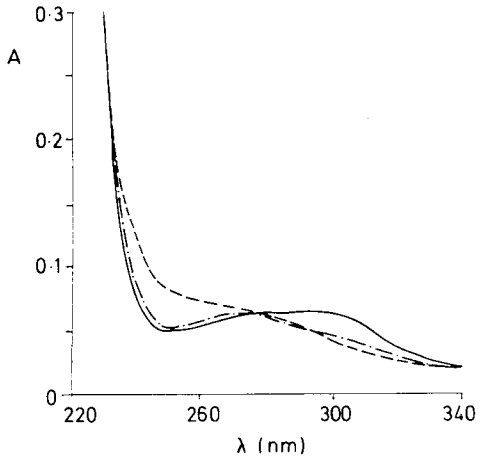

FIG. 3. Cisplatin UV spectrum in phosphate buffer without sodium chloride. —— At the time of cisplatin dissolution, — — 24 h after cisplatin dissolution, –·–·– 8 days after cisplatin dissolution.

molecular weight species which are derived from cisplatin and which change over time, depending on experimental conditions. The flameless atomic absorption spectrophotometric technique used to monitor the Pt(II) binding to HSA could not differentiate all these species, globally measured as not bound to HSA, called free Pt or Y. Thus, each one of these species may react with protein nucleophilic sites at its own rate constant, k_i. The concentrations of unbound species remaining after all these reactions constitute the test data. Moreover, it cannot be excluded that various sites with different nucleophilic character might exist on each HSA molecule; so, k_i must be considered as an average rate constant. Thus a mathematical analysis of data proved rather complex. Various models and in particular those previously published for Pt(II) plasma fraction kinetics (Repta & Long 1980) were unsuccessfully tested.

Our results were best fitted to the model previously described in Methods. Fig. 1 presents the theoretical curves. The experimental Y_0 values differed from those fitted by less than 10% (Table 1). Therefore, N_0 values were higher in the presence of sodium chloride, when intact cisplatin was the predominant species. Since the ionic strength was maintained constant, a change of HSA conformation cannot be involved, so this result would be explained by an easier accessibility to the inside of the HSA macromolecule where some nucleophilic agents might be situated, especially for intact cisplatin, the steric hindrance of which is likely to be smaller.

In the presence of sodium chloride, the number of nucleophilic sites per HSA molecule was close to 2; it was only about 1 when no sodium chloride was added. These values seem low with regard to the multiplicity of HSA potential sites that might bind Pt(II). However, the low molecular weight Pt(II) species would undergo a primary interaction with these N_0 sites, but the possibility of their also binding to another neighbouring nucleophilic site cannot be excluded.

The $\Sigma\alpha_i\ k_i$ values listed in Table 1 are related to both the average constant k_i of each species and to their concentrations. So, no easy analysis of this parameter can be made. The only conclusion that can be deduced is that it changed with respect to experimental conditions. From equation 2, it was then possible to calculate the rate of free Pt loss over time, $|dy/dt| = |v|$, as shown in Fig. 4. Curves 1 and 2 may be compared since they both were plotted at the same initial Y_0 concentration. It was clear that without sodium chloride, the loss of free Pt was initially faster.

Table 1. Kinetic parameters and number of nucleophilic sites corresponding to Fig. 1A, B, C.

	A	B	C
Y_0 exp. (10^{-4} M)	2·5	2·5	2·3
Y_0 theor. (10^{-4} M)	2·7	2·7	2·2
N_0 (10^{-4} M)	5·1	2·4	2·0
Number of sites per HSA molecule	2·0	1·0	0·8
$\Sigma k_i\alpha_i$ ($M^{-1}h^{-1}$)	316	1144	542

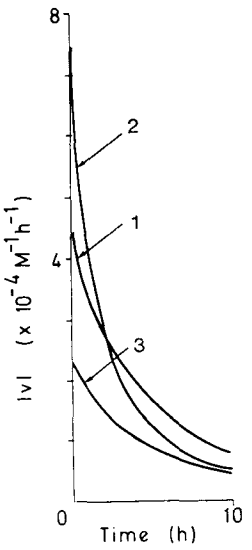

FIG. 4. Reaction rates at 37 °C of free Pt loss versus time. 1, with 0·1 M NaCl 2, without NaCl 24 h 3, without NaCl 8 days.

Sulphydryl titration

It would be interesting to know the HSA-Pt binding sites actually concerned. The possible implication of the lone sulphydryl group of HSA (CYS 34) has been previously suggested (Repta & Long 1980). So sulphydryl quantitative analysis was performed on HSA complexed by Pt(II) derivatives. In these experimental conditions, it was no longer possible to titrate free sulphydryl groups using the previously described DTNB method.

Spectrofluorometric and denaturation measurements

Normalized fluorescence emission spectra obtained from HSA-Pt(II) complexes are shown in Figs 5, 6. No difference was apparent whatever the excitation wavelength (280 or 300 nm) for the HSA-Pt(II) complex compared with HSA alone. However, addition of denaturing agents (urea 8 M or guanidine-HCl 6 M) produced an HSA unfolding which, when the excitation wavelength was 280 nm, appeared as two resolved peaks, respectively attributed to tyrosine and tryptophan residues (Fig. 5). These peaks

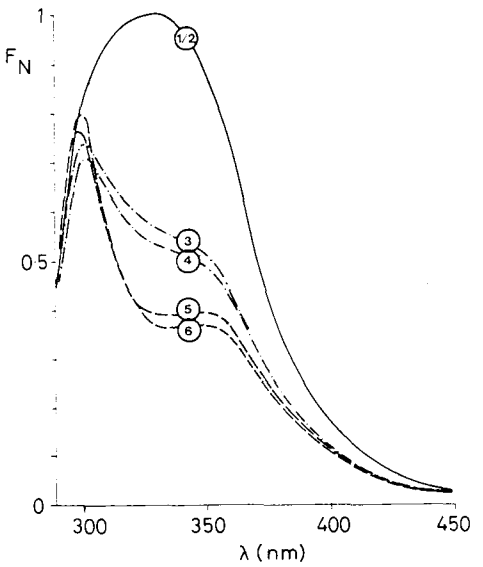

FIG. 5. Fluorescence emission spectra of HSA, normalized at 335 nm, λ_{ex} = 280 nm, θ = 25 °C. —— 1 HSA. —— 2 HSA-Pt(II). -·-·- 3 HSA-Pt(II) × urea 8 M. -·-·- 4 HSA + urea 8 M. — — 5 HSA-Pt(II) + guanidine HCl 6 M. — — 6 HSA + guanidine HCl 6 M.

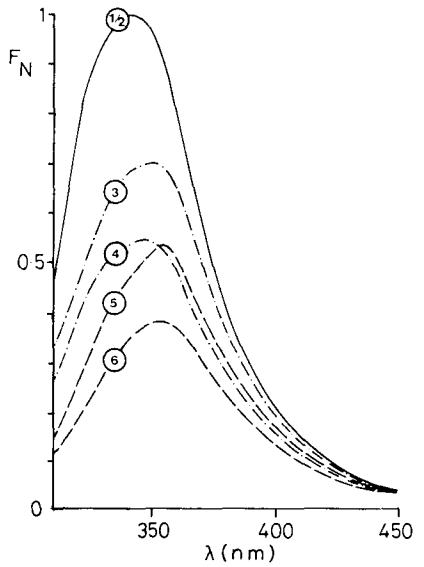

FIG. 6. Fluorescence emission spectra of HSA, normalized at 335 nm. λ_{ex} = 300 nm, θ = 25 °C. —— 1 HSA. —— 2 HSA-Pt(II). -·-·- 3 HSA-Pt(II) + urea 8 M. -·-·- 4 HSA-Pt(II) + guanidine HCl 6 M. ——6 HSA + guanidine HCl 6 M.

were still apparent in denatured HSA-Pt(II) complexes but their intensities were higher; that would be proof of a better resistance to denaturing, consequent to Pt(II) binding to HSA. When the excitation wavelength was 300 nm (Fig. 6), spectra classically showed only one peak due to tryptophan emission because there is no energy transfer from tyrosine residues. Once again, the fluorescence intensity was higher for HSA-Pt(II) complexes. So, Pt(II) binding to HSA did not significantly modify the emission spectrum and, therefore, HSA conformation. But it might stabilize HSA vis-à-vis the influence of denaturing agents.

Drug binding to HSA-Pt(II) complexes

The ability of HSA-Pt(II) complexes to bind tryptophan and warfarin was measured by equilibrium dialysis experiments as described in Materials and methods. These two molecules were chosen as reference ligands each related to a distinct HSA binding site (sites I and II), which can also react with other drugs.

No change in tryptophan binding to either the HSA-Pt(II) complex or HSA alone was obvious. Table 2 lists the binding parameters obtained by Scatchard's plots. They may be considered as equivalent with regard to experimental errors and are in good agreement with binding parameters previously published (Coassolo 1978).

Table 2. Affinities (K_a) and number of binding sites (n) of tryptophan (Trp) and warfarin on HSA and the HSA-Pt(II) complex.

	n	K_a (M^{-1})
HSA/Trp	1·2 ± 0·1	5 280 ± 300
HSA-Pt(II)/Trp	1·1 ± 0·1	5 080 ± 400
HSA/warfarin	1·3 ± 0·1	233 000 ± 8000
HSA-Pt(II)/warfarin	1·0 ± 0·1	238 000 ± 9000

On the contrary, Pt binding to HSA decreased the number of HSA binding sites to warfarin, although the apparent association constant was not significantly changed and agreed with references values (Tillement et al 1974) (Table 3). The values obtained for HSA alone would at first seem surprising. However, some authors (Tillement et al 1974; Wilting et al 1980) have considered two or more classes of binding sites, this may explain our results; here our only aim was to compare data obtained with and without Pt.

Conclusion

Our study has dealt with in-vitro interactions between various cisplatin solutions and HSA. The binding kinetics were different depending upon the chemical nature of ions in buffer solutions. Rate of loss of low molecular weight Pt(II) species is faster, at least initially, when chloride is not present. Otherwise, chloride ions increase the number of HSA binding sites. So, with a view to clinical administration, it is important to use cisplatin in solutions in which it will be protected from transformation, since the drug is often administered during long intravenous infusion. Recently, Garren & Repta (1985) have discussed situations in which cisplatin solutions were mixed with Reglan (metoclopramide, sodium metabisulphite and sodium chloride) and injected in a single parenteral fluid to prevent cisplatin-induced nausea and vomiting; they have concluded that cisplatin should not be admixed with other drug formulations unless careful and appropriate stability studies have clearly demonstrated the absence of any incompatibility. Our study showed that phosphate ions might change the rate of binding to HSA of cisplatin solutions and consequently the pharmacokinetics of this drug. Now, some drug formulations involve phosphate ions, as do a few parenteral fluids used as vehicles for long intravenous perfusion.

The lone HSA sulphydryl group would seem to be implicated in Pt(II) binding. In addition, HSA modifications induced by Pt(II) binding would result in a greater resistance to denaturing agents but

without any gross conformational changes detectable by fluorescence measurements. Pt(II) binding to HSA seems to protect the macromolecule from the unfolding effects produced by denaturing agents, i.e. this binding would stabilize the protein in a more rigid structure. Consequences which might result from this stabilization are not known as yet. The slow elimination of Pt(II) from sera in therapeutic conditions has been attributed to extensive plasma protein binding, especially to HSA. So it may be suggested that HSA molecules modified by Pt(II) binding are not metabolized much faster than native protein, though it has been mentioned that this metabolism is increased in dogs (Cole & Wolf 1980).

Lastly, the capacity of the HSA-Pt(II) complex to bind two molecules considered as typical of two distinct HSA binding sites usually called I and II (Fehske et al 1981) was checked. Cisplatin was shown not to influence tryptophan binding. On the contrary, the capacity of HSA-Pt(II) complexes to bind warfarin was slightly less because of a decrease in the number of binding sites. Nevertheless, this binding decrease seems unlikely to be of any clinical consequence because only a small fraction of total HSA would be affected following clinical use of cisplatin.

Acknowledgements

The authors thank H. Bouteille for his graphical work.

REFERENCES

Basolo, F., Pearson, R. G. (1976) in: Wiley, P. (ed.) Mechanism of Inorganic Reactions. Academic Press, New York, pp 351–453

Boze, R. N., Viola, R. E., Cornelius, R. D. (1984) J. Am. Chem. Soc. 106: 3336–3343

Campbell, T. N., Howell, S. B., Pfeifle, C. E., Wung, W. E., Bookstein, J. (1983a) J. Clin. Oncol. 1: 755–762

Campbell, B. A., Kalman, S. M. , Jacobs, C. (1983b) Cancer Treat. Rep. 67: 169–172

Casper, E. S., Kelsen, D. P., Alcook, N. W., Lewis, J. L. (1983) Ibid. 67: 235–238

Coassolo, P. (1978) Ph.D. Thesis, Marseille University, France

Cole, W. C., Wolf, W., (1980) Chem. Biol. Inter. 30: 223–235

Ellman, G. L. (1959) Arch. Biochem. Biophys. 82: 70–77

Fehske, K. J., Müller, W. E., Wollert, U. (1981) Biochem. Pharmacol. 30: 687–692

Garren, K. W., Repta, A. J. (1985) Int. J. Pharm. 24: 91–99

Gormley, P. E., Bull, J. M., Leroy, A. F., Cysyk, R. (1979) Clin. Pharmacol. Ther. 25: 351–357

Gullo, J. J., Litterst, C. L., Maguire, P. J., Sikic, B. I., Hoth, D. F., Wolley, P. V. (1980) Cancer Chemother. Pharmacol. 5: 21–26

Hincal, A. A., Long, D. F., Repta, A. J. (1979) J. Parent. Drug. Assoc. 33: 104–116

Howe-Grant, M. E., Lippard, S. J. (1980) in: Sigel, H. (ed.) Metal Ions in Biological Systems. Marcel Dekker, New York, pp 63–125

Lee, K. W., Martin, D. S. (1976) Inorg. Chim. Acta-Bioinorg. Chem. 17: 105–110

Long, O. F., Repta, A. J. (1981) Biopharm. Drug Dispos. 2: 1–16

Mavligit, G. M., Benjamin, R. S., Patt, Y. S., Jaffe, N., Chuang, V., Wallace, S., Murray, J., Ayala, A., Johnston, S., Hersh, E. M., Calvo, D. B. (1981) Cancer 48: 1–4

Patt, Y. Z., Calvoc, D. B., Benjamen, R. S., Mavligit, G. M. (1980) in: Prestakyo, A. W., Crooke, S. T., Carter, S. K. (eds) Cisplatin Current Status and New Developments. Academic Press, New York, pp 495–509

Patton, T. E., Himmerlstein, K. J., Belt, R., Bannister, S. J., Sternson, L. A., Repta, A. J. (1978) Cancer Treat. Rep. 62: 1359–1362

Petering, D. H., Antholine, W. E., Sayan, L. A. (1984) in: Ottenbrite, R. M., Butler, G. B. (eds) Anticancer and Interferon Agents. Marcel Dekker, New York, pp 203–246

Pretorius, G. R., Hacker, N. F., Berek, J. S., Ford, L. C. (1983) Cancer Treat. Rep. 67: 1085–1092

Repta, A. J., Long, D. F. (1980) in: Prestakyo, A. W., Crooke, S. T., Carter, S. K. (eds) Cisplatin Current Status and New Developments. Academic Press, New York, pp 285–304

Riley, C. M., Sternson, L. A. (1984) Pharm. Int. 5: 15–19

Rosenberg, B., Van Camp, L., Krigas, T. (1965) Nature 205: 698–699

Rosenberg, B., Van Camp, L., Trosko, J. E., Mansour, V. H. (1969) Nature 22: 385–386

Sarrazin, M., Peyrot, V., Briand, C. (1986) Inorg. Chim. Acta-Bioinorg. Chem. 124: 87–96

Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51: 660–666

Tillement, J. P., Zini, R., D'Athis, P., Vassent, G. (1974) Eur. J. Clin. Pharmacol. 7: 307–313

Wilting, J., Van der Giesen, W. F., Janssen, L. H., Weideman, M. M., Otagiri, M., Perrin, J. H. (1980) J. Biol. Chem. 255: 3032–3037

Wolpert-De Fillipes, M. K. (1980) in: Prestakyo, A. W., Crooke, S. T., Carter, S. K. (eds) Cisplatin Current Status and New Developments. Academic Press, New York, pp 183–191