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CISPLATIN METABOLITES: A METHOD FOR THEIR SEPARATION AND FOR MEASUREMENT OF THEIR RENAL CLEARANCE IN VIVO

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INTRODUCTION

After introduction into animals or biological fluids cisplatin (cis-dichlorodiammine PtII) undergoes ligand exchange reactions leading to the production of a mixture containing high molecular weight (protein bound) and soluble transformation products and unchanged cisplatin (Daley-Yates and McBrien, 1982a,b). The biotransformation products of cisplatin have not been characterised owing to the lack of a suitable procedure for their separation. A separation method which is potentially useful is high performance liquid chromatography (HPLC) but up until now the application of this technique to the study of cisplatin pharmacokinetics has been limited. Borch et al.(1979) and Bannister et al.(1979) have described methods for measuring total platinum in urine following derivatisation. In each case the derivative is passed through an HPLC column and quantified by uv absorption. The methods were devised either to permit laboratories without atomic absorption spectrophotometers (AAS) to measure total urinary platinum (Borch et al.,1979) or to eliminate matrix effects associated with urine analysis using flameless AAS (Bannister et al.,1979). Neither method distinguishes between different transformation products of cisplatin since all species are converted to the same derivative. Chang et al.(1979) applied an HPLC separation technique to plasma ultra-filtrates following in vitro incubation with cisplatin. They detected in the eluate two peaks other than the one containing cisplatin but 80% of the platinum applied to their column was not resolved into peaks (Repta and Long, 1980) and the method was principally used for the measurement of unchanged cisplatin. We here describe a technique for the HPLC separation of platinum species in urine and plasma in which all of the platinum applied to the column is resolved as peaks. We also report some preliminary measurements of the renal clearance of the metabolites, or transformation products, separated by this technique.

HPLC SEPARATION OF CISPLATIN AND ITS METABOLITES

Separation of platinum species was accomplished using ion pair chromatography. The column was generated by passing a 5mM sodium dodecylsulphate (SDS) solution through a 25cm x 5mm internal diameter Spherisorb ODS reverse phase column. A precolumn of the same material was also used. Samples of urine and plasma were deproteinised before HPLC separation by passage through Amicon CF50 membrane ultrafilters. The column was pre-equilibrated with eluant A (5mM SDS) then the sample eluted with a linear gradient of eluant B (90% acetonitrile by volume) with a flow rate of 0.5 cm^3 min^{-1}. Column effluent was monitored by passage through a uv detector operating at 225nm and then collected as 0.5cm^3 fractions for platinum analysis. Where ^{191}Pt-cisplatin was employed analysis was by gamma-counting in an LKB-Wallac Ultrogamma II spectrometer with automatic decay correction. With unlabelled samples analysis was by flameless atomic absorption spectrometry (NFAAS). The total platinum recovered from the column is equal to the amount applied. Figure 1 shows typical elution profiles for plasma and urine obtained from an animal used in renal clearance studies (see later). Peaks corresponding to unchanged cisplatin and to six transformation products or metabolites are typically seen. When NFAAS is used for platinum analysis plasma samples from animals treated with cisplatin in vivo contain platinum levels too low for efficient detection and are therefore

concentrated by lyophilisation prior to passage through the HPLC column. Elution profiles obtained from lyophilised plasma samples give similar patterns of peaks but the resolution of the peaks is poorer than that obtained with unconcentrated protein free plasma presumably because of interference from solutes present in higher concentrations. We have studied the formation of cisplatin metabolites during incubation of cisplatin with plasma in vitro and have found similar patterns of peaks in the platinum elution profiles as are obtained from in vivo experiments. Since higher concentrations of cisplatin may be used in in vitro incubations some of the peaks eluted from the HPLC column contain sufficient platinum to permit further purification and characterisation. On the basis of the following observations peak E (Fig.1) has been tentatively identified as a mono-methionine substitution complex of cisplatin. A preincubated mixture of cisplatin and methionine prepared as previously described (Daley-Yates and McBrien, 1982c) gives three peaks when separated using the HPLC technique; one peak is unchanged cisplatin, the other two are presumed to be mono-methionine and di-methionine substitution complexes of cisplatin. One of these peaks has the same retention volume, and co-chromatographs with peak E. Using thin layer chromatography as previously described (Daley-Yates and McBrien 1982a) peak E co-chromatographs with the spot identified provisionally as a mono-methionine-cisplatin substitution complex. The HPLC peak presumed to be the di-methionine-cisplatin substitution complex has a retention volume much greater than any platinum containing peak found in urine and plasma HPLC elution profiles. The peaks other than B and E are still unidentified but cysteine-cisplatin substitution complexes, which might have been present, are not found in urine or plasma.

RENAL CLEARANCE STUDIES

Work with the isolated perfused rat kidney (Daley-Yates and McBrien, 1982a) and data from human studies (Jacobs et al.,1980) have shown that there is a net tubular excretion of platinum by the kidney after dosing with cisplatin. The HPLC method described above has permitted a determination of the renal clearance of each individual platinum species present in urine. Male Wistar rats (Charles River, Essex), 300g weight, were prepared by surgical cannulation of the ureters. This procedure allowed continuous collection of urine in the conscious animal. Animals were hydrated by the administration of 2.0cm^3 0.9% saline by gastric intubation prior to experiment. At zero time ^{191}Pt-cisplatin (a gift from the Manchester Platinum Group; specific activity at T_o = 60μCi per mg, $t_{\frac{1}{2}}$ = 3.1d) 15mg kg^{-1} and Inulin, 60mg kg^{-1}, dissolved in 0.9% saline were given i.p. Sixty minutes following injection urine was collected for 10 min and at the midpoint of the period of urine collection a blood sample was withdrawn from the caudal vein. The blood samples were centrifuged,plasma and urine passed through membrane ultrafilters and samples not immediately separated by HPLC were stored frozen until required. Figure 1 shows the results obtained from one animal in such an experiment. The concentration of platinum in the separate peaks shown in Fig.1 was calculated from peak area and the plasma and urinary concentrations of inulin determined as previously described (Daley-Yates and McBrien, 1982a). From these data the renal clearance, the glomerular filtration rate (GFR clearance of inulin) and the fractional clearance were calculated and are shown in Table 1. As with the isolated perfused rat kidney the fractional clearance of total platinum is greater than unity. Compared with a value of 1.25 times the GFR with the perfused kidney (Daley-Yates and McBrien, 1982a) in three separate experiments in vivo in which the fractional clearance of total platinum has been determined the mean ± S.D. of the fractional clearance is 3.3 ± 0.9 for the 60-70 min period post dosing. Table 1 shows that cisplatin (peak B) is actively excreted by the kidney with a fractional clearance of 3.5. Species A and F are also actively excreted whilst C and G are reabsorbed. Species D and E (the possible mono-methionine-cisplatin substitution complex) have values for the fractional clearance close to unity - consistent with their being largely excreted by

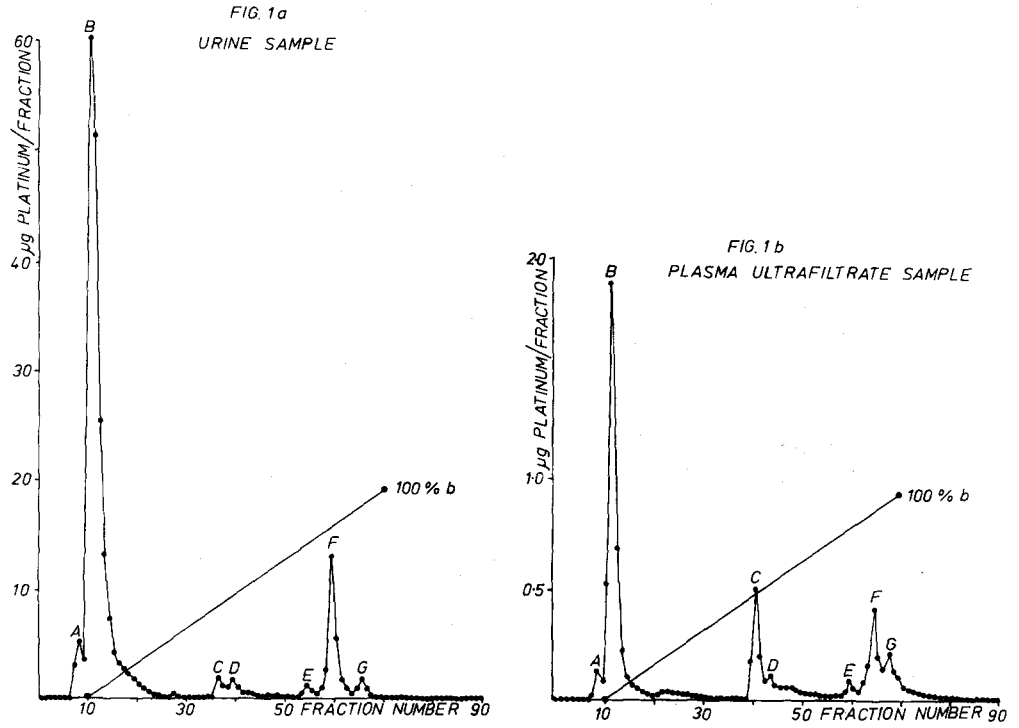

FIG.1. Elution profiles from the HPLC separation of platinum compounds formed from ^{191}Pt-cisplatin in vivo (a) Urine (10µl sample) (b) Plasma ultrafiltrate (90µl sample). The diagonal lines indicate increasing percentage of the second eluant (b).

TABLE 1. Renal Clearance of Platinum Metabolites.

Substance (x)	Clearance C(x) $\frac{U(x)}{P(x)} \times V$ ($cm^3 min^{-1}$)	Fractional Clearance C(x)/C(Inulin)	Percentage Pt species in Plasma (60-70min)	Percentage Pt species in Urine (60-70min)
Inulin	2.61	1.0	-	-
Total Platinum	6.17	2.4	100	100
A	11.08	4.2	1.9	3.4
B(cisplatin)	9.20	3.5	48.1	71.7
C	0.93	0.35	11.4	1.7
D	3.00	1.1	2.9	1.4
E	3.67	1.4	2.4	1.5
F	5.60	2.1	11.9	10.8
G	1.44	0.55	7.4	1.7

U(x) : urinary concentration of substance x
P(x) : plasma concentration of substance x
V : urine flow rate

filtration.

DISCUSSION

The use, in HPLC separation of cisplatin soluble metabolites, of a hydrophobic, anionic, ion-pairing agent such as SDS gives good separation of peaks. The method was devised in the expectation that most cisplatin metabolites would be hydrophilic and either neutral complexes or else positively charged. For example, in the case of the methionine-cisplatin substitution complexes the replacement of a chloride ligand by a methionine sulphur would yield a 1+ charged species. Substitution by two methionine molecules could yield a 2+ charged species and this would be retained by the SDS column to a greater extent than the 1+ charged species. Neutral species would be retained depending upon the extent of their Van der Waals interactions with the column. Cisplatin, a neutral complex, is observed to have a low retention volume. When a column was prepared using inorganic orthophosphate ions (PO_4^{3-}) as ion-pairing agents the platinum species were not separated by the column because of poor retention of the ion pair. This is indicative of the platinum species being hydrophilic.

The retention volumes of peaks C - G have been observed to vary slightly from run to run (e.g. compare Figs. 1a and 1b) hence monitor peaks in the uv absorption trace were used to assist in precise identification of peaks on the platinum elution profile for comparison between different runs. We did not control the temperature of the columns or the precise length of time for regeneration of the column and this may account for the variations observed

The observation that most of the platinum species present in the urine are either actively secreted or reabsorbed by cells of the renal tubules may be of significance for an understanding of the mechanism of cisplatin nephrotoxicity. Such species, in their transit across the tubular cells clearly have a greater potential for damage than the species which are simply filtered into the urine. In this respect it is notable that the parent molecule, cisplatin itself, is secreted by the kidney tubules.

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