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THE MECHANISM OF RENAL CLEARANCE OF CISPLATIN (*CIS*-DICHLORODIAMMINE PLATINUM II) AND ITS MODIFICATION BY FUROSEMIDE AND PROBENECID

PETER T. DALEY-YATES and DAVID C. H. MCBRIEN
Biochemistry Department, Brunel University, Uxbridge, Middlesex UB8 3PH, U.K.

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Abstract—When isolated rat kidneys are perfused with 30 μM cisplatin there is net tubular transport of platinum, resulting in excretion of platinum at a rate 125% of the rate attributable to glomerular filtration alone. Probenecid and furosemide are drugs which have been reported to protect against cisplatin nephrotoxicity, by unknown mechanisms. When probenecid 0.3 mM is included in the perfusate net transport of platinum is increased to 200% of that accounted for by glomerular filtration alone. Increasing the concentration of probenecid to 3.0 mM does not significantly further increase the rate of excretion of platinum. The inclusion of furosemide 0.3 mM in the perfusate has no effect on the net tubular transport of platinum. However, at 3.0 mM furosemide causes a decrease in the net platinum transport and only 93% of platinum filtered at the glomerulus appears in the urine. Thin-layer chromatography revealed the presence of at least three platinum compounds in the urine in addition to cisplatin. We conclude that the renal excretion of cisplatin and its transformation products, even in this model system, is a complex process involving glomerular filtration, tubular excretion and tubular reabsorption.

Cisplatin (*cis*-dichlorodiammine platinum II) is a cytotoxic drug used in the treatment of certain human tumours [1]. There are several side effects associated with cisplatin chemotherapy [1]. However, the major dose limiting factor is a dose dependent cumulative nephrotoxicity [2]. This nephrotoxicity can be ameliorated by maintaining the patient receiving cisplatin in extensive diuresis using either hydration and mannitol [3, 4] or furosemide [5]. In rats, furosemide [6, 7] and probenecid [8] have been reported as inhibiting the development of cisplatin nephrotoxity by mechanisms not yet elucidated. We have investigated the clearance of cisplatin by the isolated perfused rat kidney and the effects upon it of furosemide and probenecid.

MATERIALS AND METHODS

Kidneys were perfused using the method of Bowman [9]. Male Wistar rats (Charles River, Margate, U.K., 350–400 g) were used as kidney donors. All glassware was siliconised before use. The perfusate consisted of a Krebs–Henseleit bicarbonate buffer [10] containing glucose (5.5 mM); alanine (1 mM); glycine (1 mM); arginine (1 mM); creatinine (4.5 mM); inulin (0.6 mg/cm^3) and when required bovine serum albumin (Miles Laboratories, Stoke Poges, U.K., Pentex brand) 5% w/v. When albumin was not incorporated into the perfusate the calcium concentration was reduced from 2.5 mM to 1 mM. The perfusate was equilibrated at 37° with O_2/CO_2 (95:5) and the pH adjusted to 7.45 with 1 M NaOH before use. For each perfusion 40–50 cm^3 of perfusate was used. Kidneys were perfused for approximately 1 hr, urine being returned to the perfusate except for three clearance periods during which urine was collected. The clearance periods started after 20, 40 and 60 min of perfusion and lasted for 2–10 min, depending on the rate of urine flow. A sample of the perfusate was taken at the mid-point of each clearance period. When required: cisplatin (a gift from Johnson Matthey Research Centre, Sonning Common, U.K.) probenecid (Sigma Chemical Co., London, U.K.) or furosemide (a gift from DDSA Pharmaceuticals Ltd, London, U.K.) were dissolved in 0.9% w/v sodium chloride and added to the perfusate 3–4 hr prior to the start of a perfusion. The glomerular filtration rate (GFR) was determined by measuring the clearance of inulin.

Platinum was measured directly in untreated and in deproteinised perfusate samples and untreated urine samples with a Perkin–Elmer flameless atomic absorption spectrophotometer [11]. Perfusate samples were deproteinised by passage through an Amicon centriflow ultrafiltration membrane (CF25, which retains molecules larger than 25,000 molecular mass) and the filtrates and urine samples were used for the determination of inulin by the method of Heyrovsky [12].

The number of platinum containing species present in the perfusate following incubation of perfusate with cisplatin was investigated by thin layer chromotography. Silica gel plates were developed with isopropanol:formic acid:water (20:1:5 by volume). Stannous chloride was used to detect platinum containing compounds on developed plates [13].

RESULTS AND DISCUSSION

Cisplatin binds extensively to albumin and other blood proteins [14]. Since only the unbound drug is filtered at the glomerulus it is necessary to know the

Table 1. The renal clearance of platinum

Perfusate* composition	U_x/P_x			cm³/min			
	Inulin	Total platinum	Unbound platinum	Urine flow (V)	GFR‡ (clearance of inulin)	C_{Pt} (clearance of platinum)	C_{Pt}/GFR§
Control†	1.34 ± 0.17	1.65 ± 0.18	1.65 ± 0.18	0.572 ± 0.282	0.749 ± 0.342	0.912 ± 0.412	1.25 ± 0.16**
Control	3.81 ± 1.02	1.71 ± 0.04	4.95 ± 0.96	0.208 ± 0.077	0.730 ± 0.143	0.921 ± 0.127	1.29 ± 0.19**
0.3 mM Probenecid	9.65 ± 3.77	3.58 ± 1.39	18.75 ± 7.35	0.074 ± 0.052	0.544 ± 0.172	1.032 ± 0.296	1.95 ± 0.36‖
3.0 mM Probenecid	4.83 ± 3.26	1.59 ± 0.85	9.34 ± 5.94	0.206 ± 0.158	0.585 ± 0.169	1.147 ± 0.334	1.97 ± 0.14‖
0.3 mM Furosemide	2.56 ± 0.69	1.05 ± 0.47	3.16 ± 0.92	0.295 ± 0.150	0.660 ± 0.271	0.810 ± 0.331	1.24 ± 0.13¶
3.0 mM Furosemide	2.28 ± 0.01	0.48 ± 0.09	1.86 ± 0.19	0.577 ± 0.303	1.096 ± 0.536	1.024 ± 0.507	0.93 ± 0.07‖

Values are all means ±S.D., from 9 clearance periods (3 kidneys).
* All perfusates contained 30 μM cisplatin and 5% (w/v) albumin except: † no albumin.
‡ GFR for different treatments not significantly different from each other ($P = 0.01$).
§ C_{Pt}/GFR was calculated from individual values of C_{Pt} and GFR for each clearance period.
‖ Means significantly different from controls ($P < 0.01$).
¶ Mean significantly different from 3.0 mM furosemide ($P < 0.01$).
** Means for two controls (with and without albumin) not significantly different ($P = 0.01$).

concentration of free (unbound) platinum in the perfusate in order to calculate the renal clearance. When kidneys were perfused with perfusate containing albumin, half each mid-clearance period perfusate sample was immediately passed through an Amicon CF25 membrane for the determination of free platinum. Immediately prior to the first clearance period the proportion of free platinum in the perfusate was approximately 53%.

The urine samples from the isolated perfused rat kidney contained a small amount of protein; however, when these urine samples were passed through an Amicon CF25 membrane the platinum concentrations in the filtrates were not significantly different from those of whole urine.

The clearance of platinum (C_{Pt}) and inulin (glomerular filtration rate, GFR) were calculated from the formula $U_x/P_x \times V$, where U_x and P_x are the urine and perfusate concentrations of the substance and V is the urine flow rate. The concentration of free platinum was used for P_x.

The clearance of platinum was measured from perfusate containing 30 μM cisplatin either with or without the inclusion of 5% w/v albumin. The clearance of platinum from perfusates containing 30 μM cisplatin and 5% w/v albumin, together with probenecid (at 0.3 and 3 mM) or furosemide (at 0.3 and 3 mM) was also measured. The lower of the two concentrations of each drug corresponds approximately to the plasma concentration *in vivo* of a therapeutic dose immediately following intravenous administration [15]. For each treatment three kidneys were perfused and three clearance periods were investigated from each kidney. Table 1 shows the results from these experiments. The fractional clearance of platinum (C_{Pt}/C_{In}) was calculated from these data and is shown in the final column. There are large standard deviations associated with some of the GFR and C_{Pt} values shown in Table 1. However, the fractional clearance of platinum was calculated from the individual GFR and C_{Pt} values for each clearance period. This minimises the errors due to variable GFR. As can be seen from Table 1 there are significant differences between treatments for the fractional clearance of platinum but not for the glomerular filtration rate and therefore it is not possible to attribute the changes of fractional clearance in platinum merely to changes in GFR.

Our results (Table 1) show that there is a renal tubular transport component in the excretion of platinum from cisplatin. The clearance of platinum is found to be 125% of the GFR. This value is the same in kidneys perfused with or without albumin provided that the clearance is calculated from the concentration of free platinum in the perfusate. This observation also provides evidence that it is only free non-protein bound platinum which is available for excretion, whether by glomerular filtration or by tubular secretion. Further evidence for an active excretory mechanism for platinum has come from the recent observations of Jacobs *et al.* [16] who have shown that the clearance of platinum from humans given cisplatin therapeutically is greater than creatinine clearance.

Cisplatin is not the only platinum containing compound to pass into the urine of the isolated perfused

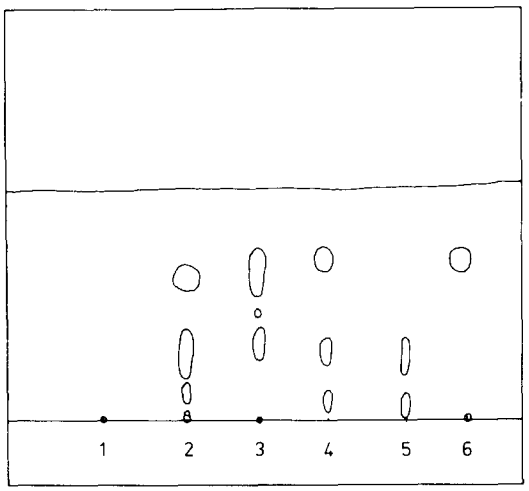

Fig. 1. The separation of platinum compounds by thin-layer chromatography. Samples: (1) urine from an isolated rat kidney perfused without cisplatin; (2) urine from an isolated rat kidney perfused with 30 μM cisplatin for 1 hr; (3–5) amino acid cisplatin mixtures (30 : 1 mole ratio), incubated at 37° for 1 hr in 0.9% w/v NaCl; (3) analanine–cisplatin mixture; (4) glycine–cisplatin mixture; (5) arginine–cisplatin mixture and (6) cisplatin, fresh solution in saline (approximately 5 μg Pt per sample applied to the plate).

kidney (Fig. 1). It is known that cisplatin can react with amino acids to form new complexes by ligand exchange reactions [17]. The perfusate contains arginine, alanine and glycine at concentrations of 1 mM. These amino acids were shown individually to react with cisplatin when incubated *in vitro* (Fig. 1). For each of these amino acids on incubation with cisplatin at least two new platinum containing species are formed. The urine from the isolated perfused kidney can be seen to contain at least three platinum species in addition to cisplatin and it is likely that a method of chromatographic separation with higher resolution would reveal the existence of even more than three. Because the perfusate contains a number of different platinum species the observed net tubular excretion of platinum could be due to a combination of different pathways involving the filtration, the secretion and the reabsorption of different platinum compounds. Since blood contains many amino acids able to undergo substitution reactions with cisplatin one would expect that *in vivo* the situation would be even more complex. Ross and Gale, who observed that probenecid inhibited development of cisplatin nephrotoxicity in the rat, postulated that cisplatin might form complexes *in vivo* with small molecules such as amino acids to form compounds with free carboxylate groups and these metabolites of cisplatin might be available for transport in the kidney by a *p*-aminohippurate (PAH) type carrier. Arginine/cisplatin substitution complexes could be examples of this type of compound since the substitution reaction probably involves the side chain nitrogen atoms of the amino acid [17] and the products would therefore have free carboxylate groups. However if the amino acid/platinum complex were transported by a PAH-type carrier then it would be expected that the direction of transport would be from the blood across the tubular cells and into the urine. The effect of probenecid as a well-known inhibitor of such a transport mechanism would then be the inhibition of tubular secretion of platinum, resulting in a reduction in its fractional clearance. However, we observe an increase in the fractional clearance of platinum in the presence of probenecid to 200% of the GFR.

Probenecid is a drug used mainly in the treatment of gout, since it blocks renal tubular reabsorption of uric acid [15] although it also acts as a competitive inhibitor of renal tubular excretion of organic anions such as *p*-aminohippurate, penicillin, furosemide and many others [18]. Since the known pharmacological effects of probenecid on the kidney arise from inhibitions of tubular transport, it seems unlikely that probenecid can be stimulating the tubular secretion of platinum and we think it more likely that the increase in fractional clearance brought about by probenecid arises from an inhibition of reabsorption of a platinum species.

Furosemide has been shown by DeSimone *et al.* to alter the tissue distribution of cisplatin but they observed no change in the rate of excretion of platinum, even though urine flow was increased [6]. However, furosemide has been shown by Ward *et al.* to reduce the nephrotoxicity of cisplatin in rats by a mechanism which is unknown [7].

In our work with the isolated perfused kidney when furosemide was used at 0.3 mM it had no effect on the clearance of platinum (Table 1). However, we observe that increasing the furosemide concentration from 0.3 to 3 mM causes a net reabsorption of platinum such that only 93% of filtered platinum appears in the urine (Table 1). This could explain why other workers [6, 19] found that rats treated with furosemide and cisplatin had more platinum in their kidneys than those treated with cisplatin alone. Furosemide is a diuretic which inhibits sodium reabsorption in the nephron, high concentrations of furosemide are attained in the tubular lumen because this drug like probenecid is excreted by active transport by the kidney [20]. Indeed probenecid blocks the tubular transport of furosemide by competing for transport sites [18]. It is surprising, therefore, that furosemide does not effect the clearance of platinum in a similar way to probenecid. Apart from active transport, passive reabsorption of compounds from the tubule fluid may occur [18]. This phenomenon being highly dependent on urine flow rate is easily modified by diuretics. It is clear from our results that passive reabsorption of platinum does not occur, because when albumin is omitted from the perfusate and urine flow is increased (Table 1), the fractional clearance of platinum is not significantly different from that in kidneys perfused with albumin. Pera and Harder [19] concluded that furosemide reduces cisplatin nephrotoxicity by lowering the platinum concentration in the urine. If this is so, then any form of diuretic would have the same effect. We propose to test this hypothesis.

Although it is not at present possible to explain satisfactorily the differences in the effects of furosemide and probenecid on the clearance of platinum, it is clear from our results that platinum enters the urine via glomerular filtration and active transport.

There also appears to be a mechanism for the reabsorption of platinum from the urine. We are currently investigating which, if any, of these processes is important in the development of platinum nephrotoxicity.

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