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An autoradiographic study of the intrarenal localisation and retention of cisplatin, iproplatin and paraplatin

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Summary. The intrarenal localisation of platinum following the intravenous administration of platinum-195m-labelled cisplatin, iproplatin and paraplatin was studied using autoradiography. Following injection of cisplatin, platinum was distributed throughout the kidney even up to 14 days after treatment. In the case of iproplatin and paraplatin rapid platinum clearance was noted from the glomeruli, blood vessels and renal medulla within 2 h of administration. Relative cortical and medullary platinum radionuclide concentrations for all three agents were determined by Chalkley grid analysis. This showed greater relative concentrations of platinum in the cortex at increasing times following iproplatin and paraplatin compared with cisplatin. It is suggested that the lower renal toxicity of iproplatin and paraplatin than of cisplatin may be due to reduced platinum retention within the pars recta.

Introduction

Paraplatin [carboplatin, CBDCA, JM8, *cis*-diammine 1, 1-cyclo butane dicarboxylate platinum (II)] and iproplatin [CHIP, JM9, *cis-trans*-dichlorodihydroxy-bis-(isopropylamine) platinum IV] are less nephrotoxic than cisplatin [CDDP, *cis*-diammine dichloroplatinum (II)] when administered with or without prehydration. In contrast, they have similar anti-tumour activity and have undergone clinical trials [2, 4, 12, 13, 14]. Paraplatin is now in routine clinical use. The reasons for the lower nephrotoxicity are unknown, but may be related to differences in the localisation of these compounds in the various target cell populations within the kidney. Compounds labelled with Pt-195m were used in the present autoradiographic study to investigate differences in their localisation and retention in the mouse kidney.

Materials and methods

Preparation of platinum radionuclides. Platinum-195m was produced by neutron irradiation of enriched Pt-194 (enrichment 96.04%; Source: National Laboratory, Oak Ridge, Tenn., USA) at a flux of 2×10^{14} ns^{-1} cm^{-2} for 72 h. The specific activity at the end of bombardment was approximately 37 MBq per mg platinum.

Pt-195m has a half-life of 4.1 days and emits several low energies of γ-rays, along with conversion and Auger electrons. There are four principal electron groups: 7 keV (intensity 1.487), 18–28 keV (intensity 1.575), 86.96 keV (intensity 0.216) and 117–127 keV (intensity 0.968). The energy distribution of these electrons is broadly similar to the spectrum of β-particles from carbon-14.

Synthesis of Pt-195m-labelled cisplatin, iproplatin and paraplatin. The radiolabelled compounds were prepared by introducing modifications to the previously published synthesis [1]. Partially soluble potassium hexachloroplatinate (K_2PtCl_6) was used instead of the soluble sodium salt, as it was easier to precipitate this and to obtain a pure product after reduction to potassium tetrachloroplatinate (K_2PtCl_4). Reduction of the hexachloroplatinate to the tetrachloroplatinate was by $N_2H_4.2HCl$ for 5 min at 0° C followed by 90 min at room temperature. During the synthesis of paraplatin, *cis*($PtNH_2I_2$) was mixed with Ag_2CBDCA for 90 min in the dark, giving *cis*($PtNH_2$.CBDCA $PtNH_2$.CBDCA) + 2 AgI. This was then evaporated to a clear solution and precipitated with acetone to give *cis*(Pt.NH_2CBDCA).

Quality control. The purity of the final products was assessed by thin-layer chromatography. The elution profile was compared with that of authentic compound obtained from Johnson Matthey. The stationary phase used was alumina/glass Merck 60F234 (Type E) 20 cm × 20 cm × 0.25 mm. The mobile phase was acetone: 0.1 *M* HCl (7:3) for cisplatin and paraplatin and acetone:ethylacetate:water (45:45:10) for iproplatin. Visualisation was by iodine absorption and UV detection.

Drug administration. Male Paterson BDF1 mice 8–10 weeks old were used in this study. In the initial experiment cisplatin (specific activity 3.08 MBq per mg) was administered s.c. via the tail vein at a dose of 10 mg/kg body weight (the maximum tolerated dose for BDF1 mice). In the second experiment higher specific activity (18.87 MBq per mg) cisplatin was used and administered i.v. at 15 mg/kg body weight (the LD_{50} for BDF1 mice). For the first series of experiments involving the second-generation compounds, iproplatin (specific activity 10.98 MBq per mg) and paraplatin (specific activity 12.40 MBq per mg) were

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administered i.v. at doses of 20.9 mg/kg and 18.5 mg/kg, respectively. These values corresponded to equiatomic amounts of platinum to that received by the second cisplatin-treated group. For the second series of experiments with these agents LD_{50} doses were used, these being 45 mg/kg for iproplatin (specific activity 1.57 MBq per mg) and 150 mg/kg for paraplatin (specific activity 2.66 MBq per mg). Again the i.v. route was used. It should be noted that although equitoxic doses were used in the second series of experiments, this refers to animal survival and not solely to kidney injury. This is important in clinical applications, where greater amounts of platinum are given in the form of the second-generation compounds, which are less nephrotoxic.

Cisplatin and iproplatin were made up in dilute saline solution (3:2, sterile water: physiological saline). Paraplatin was made up in sterile water.

Following injection, mice were housed three to six in a cage under a 12-h light/dark cycle. During the course of the experiment they were fed standard diet and water ad libitum. No parenteral hydration was given before or after injection. Control animals received injections of 0.25 ml physiological saline.

At predetermined times ranging from 5 min to 14 days after treatment, three animals per time point were sacrificed by cervical dislocation and both kidneys were rapidly excised. Each kidney was placed whole in 5 ml fixative (100:10:5, ethanol:formaldehyde:acetic acid) and fixed for histology using diffusion fixation. Immediately after being placed in fixative each kidney was counted for radioactivity in an autogamma counter. For all three compounds, correction for isotope decay was made against a sealed standard of each compound.

Preparation of autoradiographs. Following 12 h in fixative the kidneys were cut in two along the longitudinal axis and were transferred to 70% alcohol. After routine embedding and sectioning (sections 2–3 μm thick) the unstained slides were dewaxed in Histoclear (2 × 10 min) then brought down through alcohol to distilled water. The slides were then dipped six to eight times in Ilford K5 emulsion at 37° C. When dry the slides were boxed at 4° C with silica gel to maintain a dry atmosphere. Exposure time varied between 1 and 5 days depending on the specific activity of the compound used and the time between administration and sacrifice. Activities obtained per kidney ranged from 10^7 to 10^3 cpm. No attempt was made to estimate the activity in individual renal sections. During exposure the slides were interspaced by 3-mm sheets of lead in order to reduce background. Following development the slides were stained with Celestine Blue and Mayer's Haemalum.

Slides from animals treated with physiological saline were processed as above.

Analysis of autoradiographs. The renal distribution of platinum was measured using the method described by Chalkley [3]. Briefly, one eyepiece was fitted with a grid containing 25 random points of reference and the sections were examined at 1000× magnification. A section was evaluated by scanning a series of 10 fields chosen at random within the cortex, followed by 10 fields chosen at random within the medulla. If one or more autoradiographic grains was directly beneath or touching the reference points in the grid, this was termed positive. Each slide had an internal control as 10 background fields were also examined. The number of background "positives" was subtracted from the cortical and medullary counts. The net number of "positives" in the 10 fields in the cortex was divided by the number in the 10 fields in the medulla. The standard error of this ratio was calculated from the standard errors on the separate counts. Attempts at quantitative analysis using microdensitometric procedures were unsuccessful due to background absorbance by the histological stains.

Results

The renal retention curves for the three drugs were examined by non-linear regression procedures and each agent showed a biphasic loss from the kidney. The renal pharmacokinetic parameters for whole kidneys for all six experiments are given in Table 1.

At 5 min after injection the grain distribution was uniform throughout the cortex and medulla for all three agents. High concentrations could be seen in the renal pelvis, particularly following cisplatin, corresponding to the initial rapid loss from the kidney. With cisplatin there was widespread and uniform distribution of label even up to 14 days after treatment. However, by 30 min after injection of iproplatin and paraplatin, it was apparent that there was more rapid loss of platinum from the medulla relative to the cortex. By 2 h after treatment, in the case of iproplatin there was a sharp partition between cortical and medullary grain distributions, with grain concentrations in the medulla approaching background levels. A similar effect was seen with paraplatin. This partitioning occurred at the junction of the cortex and the outer stripe of the outer medulla. The results of the Chalkley grid analyses of these differences are shown in Fig. 1.

No preferential localisation of labelled cisplatin was noted within the nucleus (Fig. 2), although this was not quantitated. After injection of each agent the glomeruli

Table 1. Renal retention data recorded with Pt-195m-labelled platinum anti-tumour agents

(*A*) Renal half-lives (± 1 SE)

Compound	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (min)
Cisplatin (10 mg/kg)	2.9 ± 0.8	3830 ± 290
Cisplatin (15 mg/kg)	3.1 ± 0.1	6010 ± 900
Paraplatin (18.5 mg/kg)	13.3 ± 0.5	2670 ± 120
Paraplatin (150 mg/kg)	16.0 ± 0.70	1550 ± 180
Iproplatin (20.9 mg/kg)	23.7 ± 1.1	5780 ± 180
Iproplatin (45 mg/kg)	126 ± 13	3890 ± 390

(*B*) Area under the (renal concentration × time) curve (disintegrations × 10^6 ± 1 SE)

Compound	AUC	AUC (corrected[a])
Cisplatin (10 mg/kg)	1.5 ± 0.1	9.18 ± 0.60
Cisplatin (15 mg/kg)	23.8 ± 3.6	23.80 ± 3.6
Paraplatin (18.5 mg/kg)	3.6 ± 0.1	5.47 ± 0.10
Paraplatin (150 mg/kg)	14.1 ± 2.4	100 ± 17
Iproplatin (20.9 mg/kg)	16.2 ± 0.4	27.80 ± 0.70
Iproplatin (45 mg/kg)	24.5 ± 1.7	294 ± 20

[a] Corrected for differences in specific activity relative to the second cisplatin (15 mg/kg) experiment

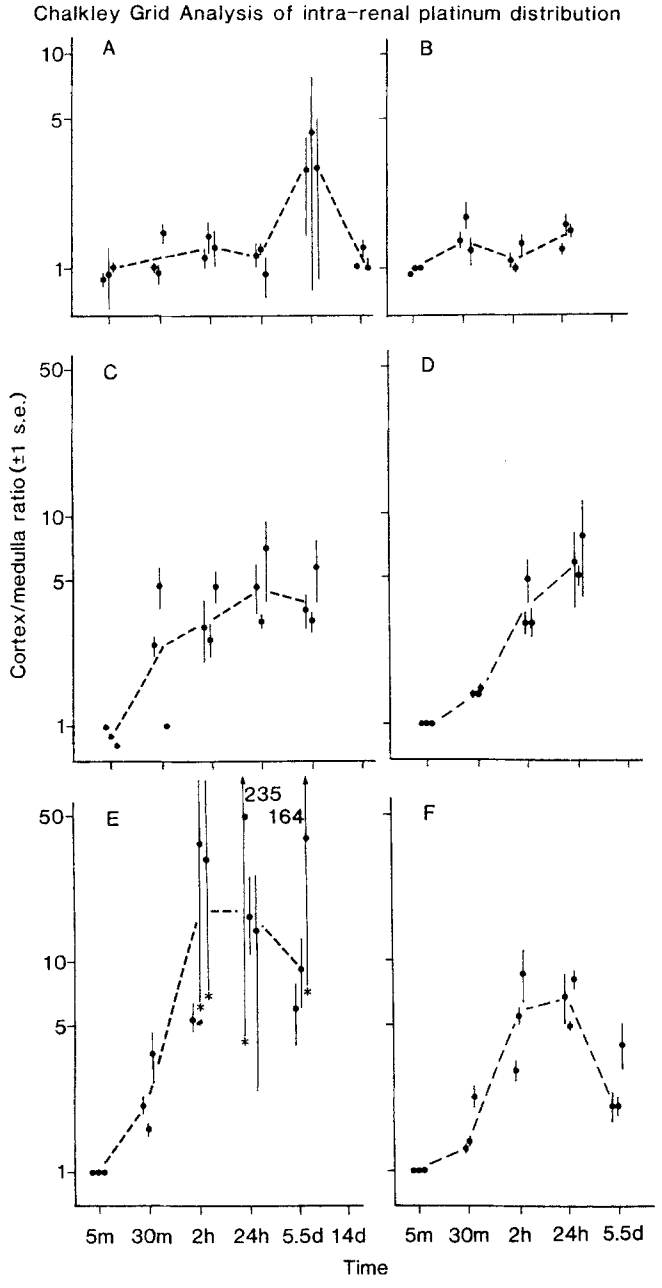

Fig. 1 A–F. Chalkley grid determination of the intrarenal localisation of platinum following cisplatin (**A** 10 mg/kg; **B** 15 mg/kg), paraplatin (**C** 18.5 mg/kg; **D** 150 mg/kg) and iproplatin (**E** 20.9 mg/kg; **F** 45 mg/kg). In **B** and **D** data not available beyond 24 h due to death of animals. *Asterisk* on the iproplatin data indicates points where medullary counts were not significantly above background

and blood vessels were cleared of label relative to the surrounding tubules. This was evident within a few hours following treatment with iproplatin and paraplatin, but was not apparent until 5–14 days after treatment with cisplatin (Fig. 3).

The consistent differences between cisplatin and the second-generation compound which was independent of dose (equiatomic or equitoxic) was the more rapid loss of platinum from the medulla. The effect seen was not a gradual decrease in grain density moving from the cortex to the inner medulla, but was the result of a sharp partition in

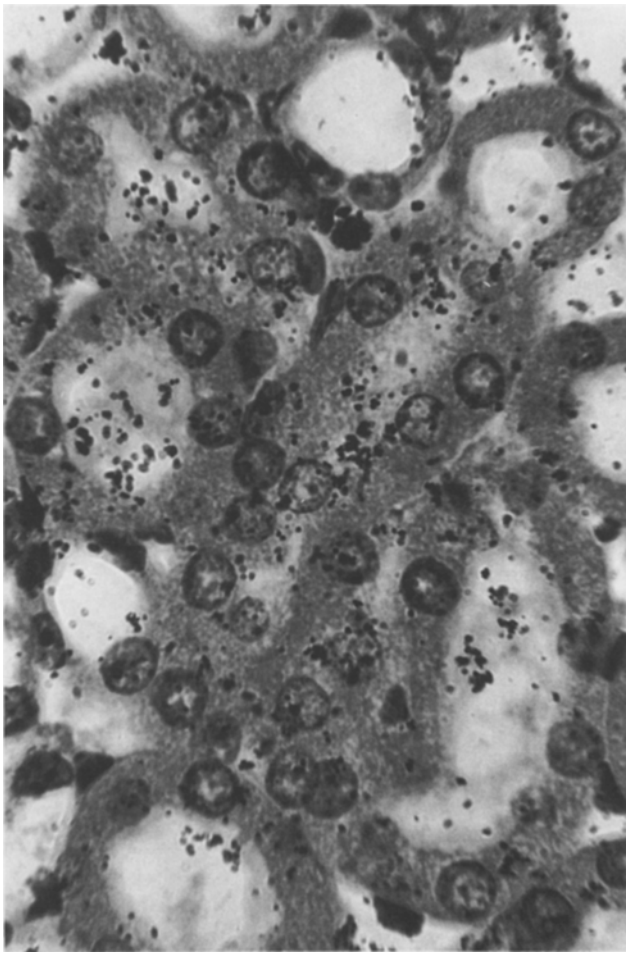

Fig. 2. Platinum distribution at 6 h after administration of cisplatin (10 mg/kg). The labelling is not limited to the nucleus. ×1000

grain concentration between the cortex and the outer stripe of the outer medulla (Fig. 4).

Discussion

The data in Table 1 show no consistent difference in the α or β retention half-life for the three compounds. This was not reported to be the case in the rat, where platinum was removed faster from the kidney following iproplatin [9]. In the present study comparison of the corrected values for the area under the (concentration × time) curves at LD_{50} doses shows a 4- to 10-fold increase when iproplatin or paraplatin is compared with cisplatin. Also, at the LD_{50} for iproplatin and paraplatin there is little renal toxicity [8, 12], and hence the AUC comparisons are not in terms of equitoxic doses for the kidney. The differential renal toxicity of these agents cannot be explained in terms of more rapid loss from the whole kidney. This is in agreement with other reports [17].

In the present study we have observed equal distribution of platinum between the cortex and medulla following cisplatin. This is in agreement with reports of equal intrarenal distribution in the rat [6]. However, other workers using autoradiographic procedures [16] have shown higher platinum concentrations in the outer stripe of the medulla. The reasons for these differences are unclear, but may be

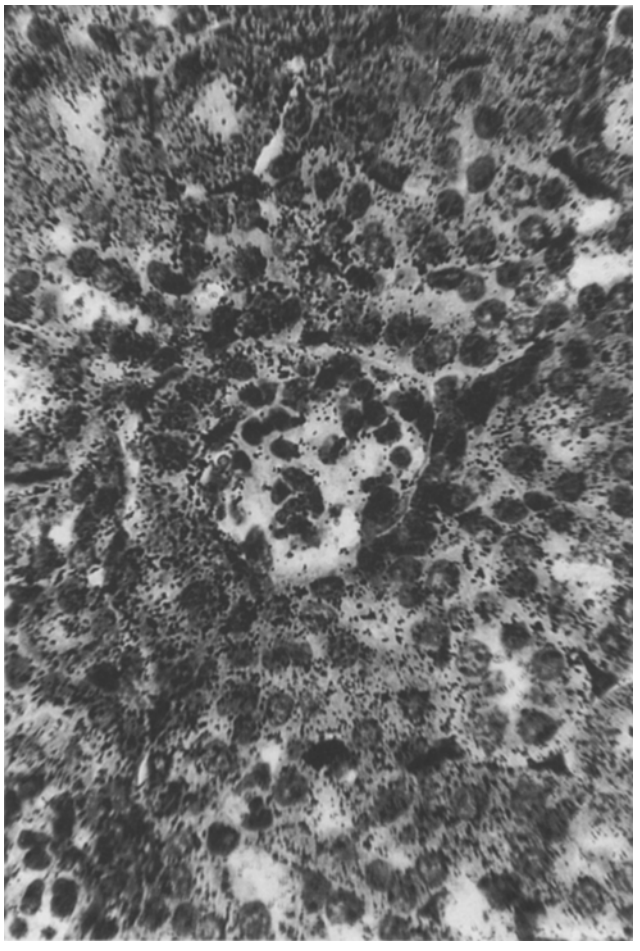

Fig. 3. Platinum distribution 14 days after administration of cisplatin (10 mg/kg). The glomerulus in the centre has been cleared of label relative to the surrounding tubules. ×400

related to technique. Saline flushing to reduce background activity in vascular and urinary spaces followed by perfusion fixation in vivo [16] will decrease platinum retention. Conversely, diffusion fixation, as used in this study, may retain previously bound platinum trapped within the tubule and could thus itself influence the distribution pattern.

There was no evidence of preferential localisation of labelled cisplatin within nuclei (Fig. 2). This is in agreement with other reports that platinum is localised mainly in the cytoplasm [15, 17].

Proximal and distal convoluted tubules can be distinguished using Periodic-acid-Schiff stain (P.A.S.) for the brush border of the proximal convoluted tubule. However, pilot studies had shown that the procedures necessary to produce good differentiation between proximal and distal convoluted tubules (P.A.S. 10 min, Feulgen stain 25 min, and dehydration to xylene) removed platinum from the sections. The sections were stained with Celestine Blue and Mayer's Haemalum as this method was fast and simple and did not remove platinum. Although this stain did not differentiate between proximal and distal convoluted tubules, widespread distribution of label throughout the cortex following all three agents did not suggest preferential binding to either of these regions of the nephron.

In mice and other species the kidneys have a single pyramid and the various segments of the nephron tend to be in register. This regularity of arrangement is reflected in a segmentation within the organ that is detectable on inspection with the naked eye of the cut surface. This effect is even more apparent under low-power microscopy and is not dependent on the use of any specific stains. The junction between the cortex and the medulla represents the transition point between the pars convoluta and the pars recta [7, 10, 18]. It is precisely at this junction that differ-

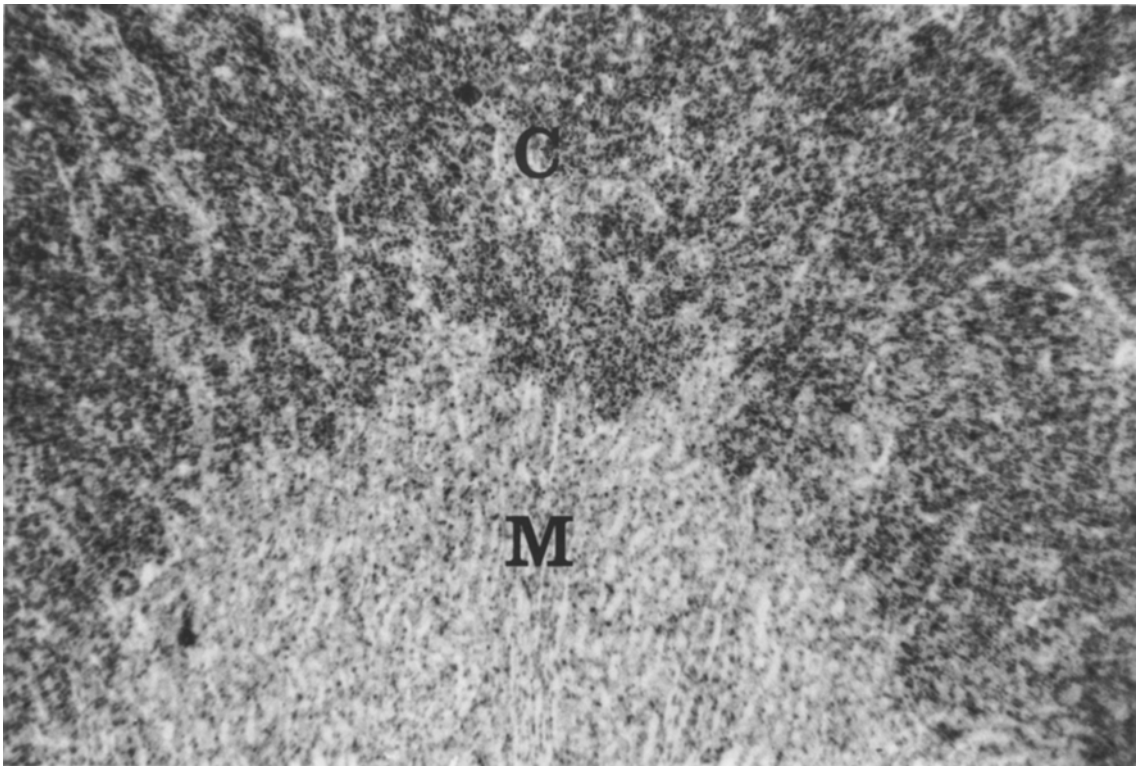

Fig. 4. Platinum distribution 2 h after the administration of iproplatin (20.9 mg/kg). The concentration difference between the cortex (*C*) and the medulla (*M*) is evident. ×100

ences in the platinum binding are seen between cisplatin and the two analogues. It is the pars recta that has been consistently identified as one of the principal targets for cisplatin nephrotoxicity using morphological and autoradiographic endpoints [5, 6, 11, 16, 19].

The present study does not give quantitative data concerning absolute platinum concentrations in the various regions of the kidney. However, 2 h after treatment, consistent differences of between 3-fold and 8-fold in relative *intrarenal* concentrations were identified when comparing LD_{50} doses of cisplatin with iproplatin and paraplatin (Fig. 1). It has been reported that despite 10- to 20-fold differences in doses used there was no difference in platinum concentrations found in the murine kidney when paraplatin was compared with cisplatin [17]. Therefore it is possible that the lesser renal toxicity of the second-generation platinum analogues or their metabolites is due, at least in part, to differences in intrarenal localisation and retention resulting in less platinum accumulation in the pars recta.

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