(Fig. 2b) suggest that the cellular UDPGA level increases with time as reported by Moldeus *et al.* [2] and its biosynthesis rate attains a steady state after 20 min preincubation (Figs 1b and 2b). Since the ratio of glucuronide to the total conjugates increases with substrate concentration (Figs 1a and b), the effect of preincubation time may be greater at higher substrate concentrations.

In summary, our data indicate the effects of preincubation time on the glucuronidation and sulfation rates of acetaminophen in isolated rat hepatocytes. The preincubation time had hardly any effect on $V_{max,S}$, but $K_{m,S}$ tended to decrease with preincubation time, reaching a constant value in 10 min. $K_{m,G}$ decreased with preincubation time in the same way as $K_{m,S}$. More than 20 min were required to attain a constant $V_{max,G}$. These results indicate that the biosynthesis rate of UDPGA to attain a steady state may be slower than that of PAPS. Thus, strictly speaking, for the conjugation reaction in hepatocytes, a preincubation period exceeding 20 min is preferable.

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Cisplatin-induced alteration of the copper and zinc content of the rat kidney

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The administration of Cd^{2+} to rats results in an increase in renal Cu concentration [1, 2] which is probably stimulated by the Cd^{2+}-induced synthesis of renal metallothionein, the low molecular weight metal-binding protein which normally functions in Zn and Cu homeostasis [3]. Treatment of rats with Hg^{2+}, Bi^{2+} [2], Au(I) [4] and Au(III) salts [5] has a similar effect on kidney Cu concentrations. In contrast, the anticancer drug *cis*-dichlorodiammine platinum II (cisplatin), which accumulates in the kidneys and produces proximal tubular necrosis [6, 7], reduces the concentrations of Cu and Zn in renal metallothionein and other soluble proteins in the rat kidney [8]. In this paper we have further investigated this interaction by comparing the time course and dose dependence of the cisplatin-induced change in renal Cu and Zn content with the renal uptake and toxicity of Pt in the rat.

Materials and methods

Cisplatin was synthesized by the method of Kauffman and Cowan [9]. Solutions of the drug were prepared in isotonic saline immediately before use. Six groups of male Wistar rats (N = 4, 130–150 g) received a s.c. injection of 5 mg cisplatin/kg (0.2 ml/100 g). Saline-treated controls (N = 4) were included with each group and the animals weighed and killed by decapitation 1, 2, 4, 8, 17 or 27 days after the administration of cisplatin. The kidneys were excised, weighed and a portion analysed for Pt, Cu and Zn, as described below. In a second experiment, 4 groups of rats (N = 4) were given a s.c. injection of 0, 1, 3 or 5 mg cisplatin/kg and then killed 7 days after the administration of cisplatin. Blood was collected from the severed cervical vessels, the serum creatinine concentration determined [10] and the kidneys removed.

Samples of whole kidney (50–100 mg) were digested to dryness with 0.5 ml conc. nitric acid (Aristar grade, BDH Chemicals Ltd, Poole, U.K.) and then analysed in 5 ml of 5% HCl (Aristar grade, BDH Chemicals Ltd) for Pt, Cu and Zn by flameless (Pt) or flame atomic absorption spectrometry (Cu and Zn).

Results and discussion

Cisplatin treatment produced a decrease in the Cu and Zn content of the kidney in relation to controls of the same age (Fig. 1). Thus, in the controls the Cu and Zn content of the kidney increased with age but decreased slightly in the cisplatin-treated animals. The percentage change in total renal Cu content was significantly greater than that of Zn. For example, 27 days after the administration of cisplatin, total renal Zn content in the cisplatin-treated animals was 63 ± 7% of the corresponding age-matched control level, whereas the total Cu content was only 28 ± 6% of the control value.

The total Pt content of the kidney increased to a maximum after 2 days and then declined slowly (Fig. 2A). The relative kidney weights of the cisplatin-treated animals were similar to those of the controls until 2 days after which they increased to a maximum at 8 days and then decreased slowly to the control value at 27 days (Fig. 2B). The

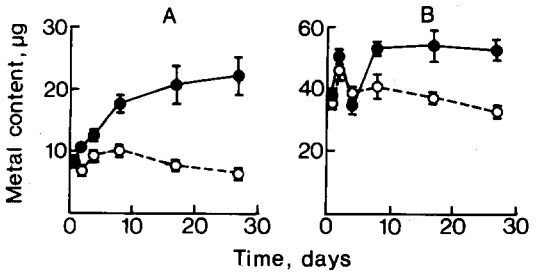

Fig. 1. Content of renal Cu (A) and Zn (B) as a function of time after the treatment of rats with 5 mg cisplatin/kg, s.c.: ●——●, controls; ○- - -○, cisplatin-treated.

cisplatin-treated animals failed to gain weight until after 8 days when their body weights started to increase at a similar rate to that of the controls (Fig. 2C).

Seven days following a dose of 1 mg cisplatin/kg, there was no significant difference in body weight gain, relative kidney weight and serum creatinine concentration in the control and cisplatin-treated groups (Table 1). At the higher doses of 3 and 5 mg/kg there was a significant decrease in the rate of body weight gain and a dose-dependent increase in relative kidney weight and serum creatinine concentration. In relation to the age-matched controls, cisplatin treatment also produced a dose-related decrease in total renal Cu content which correlated with the increase in relative kidney weight and serum creatinine concentration. Cisplatin had no significant effect on total renal Zn content (Table 1).

The immediate effect of cisplatin on body weight gain (Fig. 2C) suggests that Pt may impair the absorption of Cu and Zn by reducing the intake of food [11, 12]. However, the observation that the effect of cisplatin on the renal content of these metals continues after the animals have recovered their normal growth rate (Fig. 2C) and relative kidney weight (Fig. 2B) suggests that this factor is not significant, particularly at later times. It is also possible that the uptake of Pt by the intestinal mucosa may lead to the inhibition of the absorption mechanisms of Cu and Zn, particularly over the early stages of the experiment. This mechanism would be unlikely to explain the long-term effects of the drug since intestinal cellular necrosis, which is related to the concentration of Pt in the intestinal mucosa, is maximal at 1–2 days and absent by 7 days [12].

It is evident that the failure of the kidneys to accumulate Cu and Zn is related to the presence of Pt in the kidneys (Table 1), but the observation that the fall in the Cu and Zn content (Fig. 1) is not reversed as the renal Pt content decreases suggests that additional factors are involved in the interaction. The effect may also be related, in part, to the development of kidney damage (Table 1) but since there is little change in the Cu and Zn content of the kidney (Fig. 1) when the relative kidney weight is returning to normal (Fig. 2B and see ref. 13), it seems likely the effect does not depend on the degree of renal damage.

The failure of the kidneys of the cisplatin-treated animal to accumulate Cu and Zn suggests that the drug may block the tubular reabsorption of the cations. Also, since a primary determinant of the renal metabolism of Cu and Zn is the ability of the kidney to synthesize metallothionein, it is possible that the inhibition of metallothionein synthesis by Pt [8] could lead to the impairment of the renal uptake mechanisms of Cu and Zu. These hypotheses are consistent with the relatively long renal half-life of Pt and the persistent effects of the drug.

In conclusion, this study shows that cisplatin inhibits the normal accumulation of Cu and Zn in the rat kidney. The effect is unlikely to be mediated by impairment of the intestinal absorption of these metals and is not related simply to the Pt content of the kidney or the degree of renal damage. It is suggested that the interaction may result from inhibition of the tubular reabsorption of Cu and Zn and/or the inhibition of the synthesis of renal metallothionein by cisplatin. Experiments to test these hypotheses are in progress.

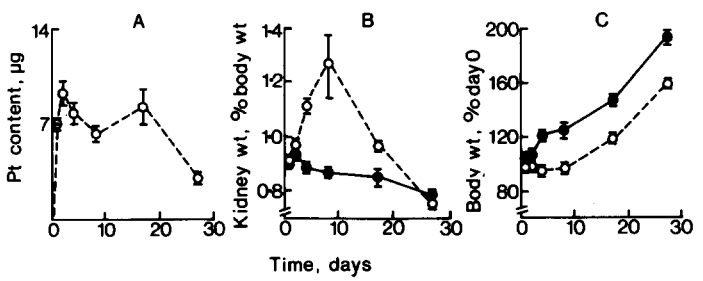

Fig. 2. Total kidney Pt content (A), relative kidney weight (B) and body weight (C) as a function of time after the treatment of rats with 5 mg cisplatin/kg, s.c.: ●——●, controls; ○- - -○, cisplatin-treated.

Table 1. Dose dependence of the cisplatin-induced change in renal Cu and Zn content in the rat

Dose of cisplatin (mg/kg)	Metal content (µg/kidney) Cu	Zn	Pt	Body wt (% day 0)	Kidney wt (% body wt)	Serum creatinine (µmoles/l)
0	14.4 ± 3.0	48.0 ± 4.6		130 ± 5	0.86 ± 0.01	10.9 ± 1.0
1	11.9 ± 2.7	37.7 ± 4.7	2.70 ± 0.03	130 ± 1	0.88 ± 0.02	12.2 ± 0.5
3	7.0 ± 0.9	41.5 ± 3.5	3.73 ± 0.18	122 ± 1	0.97 ± 0.01	14.3 ± 0.8
5	4.8 ± 0.6	39.4 ± 4.3	5.85 ± 0.66	103 ± 5	1.22 ± 0.08	24.7 ± 5.9

Four groups of male rats (N = 4) were given a s.c. injection of 0, 1, 3 or 5 mg cisplatin/kg and then killed 7 days after the administration of cisplatin.

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