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Amelioration of cisplatin-induced acute renal failure with 8-cyclopentyl-1,3-dipropylxanthine

R.J. Knight, *M.G. Collis, M.S. Yates & [1]C.J. Bowmer

Department of Pharmacology, Medical & Dental Building, The University of Leeds, Leeds LS2 9JT and *Bioscience II, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG

1 The effect of the selective adenosine A_1-receptor antagonist, 8-cyclopentyl-1,3-dipropylxanthine (CPX), on the development of cisplatin-induced acute renal failure was investigated in the rat.

2 CPX at doses of 0.03, 0.1 and 0.3 mg kg^{-1}, i.v. caused increasing degrees of antagonism of adenosine-induced bradycardia in anaesthetized rats. The magnitude of antagonism was not directly proportional to the increment in dose, but for each dose, it was similar in rats injected with either saline or cisplatin. CPX at a dose of 0.03 mg kg^{-1} significantly antagonized adenosine-induced bradycardia for up to 2.5 h, while doses of 0.1 and 0.3 mg kg^{-1} produced significant blockade for periods longer than 5 h.

3 Administration of cisplatin (6 mg kg^{-1}, i.v.) caused acute renal failure characterized by decreased inulin and *p*-aminohippurate clearances, increased urine volume but decreased excretion of Na^+, K^+ and Cl^- ions and by increased plasma levels of urea and creatinine. Kidney weight was increased in cisplatin-treated rats and renal tubule necrosis occurred.

4 Administration of CPX (0.03 mg kg^{-1}, i.v.; twice daily for two days) to rats given cisplatin did not reduce the severity of the resultant renal failure. However, treatment with 0.1 mg kg^{-1} CPX attenuated the increases in plasma creatinine/urea levels observed in rats on days 3 and 7 after induction of renal failure. In addition, this dose significantly reduced renal tubule damage and increased inulin and *p*-aminohippurate clearances. A similar pattern of protection was noted with CPX at a dose of 0.3 mg kg^{-1} although the increase in inulin clearance was not statistically significant. However, this higher dose of CPX significantly increased Na^+ and K^+ excretion compared to vehicle-treated rats.

5 CPX at doses of 0.03, 0.1 and 0.3 mg kg^{-1} produced blockade of an A_1-receptor mediated response i.e. adenosine-induced bradycardia, but only treatment with the higher doses of CPX (0.1 and 0.3 mg kg^{-1}) ameliorated nephrotoxicity produced by cisplatin. The lack of any protective effect afforded by the lowest dose of CPX could be a result of its shorter duration of action.

6 This study indicates that adenosine plays a significant role in the pathophysiology of cisplatin-induced acute renal failure.

Keywords: Cisplatin; acute renal failure; adenosine antagonism; 8-cyclopentyl-1,3-dipropylxanthine

Introduction

Cisplatin (cis-diamminedichloroplatinum II) is an inorganic complex of platinum whose cytotoxicity was first discovered by Rosenberg *et al.* in 1965. Cisplatin is one of the most potent anti-cancer drugs currently in use and is effective against a wide spectrum of human tumours. Its efficacy appears to increase with dose (Ozols *et al.*, 1988), but high doses of cisplatin are associated with severe adverse effects and in particular, with nephrotoxicity. This latter condition is often the dose-limiting factor in therapy. In order to reduce renal damage, and thereby enhance the efficacy of cisplatin, the pathophysiological mechanisms which underlie cisplatin-induced nephrotoxicity need to be investigated.

Winston & Safirstein (1985) have shown that renal blood flow, superficial nephron glomerular filtration rate, transglomerular pressure and effective filtration pressure at the afferent portion of the glomerulus were all decreased 72 h after a single dose of cisplatin. The reduction of renal blood flow was substantial (about 36%) and occurred even though mean arterial blood pressure was the same in control and cisplatin-injected rats (Winston & Safirstein, 1985). Consequently diminished renal blood flow must stem from increased renal vascular resistance. Moreover, the reductions in transglomerular pressure and effective filtration pressure at the afferent portion of the glomerulus, suggest that the increase in vascular resistance is of pre-glomerular origin (Winston & Safirstein, 1985). These observations of reduced renal blood flow and increased vascular resistance are also early features of cisplatin-induced renal failure in both dog and man (Offerman *et al.*, 1984; Daugaard *et al.*, 1987).

The mediator(s) of increased vascular resistance in cisplatin-induced acute renal failure is unknown, but one possible candidate is adenosine. This purine has been proposed to modulate renal haemodynamics under physiological conditions (Osswald, 1984) and in pathophysiological states such as acute renal failure (Churchill & Bidani, 1982). Steady-state infusion of adenosine reduces glomerular filtration rate (GFR) and the mechanism for this appears to be sustained afferent arteriolar constriction in the outer renal cortex (Osswald, 1984). These effects of exogenous adenosine are analogous to the changes in GFR and vascular resistance found in cisplatin-induced renal failure (Winston & Safirstein, 1985). Recently, Heidemann *et al.* (1989) have shown that theophylline, a non-selective adenosine antagonist and phosphodiesterase inhibitor, was able to reduce the severity of cisplatin-induced acute renal failure in the rat. They attributed this effect to the ability of theophylline to block adenosine receptors in the renal vasculature.

8-Cyclopentyl-1,3-dipropylxanthine (CPX; Figure 1) has been shown both *in vitro* (Haleen *et al.*, 1987) and *in vivo* (Kellett *et al.*, 1989) to be an A_1-selective adenosine antagonist. Since there is evidence that activation of A_1-receptors evokes constriction of the afferent arteriole (Holz & Steinhausen, 1987), we have studied the potential of this compound to protect kidney function from the deleterious effects of cisplatin. We have previously shown that CPX ameliorates glycerol-induced acute renal failure and that the selective A_1-adenosine receptor blockade produced by CPX, might be more beneficial in renal failure than non-selective antagonism

[1] Author for correspondence.

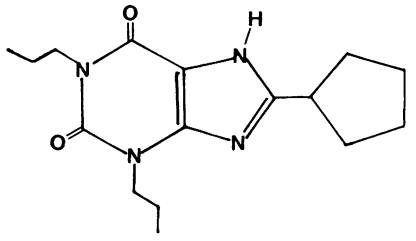

Figure 1 Structure of the selective A_1-adenosine receptor antagonist 8-cyclopentyl-1,3-dipropylxanthine (CPX).

of both A_1- and A_2-receptors (Kellett *et al.*, 1989). If adenosine is an important mediator of the changes in pre-glomerular vascular resistance which occur in cisplatin-induced renal failure, then treatment with CPX at doses which block the effects of adenosine, should abrogate this form of renal dysfunction. A preliminary account of this work has been given (Knight *et al.*, 1990).

Methods

Evaluation of 8-cyclopentyl-1,3-dipropylxanthine as an adenosine antagonist

This was carried out by determining the blockade of adenosine-induced changes in heart rate (predominantly mediated by A_1-receptors; Kellett *et al.*, 1989). Male Wistar albino rats (200 to 250 g) were anaesthetized with thiobutabarbitone (180 mg kg^{-1}, i.p.) and cannulae were inserted into: the trachea to allow artificial respiration (80 strokes min^{-1}, 10 ml kg^{-1} stroke volume); the left jugular vein for drug delivery and the right carotid artery for measurement of blood pressure. Body temperature was monitored with a rectal thermometer and maintained at 37–37.5°C by heat lamps. Systemic blood pressure was recorded via a pressure transducer (Druck PDCR 75) and heart rate was obtained electronically from the blood pressure signal. Thirty minutes after the completion of surgery, experiments were conducted to: (1) determine dose-response curves for the negative chronotropic effects of adenosine in the presence of CPX and (2) assess the duration of blockade produced by various doses of CPX on adenosine-induced falls in heart rate. These experiments were performed on rats 48 h after an i.v. injection of either 0.9% w/v NaCl (saline, 3 ml kg^{-1}) or cisplatin (6 mg kg^{-1}, 2 mg ml^{-1} in saline).

Dose-response curves Dose-response curves to adenosine dissolved in saline (0.02 to 12 mg kg^{-1}, i.v. administered every 3 min) were repeated in the presence of 0.03, 0.1 and 0.3 mg kg^{-1} CPX; dissolved in 1% v/v dimethyl sulphoxide, 0.75% v/v 1 M NaOH in saline. The vehicle for CPX had no significant effect on adenosine-induced changes in heart rate. Doses of CPX were achieved by cumulative addition every 30 min i.e. an initial dose of 0.03 mg kg^{-1} followed by doses of 0.07 and 0.2 mg kg^{-1}, respectively. In recent experiments we have administered [^{3}H]-CPX i.v. and determined the subsequent decline in plasma levels of radioactivity 2 days after administration of saline or cisplatin. These pilot experiments showed that the elimination half-life for radioactivity was 9.6 $\pm$ 2.2 h (n = 4) in saline-injected rats and 4.9 $\pm$ 1.6 h in rats injected with cisplatin (n = 4) (unpublished data). These data suggest that 30 min after the administration of one dose of CPX the fraction remaining of the original dose is 0.96 in saline-injected rats and 0.93 in cisplatin-injected rats. In view of this, the doses of CPX were given in a cumulative manner as indicated above.

Duration of adenosine blockade Rats received 3 injections of adenosine (1.2 mg kg^{-1}, i.v.) with a 3 min interval between doses. The mean response to these injections was taken as the control response. Following recovery from the third dose of adenosine, CPX (0.03, 0.1 or 0.3 mg kg^{-1}) or vehicle (1.0 ml kg^{-1}) was administered i.v. after which the same dose of adenosine (1.2 mg kg^{-1}) was repeatedly administered every 30 min for up to 5 h after injection of CPX or vehicle.

Evaluation of 8-cyclopentyl-1,3-dipropylxanthine in cisplatin-induced acute renal failure

Experimental protocol Male Wistar albino rats (250–350 g) were placed in metabolic cages for 24 h and urine was collected. At the end of this period rats were lightly anaesthetized with ether and a control (day 0) blood sample (about 0.75 ml) was taken from the tail vein. Rats were then injected i.v. with either cisplatin (6 mg kg^{-1}, 2 mg ml^{-1}) or its vehicle (saline, 3 ml kg^{-1}). Immediately after injection of cisplatin, rats received i.v. one of four treatments: CPX (0.03, 0.1 or 0.3 mg kg^{-1}) or the vehicle for CPX (1 ml kg^{-1}; 1% v/v dimethyl sulphoxide, 0.75% v/v 1 M NaOH in saline). This first injection of CPX or vehicle was made between 09 h 00 min to 10 h 00 min and additional i.v. doses of CPX or its vehicle were given 12, 24 and 36 h later. One group of cisplatin-injected rats received no treatment. Animals given saline were treated i.v. with either saline (1 ml kg^{-1}) or CPX (0.1 or 0.3 mg kg^{-1}) using the same dosage regimen employed for the cisplatin-injected groups. The saline-injected saline treated rats are referred to as normal animals.

A second blood sample (0.75 ml) was taken from the tail vein 3 days after injection of cisplatin or saline and rats were then placed in metabolic cages for a second 24 h urine collection. Seven days after the initial injection, rats were anaesthetized and the clearances of [^{3}H]-inulin (C_{IN}) and *p*-[^{14}C]-aminohippuric acid (C_{PAH}) determined. At the end of the experiment, a final blood sample (1 ml) was taken from the carotid artery and rats were killed with an overdose of anaesthetic. The kidneys were removed and prepared for histological examinination.

Estimation of [^{3}H]-inulin and p-[^{14}C]-aminohippuric acid clearances Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and cannulae were inserted into the trachea, left jugular vein and right carotid artery. Animals were injected with heparin (500 units kg^{-1}) and the clearances of [^{3}H]-inulin (100 mg kg^{-1}, 20 μCi kg^{-1}, i.v.) and *p*-[^{14}C]-aminohippuric acid acid (40 mg kg^{-1}, 4 μCi kg^{-1}, i.v.) dissolved in saline, were then determined simultaneously by the single injection method of Hall *et al.* (1977).

Measurement of urinary electrolytes Sodium and potassium concentrations were measured by use of a Corning 480 flame photometer whilst chloride levels were estimated with a Corning 925 chloride analyzer.

Measurement of plasma creatinine and urea concentrations Levels of these nitrogenous metabolites were assayed by standard colorimetric procedures: creatinine by reaction with picrate in alkaline solution and urea by reaction with diacetyl monoxime.

Kidney histology At the end of the clearance experiment, both kidneys were removed, cleared of adipose tissue and weighed. Kidneys were washed in saline, bisected longitudinally and preserved in formal saline (BDH). A longitudinal section was cut from one kidney of each rat and stained with haematoxylin and eosin. Sections were evaluated by a pathologist who was unaware of the treatment each donor animal had received. Renal damage was largely confined to proximal tubule necrosis (S_3 segment) and the extent of damage was scored out of four using the following scoring system:
0 = normal; 1 = mild tubular basophilia and dilatation; 2 = mild tubular necrosis, evidence of extensive regeneration and some hyaline and granular casts in the tubular lumen; 3 = moderate tubular necrosis with evidence of regeneration,

numerous casts; 4 = severe late stage tubular and papillary necrosis, little evidence of regeneration and numerous casts.

Statistical methods

Results are given as mean ± s.e.mean. Statistical analyses of functional and biochemical data were done by use of either Student's non-paired t test or where appropriate by one way analysis of variance (ANOVA) with means compared by Scheffe's test. Analysis of the histology scores was by a two-sided Mann-Whitney test. Dose-ratios for adenosine-induced bradycardia were calculated at a response level equivalent to a fall in heart rate of 140 beats min^{-1}.

Materials

Adenosine (free base) and cisplatin were purchased from Sigma Chemical Co. [^{3}H(G)]-inulin (355.5 mCi g^{-1}) and p-[glycyl-1-^{14}C]-aminohippuric acid (60 mCi $mmol^{-1}$) were obtained from NEN Research Products. The stated radiochemical purity of each isotope was greater than 98%. CPX was synthesized by Dr R. James of ICI and reagent kits for the assay of creatinine and urea were bought from Pierce & Warriner and BDH Ltd, respectively.

Results

Evaluation of adenosine antagonism

There was no significant ($P > 0.05$) difference in basal heart rate or mean arterial blood pressure between saline-injected rats (374 ± 8 beats min^{-1}; 71 ± 7 mmHg, $n = 5$) and cisplatin-injected rats (370 ± 5 beats min^{-1}; 80 ± 3 mmHg, $n = 5$). Administration of CPX at any dose (0.03 to 0.3 mg kg^{-1}) had no significant ($P > 0.05$) effects on basal heart rate or mean arterial blood pressure in either saline- or cisplatin-injected rats. All three doses of CPX (0.03, 0.1 and 0.3 mg kg^{-1}, i.v.) antagonized the bradycardia induced by adenosine (0.02 to 12 mg kg^{-1}, i.v.) in both groups of animals and Figure 2 illustrates the results for saline-injected rats. The mean log dose-ratios in saline-injected animals were similar to those obtained for rats given cisplatin (Table 1). In both groups of rats, however, the displacement of the agonist dose-response curve in the presence of CPX was not proportional to the increment in antagonist dose (Table 1, Figure 2). Adenosine at doses from 0.02 to 12 mg kg^{-1} evoked decreases in mean arterial blood pressure of 6 to 40 mmHg in both saline- and cisplatin-injected rats. As found previously (Kellett *et al.*, 1989), CPX was less potent in antagonizing the hypotensive responses produced by adenosine than the reductions in heart rate. There was no antagonism of the hypotensive responses to lower doses of adenosine with statistically significant ($P < 0.05$) reductions in response only occurring at doses of adenosine > 0.60 mg kg^{-1}, when the dose of CPX was either 0.03 or 0.1 mg kg^{-1}, and >0.40 mg kg^{-1} when the dose of CPX was 0.3 mg kg^{-1}.

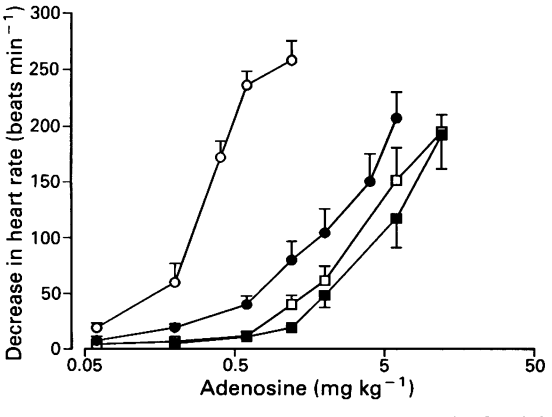

Figure 2 Adenosine-induced bradycardia in rats 48 h after injection with saline (3 ml kg^{-1}, i.v.) in the absence (○) and presence of 8-cyclopentyl-1,3-dipropylxanthine (CPX) 0.03 mg kg^{-1} (●), 0.1 mg kg^{-1} (□) and 0.3 mg kg^{-1} (■), i.v. Results are given as mean and vertical lines indicate s.e.mean ($n = 5$).

Table 1 Log dose-ratios for antagonism by 8-cyclopentyl-1,3-dipropylxanthine (CPX) of adenosine-induced bradycardia in anaesthetized rats

	Log dose-ratios	
CPX dose (mg kg^{-1})	*Saline-injected rats* ($n = 5$)	*Cisplatin-injected rats* ($n = 5$)
0.03	0.92 ± 0.09	0.90 ± 0.09
0.1	1.2 ± 0.1	1.1 ± 0.1
0.3	1.4 ± 0.1	1.3 ± 0.1

Mean ± s.e.mean and number of rats in parentheses.

The duration of blockade of adenosine-induced bradycardia produced by the various doses of CPX in saline-injected animals is shown in Figure 3. The blockade produced by a dose of 0.03 mg kg^{-1} of CPX lasted for 2.5 h since by 3 h the fall in heart rate produced by 1.2 mg kg^{-1} of adenosine was not significantly different ($P > 0.05$) from the response to adenosine following vehicle administration. By contrast, the blockade produced by 0.1 and 0.3 mg kg^{-1} of CPX persisted for longer than 5 h as the responses to adenosine showed little recovery over this period (Figure 3). The duration of adenosine blockade produced by the various doses of CPX in cisplatin-injected rats was identical to that in saline-injected animals.

Effect of CPX treatment on saline-injected rats

In saline-injected rats given 0.1 mg kg^{-1} CPX every 12 h for 2 days, there were no significant ($P > 0.05$) changes in plasma creatinine/urea concentrations (data not shown), C_{IN}/C_{PAH} (Figure 4), histology score (Table 2) or 24 h urine output/inorganic ion excretion (data not shown) compared to data for normal animals. These results agree with previous work (Kellett *et al.*, 1989). Similar results were obtained when the dose was increased to 0.3 mg kg^{-1}. However, in comparison to normal rats, there was a small but significant ($P < 0.05$) elevation of plasma creatinine levels on day 3 (0.73 ± 0.07 vs. 0.59 ± 0.04 mg 100 ml^{-1}; $n = 8$) and day 7 (0.73 ± 0.10 vs.

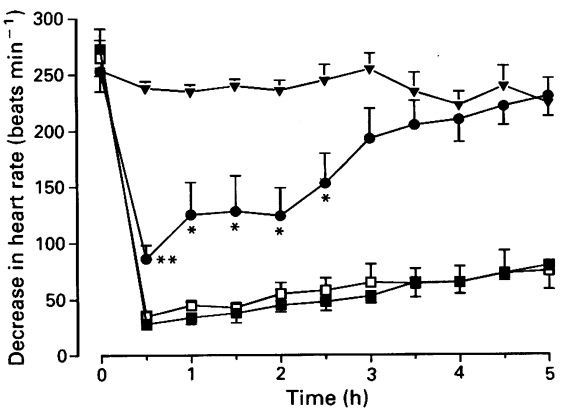

Figure 3 The bradycardia produced by repeated bolus i.v. administration of adenosine (1.2 mg kg^{-1}) in saline-injected rats before (0 h) and at various times after the i.v. administration of 8-cyclopentyl-1,3-dipropylxanthine (CPX) 0.03 mg kg^{-1} (●), 0.1 mg kg^{-1} (□) and 0.3 mg kg^{-1} (■) or vehicle (1.0 ml kg^{-1}, ▼). Results are given as mean and vertical lines indicate s.e.mean ($n = 4$). *$P < 0.05$, **$P < 0.001$, compared to the response in the vehicle-injected group at the same time (t test). All the responses to adenosine following administration of 0.1 and 0.3 mg kg^{-1} of CPX were significantly different ($P < 0.001$ t test) from the response in the vehicle-injected group at the equivalent time point.

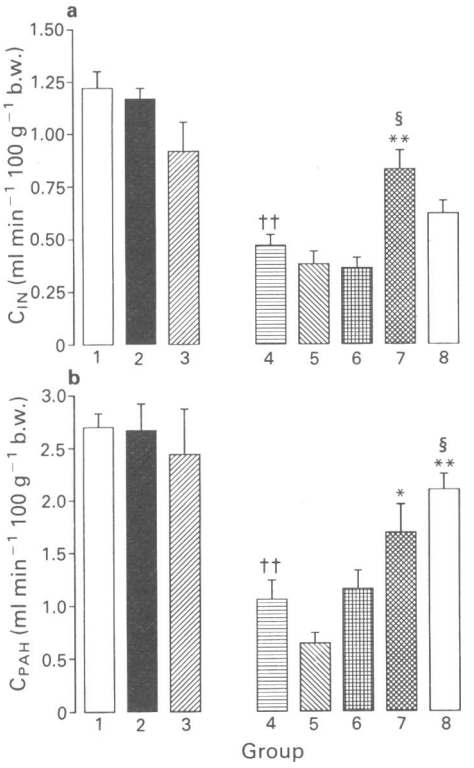

Figure 4 Clearances of (a) [^{3}H]-inulin (C_{IN}) and (b) *p*-[^{14}C]-aminohippuric acid (C_{PAH}) determined in rats seven days after i.v. injection of either saline or cisplatin. Rats were treated with either 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.03, 0.1 or 0.3 mg kg^{-1}, i.v.) or its vehicle (1.0 ml kg^{-1}, i.v.) twice daily for two days. Key to group: (1) saline-injected saline-treated ($n = 8$); (2) saline-injected + CPX 0.1 mg kg^{-1} ($n = 8$); (3) saline-injected + CPX 0.3 mg kg^{-1} ($n = 8$); (4) cisplatin-injected ($n = 15$); (5) cisplatin-injected vehicle treated ($n = 15$); (6) cisplatin-injected + CPX 0.03 mg kg^{-1} ($n = 15$); (7) cisplatin-injected + CPX 0.1 mg kg^{-1} ($n = 15$) and (8) cisplatin-injected + CPX 0.3 mg kg^{-1} ($n = 25$). Columns represent mean and vertical bars s.e.mean. ††$P < 0.001$ relative to group 1 (*t* test). *$P < 0.05$, **$P < 0.001$ relative to group 5 (ANOVA). §$P < 0.01$ relative to group 6 (ANOVA).

0.44 ± 0.04 mg 100 ml^{-1}; $n = 8$). Moreover, there was a 25% decrease in C_{IN} (Figure 4a), although this latter change was not statistically significant ($P > 0.05$).

Effect of cisplatin on renal function

A single intravenous dose of cisplatin (6 mg kg^{-1}) produced renal failure in all rats studied. Mean plasma concentrations of creatinine and urea were substantially increased at both 3 and 7 days after injection of drug (Table 3). Comparison of urine from cisplatin-injected rats and normal animals indicated a significant ($P < 0.001$) fall in 24 h excretion of Na^+ (0.25 ± 0.03; $n = 15$ vs. 0.76 ± 0.06 mmol 100 g^{-1} 24 h^{-1}; $n = 8$), K^+ (0.44 ± 0.05; $n = 15$ vs. 1.1 ± 0.1 mmol 100 g^{-1} 24 h^{-1}; $n = 8$) and Cl^- (0.30 ± 0.03; $n = 15$ vs. 0.99 ± 0.07 mmol 100 g^{-1} 24 h^{-1}; $n = 8$). Reduced inorganic ion excretion, 3–4 days after injection of cisplatin, was accompanied by a substantial increase in urine output (14.4 ± 1.7; $n = 15$ vs. 3.3 ± 0.3 ml 100 g^{-1} 24 h^{-1}; $n = 8$). In addition, Figure 4 shows that on day 7 C_{IN} and C_{PAH} were both reduced by about 60% ($P < 0.001$) relative to their values in normal animals. Cisplatin increased total wet kidney weight by about 35% ($P < 0.001$) and caused extensive tubule necrosis when compared to data for normal rats (Table 2).

Effect of treatment with either CPX or its vehicle on cisplatin-induced acute renal failure

There were no significant differences between rats given cisplatin alone and those injected with cisplatin but treated with vehicle in any of the indices of renal function, other than a small reduction ($P < 0.01$) in tubular damage in vehicle-treated animals (Table 2).

When compared to cisplatin-injected vehicle treated rats, treatment with CPX, at a dose of 0.03 mg kg^{-1} every 12 h for 2 days, did not significantly reduce plasma levels of the nitrogenous compounds creatinine and urea (Table 3). By contrast, cisplatin-injected rats treated with either 0.1 or 0.3 mg kg^{-1} CPX had lower ($P < 0.01$) plasma levels of creatinine and urea on days 3 and 7 than vehicle-treated animals (Table 3). Moreover, treatment with either 0.1 or 0.3 mg kg^{-1} CPX resulted in a significant reduction of creatinine levels on both days 3 and 7 compared to treatment with 0.03 mg kg^{-1} (Table 3). Table 3 shows that although mean urea levels on days 3 and 7 were lower in rats given 0.1 mg kg^{-1} compared to animals treated with 0.03 mg kg^{-1}, these differences were not statistically significant. However, rats dosed with 0.3 mg kg^{-1} of CPX had lower ($P < 0.05$) urea concentrations than animals given 0.03 mg kg^{-1}. Creatinine and urea concentrations were not significantly different in animals treated with either 0.1 or 0.3 mg kg^{-1} CPX (Table 3).

Figure 4 illustrates that treatment with CPX at a dose of 0.03 mg kg^{-1} did not result in a statistically significant increase in C_{IN} or C_{PAH} when compared to cisplatin-injected vehicle-treated rats. At 0.1 mg kg^{-1}, CPX significantly improved both C_{IN} and C_{PAH}; but at a dose of 0.3 mg kg^{-1} only C_{PAH} was significantly greater than the corresponding mean value for cisplatin-injected vehicle-treated rats (Figure 4). C_{IN} at 0.1 mg kg^{-1} and C_{PAH} at 0.3 mg kg^{-1} were significantly greater than in rats treated with 0.03 mg kg^{-1} CPX. Polyuria was not attenuated by any of the doses used (Table 4). In fact, Table 4 shows that there was a tendency for increased polyuria in all groups of cisplatin-injected rats treated with CPX, although these increases were not statistically significant. Inorganic ion excretion was largely unaffected by CPX treatment (Table 4). However, the 0.3 mg kg^{-1} dose did increase ($P < 0.05$) excretion of Na^+ and K^+ com-

Table 2 Total wet kidney weight and histological damage score 7 days after injection with saline or cisplatin

Group	n	*Total kidney weight* (g)	*Damage score*
Saline-injected, saline treated	8	2.16 ± 0.06	0.3 ± 0.2
Saline-injected, CPX treated (0.1 mg kg^{-1})	8	2.35 ± 0.09	0.1 ± 0.1
Saline-injected, CPX-treated (0.3 mg kg^{-1})	8	2.36 ± 0.06	0.7 ± 0.5
Cisplatin-injected, no treatment	15	2.92 ± 0.07***	4.0 ± 0.0
Cisplatin-injected, vehicle treated	15	2.61 ± 0.10***	3.3 ± 0.2#
Cisplatin-injected, CPX-treated (0.03 mg kg^{-1})	15	2.86 ± 0.10***	2.1 ± 0.2†
Cisplatin-injected, CPX-treated (0.1 mg kg^{-1})	15	2.75 ± 0.07***	2.5 ± 0.1†
Cisplatin-injected, CPX-treated (0.3 mg kg^{-1})	25	2.73 ± 0.08***	2.3 ± 0.2‡

Mean ± s.e.mean.
*** $P < 0.001$ relative to saline-injected saline treated (*t* test).
# $P < 0.01$ relative to cisplatin-injected no treatment (Mann-Whitney test).
† $P < 0.05$; ‡ $P < 0.001$ relative to cisplatin-injected vehicle treatment (Mann-Whitney test).
CPX, 8-cyclopentyl-1,3-dipropylxanthine.

Table 3 Plasma creatinine and urea concentrations in cisplatin-injected rats treated by i.v. injection with either vehicle (1.0 ml kg^{-1}) or 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.03, 0.1 and 0.3 mg kg^{-1}) twice daily for 2 days

Day	*No treatment* ($n = 15$)	*Vehicle* ($n = 15$)	*CPX* 0.03 mg kg^{-1} ($n = 15$)	*CPX* 0.1 mg kg^{-1} ($n = 15$)	*CPX* 0.3 mg kg^{-1} ($n = 25$)
(a) Creatinine (mg 100 ml^{-1})					
0	0.60 ± 0.02	0.61 ± 0.06	0.54 ± 0.3	0.60 ± 0.04	0.66 ± 0.02
3	2.0 ± 0.2	2.7 ± 0.3	2.1 ± 0.2	1.1 ± 0.1**#	1.2 ± 0.1**#
7	1.8 ± 0.3	2.3 ± 0.4	2.6 ± 0.3	1.3 ± 0.2**#	1.3 ± 0.1**#
(b) Urea (mg 100 ml^{-1})					
0	33 ± 2	43 ± 3	35 ± 1	35 ± 2	35 ± 1
3	126 ± 12	152 ± 10	114 ± 13	83 ± 9***	70 ± 5***#
7	148 ± 20	217 ± 25	153 ± 19	98 ± 11***	72 ± 6***#

Mean ± s.e.mean and number of rats in parentheses.
** $P < 0.01$; *** $P < 0.001$ relative to cisplatin-injected vehicle treated group (ANOVA).
# $P < 0.05$ relative to cisplatin-injected CPX (0.03 mg kg^{-1})-treated group (ANOVA).

pared to vehicle-treated animals (Table 4). All three doses of CPX significantly reduced the renal damage score relative to vehicle-treated rats, but the reduction in tubule necrosis was not dose-dependent and CPX treatment did not reverse the increase in kidney weight caused by cisplatin (Table 2).

Discussion

The results show that a single 6 mg kg^{-1} i.v. injection of cisplatin produced acute renal failure in the rat, and the changes in renal function were characterized by increases in plasma creatinine and urea concentration; reduced C_{IN} and C_{PAH} as well as polyuria. Cisplatin also caused an increase in kidney weight, necrosis to the S_3 segment of the proximal tubule and some cast formation. Treatment of cisplatin-injected rats with the vehicle for CPX (alkaline dimethyl sulphoxide) resulted in a small improvement in kidney morphology, but overall the vehicle did not reduce the severity of renal failure and this finding agrees with previous work using CPX vehicle in the glycerol model of acute renal failure (Kellett *et al.*, 1989). By comparison to vehicle-treated rats, animals given all three doses of CPX had reduced tubule necrosis, but with the 0.03 mg kg^{-1} dose, there were no other beneficial effects of therapy. However, increasing the dose to 0.1 mg kg^{-1} resulted in significant reductions in plasma creatinine and urea levels as well as marked improvements in C_{IN} and C_{PAH}. The highest dose of CPX, 0.3 mg kg^{-1}, also ameliorated renal dysfunction (including increases in Na^+ and K^+ excretion) but the increase in C_{IN}, about 75% relative to vehicle-treated group, was not statistically significant. There was some evidence in saline-injected rats that the 0.3 mg kg^{-1} dose of CPX depressed C_{IN}, which might account for the lack of a significant improvement of this variable in cisplatin-injected CPX-treated rats.

In the rat the polyuric effect of cisplatin is biphasic. The first phase occurs 1 to 2 days after exposure to the drug and is associated with normal GFR (Safirstein *et al.*, 1986). This phase usually abates spontaneously and seems to be due to cisplatin-induced inhibition of anti-diuretic hormone release or synthesis (Clifton *et al.*, 1982; Gordon *et al.*, 1982). The second phase, however, occurs 3 to 4 days after administration of drug and develops despite a persistent reduction of GFR. This polyuria results from a direct effect of cisplatin on the kidney, probably as a consequence of reduced medullary hypertonicity brought about by inhibition of urea recycling in the renal medulla (Safirstein *et al.*, 1981; Gordon *et al.*, 1982). In the present study, urine was collected 3 to 4 days after injection of cisplatin, so the polyuria detected was likely to be the second phase. There was no attentuation by CPX of the polyuria, in fact urine output was increased by about 4 ml 100 g^{-1} 24 h^{-1} in the three groups of CPX-treated rats. Although this increase was not statistically significant, it may be an indication of improved renal function. By antagonizing adenosine-induced vasoconstriction, CPX might elevate GFR and so increase the flow of filtrate entering the proximal tubule. As a consequence of the urine concentrating defect (Safirstein *et al.*, 1981; Gordon *et al.*, 1982) the kidney is unable to conserve this fluid, resulting in enhanced polyuria.

Neither inhibition of angiotensin II formation with captopril (Safirstein *et al.*, 1986) nor blockade of α-adrenoceptors (Daugaard & Abildgaard, 1989) improves cisplatin-induced nephrotoxicity. These findings suggest that activation of the renin-angiotensin system and increased sympathetic activity are not major pathogenic factors in the development of this type of renal damage. However, the observations that CPX as well as theophylline (Heidemann *et al.*, 1989) ameliorate nephrotoxicity imply that adenosine mediates, at least in part, the increase in renal vascular resistance seen during the development of cisplatin-induced renal failure. The effects of exogenous adenosine on the renal vasculature involve both

Table 4 Urine output and inorganic ion excretion 3 to 4 days after cisplatin injection in rats treated by i.v. injection with either vehicle (1.0 ml kg^{-1}) or 8-cyclopentyl-1,3-dipropylxanthine (CPX, 0.03, 0.1 and 0.3 mg kg^{-1}) twice daily for 2 days

	No treatment ($n = 15$)	*Vehicle* ($n = 15$)	*CPX* 0.03 mg kg^{-1} ($n = 15$)	*CPX* 0.1 mg kg^{-1} ($n = 15$)	*CPX* 0.3 mg kg^{-1} ($n = 25$)
Urine volume (ml 100 g^{-1} 24 h^{-1})	14.4 ± 1.7	13.3 ± 1.5	16.9 ± 2.1	18.9 ± 2.3	15.8 ± 1.6
Na^+ (mmol 100 g^{-1} 24 h^{-1})	0.25 ± 0.03	0.23 ± 0.02	0.28 ± 0.03	0.31 ± 0.04	0.39 ± 0.04**
K^+ (mmol 100 g^{-1} 24 h^{-1})	0.44 ± 0.05	0.36 ± 0.03	0.42 ± 0.04	0.46 ± 0.04	0.60 ± 0.05*
Cl^- (mmol 100 g^{-1} 24 h^{-1})	0.30 ± 0.03	0.31 ± 0.03	0.37 ± 0.04	0.34 ± 0.04	0.35 ± 0.03

Mean ± s.e.mean and number of rats in parentheses.
* $P < 0.05$; ** $P < 0.01$ relative to cisplatin-injected vehicle-treated group (ANOVA).

constriction of vessels in the outer cortex and vasodilatation in the inner cortex/medulla (Osswald, 1984). Vasoconstriction is likely to be mediated via A_1-adenosine receptors, whereas activation of A_2-receptors evokes vasodilatation (Murray & Churchill, 1984; Holz & Steinhausen, 1987). A_1-selective adenosine antagonists may be more beneficial than their non-selective counterparts, such as theophylline, since they will help to sustain GFR yet maintain vasodilatation and, therefore, provide better perfusion to the tubules. This selective effect could explain why treatment with CPX not only increased GFR but also reduced damage to the S_3 segment of the tubule.

The beneficial effects of CPX treatment were dose-dependent in as much as there was a clear differentiation between the improvements in renal function noted with the $0.03\,mg\,kg^{-1}$ dose and those obtained with the 0.1 and $0.3\,mg\,kg^{-1}$ doses. Increasing the dose from 0.1 to $0.3\,mg\,kg^{-1}$ did not lead to increased protection, so it is probable that the maximum beneficial effect had been reached with the $0.1\,mg\,kg^{-1}$ dose. Unfortunately it was not possible to confirm this by testing doses greater than $0.3\,mg\,kg^{-1}$ because of the poor aqueous solubility of CPX. All three doses of CPX antagonized adenosine-induced bradycardia in control rats, which results from A_1-receptor stimulation (Kellett *et al.*, 1989). These doses also blocked bradycardia in cisplatin-injected animals and there appeared to be no difference in the magnitudes of blockade seen in the two groups of rats. On increasing the dose of CPX, the displacement of the dose-response curve was not proportional in both groups of rats. The reason for this is not clear, but it could be explained by non-equilibrium conditions existing at the receptor sites. Such conditions might be produced by dose-dependent clearance of CPX and/or adenosine. In the latter case it is interesting that Vidrio *et al.* (1987) found that repeated administration of adenosine resulted in an enhancement of its cardiovascular effects. These authors concluded that this self-potentiation might be due to saturation or inhibition of the cellular uptake of adenosine.

Treatment of cisplatin-injected rats with $0.03\,mg\,kg^{-1}$ dose of CPX had little beneficial effect, yet this dose attenuated adenosine-induced bradycardia. This raises the possibility that another pharmacological property of CPX might be responsibile for its protective effects in renal failure. Alkylxanthines inhibit phosphodiesterases to different degrees (Amer & Kreighbaum, 1975) and there is evidence that CPX can inhibit these enzymes in cultured opossum kidney cells (Coulson & Scheinman, 1989). However, we have recently shown that in rats with glycerol-induced acute renal failure that neither 0.1 or $0.3\,mg\,kg^{-1}$ CPX reduced total renal phosphodiesterase activity for up to 6 h after i.v. administration (Panjehshahin *et al.*, 1992). Furthermore, in the same study we showed that repeated administration of CPX, twice daily for two days, at either 0.1 or $0.3\,mg\,kg^{-1}$ was also without effect on renal phosphodiesterase activity. This evidence and the finding by Heidemann *et al.* (1989) that enprofylline, an alkylxanthine which inhibits phosphodiesterase (Fredholm, 1985) but has a low affinity for adenosine receptors (Collis *et al.*, 1984), does not ameliorate cisplatin-induced renal failure, suggests that inhibition of renal phosphodiesterases does not account for the protective effects of CPX. The lack of a beneficial effect of the $0.03\,mg\,kg^{-1}$ dose, despite evidence of adenosine blockade, might be due to inadequate blockade of adenosine receptors within the kidney or to a shorter duration of adenosine blockade with this dose. This latter possibility is supported by the finding that the duration of blockade of adenosine-induced bradycardia with $0.03\,mg\,kg^{-1}$ was considerably shorter than that produced by either 0.1 or $0.3\,mg\,kg^{-1}$ CPX.

In conclusion, the results of this study demonstrate that the A_1-selective adenosine receptor antagonist CPX at doses of 0.1 and $0.3\,mg\,kg^{-1}$, i.v., is able to reduce significantly the severity of cisplatin-induced nephrotoxicity. These renal protective effects occur at doses which significantly antagonize adenosine-induced bradycardia. Our results support the work of Heidemann *et al.* (1989) and imply that adenosine plays a significant role in the pathogenesis of cisplatin-induced acute renal failure. The administration to patients of an adenosine antagonist with cisplatin should reduce the subsequent risk of nephrotoxicity and may allow higher doses of cisplatin to be employed in cancer chemotherapy.

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