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Long-term studies of cisplatin-induced reductions in porcine renal functional reserve*

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Summary. Mature Large White female pigs aged approx. 10 months received single intravenous doses of 1.5, 2, or 2.5 mg/kg cisplatin. The glomerular filtration rate (GFR) and the effective renal plasma flow (ERPF) in individual kidneys were measured prior to and at 4-week intervals for up to 24 weeks after cisplatin administration by renography using [^{99m}Tc]-diethylenetriamminepentaacetic acid (DTPA) and iodohippurate sodium I 131, respectively. The left kidney of each cisplatin-treated pig and that of three age-matched control pigs was then removed, and GFR and ERPF values were measured in the remaining kidney at 4-week intervals for a further 24 weeks after unilateral nephrectomy (UN). Pigs treated with cisplatin showed no significant reduction in GFR or ERPF for up to 24 weeks after drug infusion. As measured using inductively coupled plasma mass spectrometry, the mean renal platinum concentration in the left kidney removed at UN was 77.5 ± 9.1 ng/g kidney per mg/kg cisplatin. Histological evaluation of these kidneys revealed narrow interconnecting rays of interstitial fibrosis in the deep cortex and medulla; in these areas, glomeruli exhibited thickened Bowman's capsules and occasionally shrunken sclerotic capillaries. In cisplatin-treated pigs, UN was associated with a marked reduction in the ability of the remaining kidney to increase its function in terms of GFR and, to a lesser extent, of ERPF. The increase seen in GFR following UN in the cisplatin-treated pigs was only ca. 50%–70% of that seen in age-matched UN controls. Histologically, these kidneys revealed resolution of the peritubular fibrosis observed at UN; occasional sclerotic glomeruli were also evident. Platinum remained detectable in these kidneys, the mean levels being 18.8 ± 4.9 ng/g kidney per mg/kg cisplatin. These findings confirm previous observations and illustrate the need for caution in considering further treatment of patients who have previously received cisplatin along with a second potentially nephrotoxic agent.

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Introduction

cis-Diamminedichloroplatinum (II), or cisplatin, is an extremely effective anticancer agent in the treatment of a number of neoplasms [23]. For malignant germ-cell tumours in particular, its use in chemotherapy regimens is associated with cure rates approaching 100% in patients presenting with stage 1 disease [18]. In subjects suffering from metastatic disease, cure rates are about 80% [19]. Unfortunately, cisplatin is also highly toxic, its dose-limiting toxicity being nephrotoxicity [25].

Although some initial studies of the drug's nephrotoxicity appeared to indicate an acute but reversible reduction in renal function [17], later findings indicated that cisplatin could cause a permanent reduction in renal function as assessed in terms of either the glomerular filtration rate (GFR) [7, 12, 16, 24] or the effective renal plasma flow (ERPF) [1]. These findings show that cisplatin exerts a chronic nephrotoxic effect, i.e. it reduces renal function at least subclinically for up to several years after the initial treatment. Thus, it may well be that patients initially given the drug may be at risk of exhibiting clinical renal damage if they are subsequently treated with an additional nephrotoxic treatment modality. Such an enhanced nephrotoxic response following treatment with cisplatin has been reported in patients receiving methotrexate [14], ifosfamide [15], and radiotherapy [1]. Similar results have been obtained in experimental studies [11, 28].

We have previously reported that treatment with single doses of 2 or 2.5 mg/kg cisplatin at 4 weeks prior to unilateral nephrectomy (UN) resulted in a dose-related reduction in renal reserve [29]. In addition, cisplatin appeared to

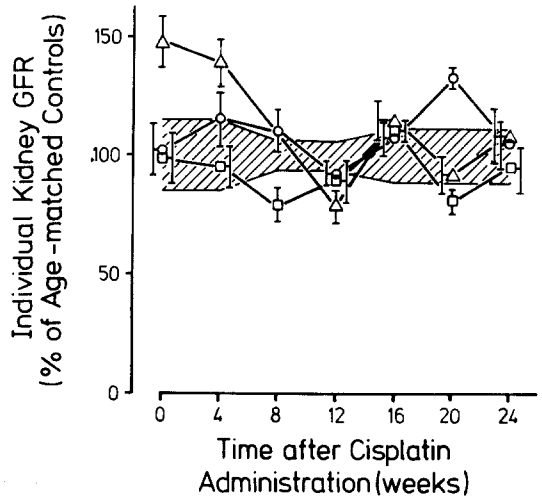

Fig. 1. Time-related changes in the individual-kidney GFR of mature pigs following the infusion of 1.5 (○), 2 (□), and 2.5 (△) cisplatin. The *hatched area* represents the 95% confidence limits on GFR values for individual kidneys in intact, age-matched control animals. Bars indicate the standard error

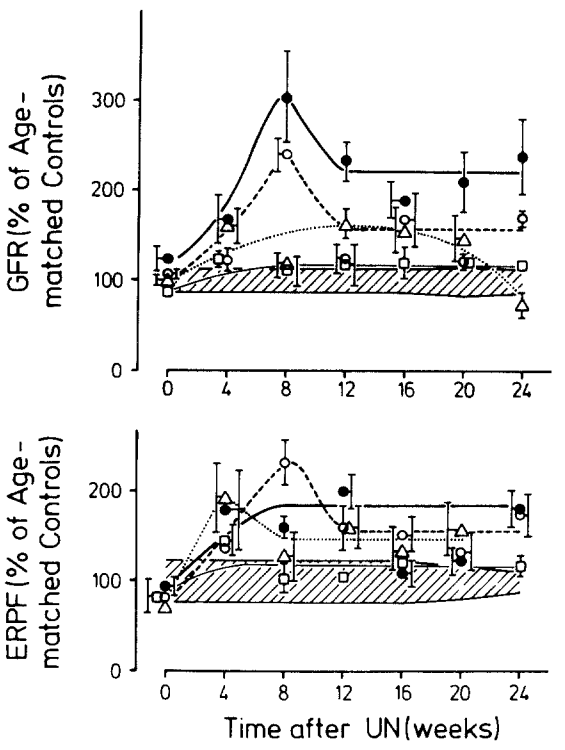

Fig. 2. Time-related changes in the GFR (*upper panel*) and ERPF (*lower panel*) in the right kidney of mature pigs following UN performed at 24 weeks after the infusion of cisplatin. ●——●, Changes in right-kidney function in age-matched control animals after UN. Symbols are defined as shown in Fig. 1

cause glomerular lesions, which progressed to glomerular hyalinization following UN. These findings were observed in the pig, which appears to be a useful model of clinically relevant cisplatin nephrotoxicity due to its marked renal structural and functional similarities with man [31, 33]. Since no data were available on the long-term effects of cisplatin in otherwise untreated pigs, it was not possible to determine whether the chronic glomerular lesions seen in the pig kidney were a direct result of cisplatin infusion alone. Moreover, the question as to whether prolongation of the interval between cisplatin administration and UN would reduce the drug-induced diminution in renal reserve remained unanswered.

To address these issues, we investigated the long-term effects of cisplatin alone on pigs following the infusion of single doses of cisplatin by measuring both the GFR and the ERPF for up to 24 weeks after drug treatment. UN was then carried out, and the functional status of the remaining kidney was determined for a further 24 weeks. The platinum content of both the left kidney removed at UN and the right kidney removed at the completion of the study was determined using inductively coupled plasma mass spectrometry (ICP-MS), a technique that enables the measurement of platinum concentrations of <1 ng/g tissue [34]. It was hoped that a study of the long-term retention of platinum might provide an insight into the mechanism(s) responsible for the chronic nephrotoxic action of cisplatin.

Materials and methods

A total of 12 mature female Large White pigs aged approx. 10 months were used in this study. Of these, 9 received a single intravenous infusion of 1.5, 2, or 2.5 mg/kg (i.e. ~90, ~120, and ~150 mg/m^2) cisplatin, with 3 pigs being tested at each dose. All procedures were carried out in anaesthetized animals maintained using a mixture comprising 2%–3% halothane, ~30% nitrous oxide, and ~70% oxygen [8]. Prior to the infusion of cisplatin, each pig was hydrated via an ear vein with 2 l saline at a rate of 1.31 l/h. Cisplatin was then infused in 1 l saline to which an anti-emetic agent (Maxolon, 1.5 mg/kg) had been added. Additional saline (1 l) was subsequently infused.

Prior to cisplatin administration and at 4-week intervals for up to 24 weeks thereafter, the GFR and the ERPF in individual kidneys were determined by renography using [^{99m}Tc]-diethylenetriamminepentaacetic acid (DTPA) and iodohippurate sodium I 131, respectively. Renograms were carried out as previously described [29]. The relative contribution of each kidney to the total function was assessed by determining the uptake function of each tracer in each kidney [27]. The total GFR and ERPF values were determined from the [^{99m}Tc]-DTPA and iodohippurate sodium I 131 plasma disappearance curves based on measurements from serial blood samples [26]. GFR and ERPF values (mean ± SE) measured in individual kidneys were compared with those previously obtained in historically age-matched control animals [29]. The statistical significance of the difference between these mean values was evaluated using Student's *t*-test; a value of $P < 0.05$ was considered to be significant.

At 24 weeks after cisplatin infusion, the left kidney of each treated animal as well as that of the three age-matched, untreated control pigs was removed surgically. After UN, each pig received analgesics (1 ml Temgesic; Reckitt and Colman) intramuscularly twice daily for 4 days. All animals recovered well from the surgical procedure. GFR and ERPF values were subsequently assessed in the remaining kidney at 4-week intervals for periods of up to 24 weeks after UN. Tissue samples from the surgically removed left kidney and the right kidney, which was removed at post-mortem examination, were taken and frozen at –20° C prior to determination of the platinum content using ICP-MS [34]. The remaining tissue was fixed in a solution of 1% acetic acid in 10% formol saline. Several renal tissue samples were then dehydrated, cleared, and embedded in paraffin wax. A histological evaluation was carried out on 5-μm-thick sections stained with periodic acid-Schiff's reagent (PAS).

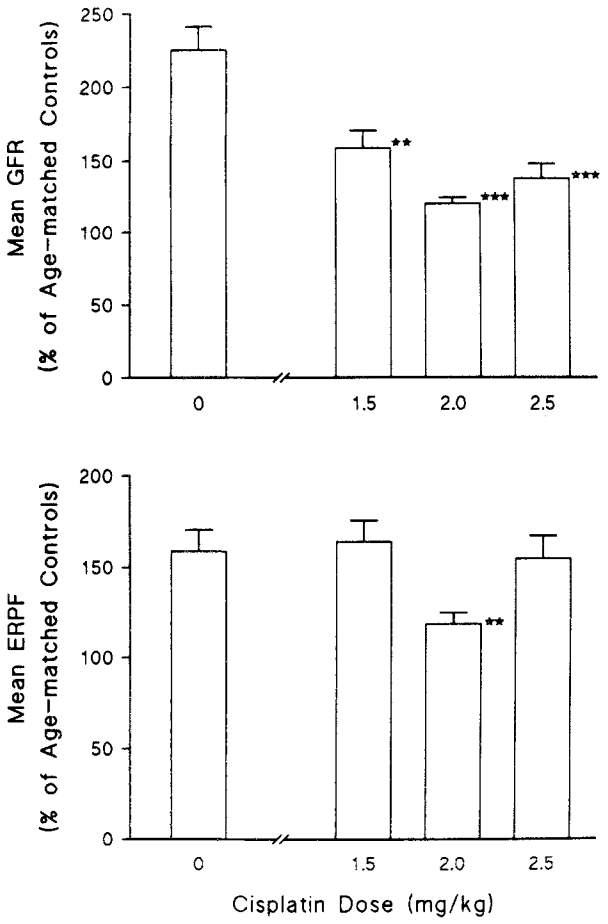

Fig. 3. Dose-related changes in the mean GFR (*upper panel*) and ERPF (*lower panel*) values as assessed over the period of 4–24 weeks after UN. Animals received a single dose of cisplatin at 24 weeks prior to UN. Bars indicate the standard error. ** $P < 0.01$; *** $P < 0.001$

Results

Effect of cisplatin on renal haemodynamics

The time-related changes in the individual-kidney GFR following the infusion of cisplatin in mature pigs are shown in Fig. 1. Although there was a tendency for the GFR to be reduced as compared with that in age-matched control animals at between 8 and 12 weeks after cisplatin administration, there was little evidence of a significant cisplatin-induced reduction in GFR over the 24-week period following drug treatment. Similarly, there was little change in the individual-kidney ERPF during this period (data not shown).

Effect of UN on renal haemodynamics

At 24 weeks after the infusion of cisplatin, the left kidney of each pig was surgically removed and the GFR and ERPF values in the remaining kidney were determined for periods of up to 24 weeks after UN. The left kidney was also removed from three age-matched control pigs. The effects of prior treatment with cisplatin on the functional status of the remaining kidney are shown in Fig. 2.

Following UN, the GFR in the remaining kidney of the age-matched control animals increased markedly such that within 8 weeks of UN, the mean GFR was around 3-fold that seen in individual kidneys of intact age-matched controls. By week 12, the GFR had declined slightly but remained >2-fold that measured in individual kidneys of intact control animals (Fig. 2a). A similar pattern of response was seen in the remaining kidney of pigs that had received a single dose of 1.5 mg/kg cisplatin at 24 weeks prior to UN. However, the extent of this increase was significantly lower than that seen in the age-matched UN controls, particularly over the 12- to 24-week period following UN. An even more severe reduction in the ability of the remaining kidney to increase its GFR following UN was observed in pigs that had been treated with single doses of $\geqq 2$ mg/kg cisplatin. In those that had received 2 mg/kg cisplatin, only a slight increase in GFR occurred after UN. However, in pigs that had been given 2.5 mg/kg cisplatin the GFR in the remaining kidney increased to ~1.5-fold that observed in individual kidneys of intact control pigs at 12–16 weeks after UN, but by 24 weeks after UN the GFR had markedly declined to a mean value that was not significantly different from that seen in the individual kidneys of intact animals (Fig. 2a).

The pattern of response of the ERPF in age-matched controls following UN was qualitatively similar to that seen for the GFR, although the absolute increase was smaller (Fig. 2b). In contrast, the effect of prior infusion of cisplatin on the ERPF of the remaining kidney following UN was different. Doses of 1.5 and 2.5 mg/kg cisplatin did not appear to significantly reduce the marked increase in ERPF that resulted from UN. However, in the animals that had received a dose of 2 mg/kg cisplatin at 24 weeks prior to UN, a significant reduction ($P < 0.01$) in the degree of increase in ERPF seemed to occur following UN in control pigs.

We further analyzed these data by calculating for each treatment group the mean GFR and ERPF value for the remaining kidney as measured at 4–24 weeks after UN; the results were expressed as a percentage of the individual kidney function in age-matched intact controls (Fig. 3). These findings clearly demonstrated that prior treatment with cisplatin severely compromised the ability of the remaining kidney to increase its function in terms of GFR following UN (Fig. 3a). Thus, whereas the mean GFR value found for age-matched UN control pigs was $224\% \pm 16.1\%$, that measured in pigs initially treated with 1.5 mg/kg cisplatin was significantly lower ($158.2\% \pm 11.8\%$, $P < 0.01$). However, there did not appear to be a clear dose-related response, as the mean GFR observed in pigs that had been infused with 2.5 mg/kg cisplatin prior to UN was not significantly different from that seen following a dose of 1.5 mg/kg cisplatin (Fig. 3a).

A different conclusion was reached when renal function was expressed in terms of ERPF. A reduction in mean ERPF values as compared with those seen in the age-matched UN controls was observed only in pigs that had been infused with 2 mg/kg cisplatin ($158.9\% \pm 11.6\%$ and $118.6\% \pm 6.4\%$, respectively; $P < 0.01$). The administra-

Table 1. Renal weights and platinum concentrations of kidneys removed from pigs at 24 and 48 weeks after the infusion of 1.5–2.5 mg/kg cisplatin

	Time after cisplatin infusion (weeks)		
	24		48
Kidney weight (g)	235.3± 11.1	P <0.001	401.8±32.6
Renal platinum concentration (ng/g kidney per mg/kg cisplatin)	77.5± 9.1	P <0.001	18.8± 4.9
Renal platinum content (μg/kidney)	18.2± 2.5	P <0.001	7 ± 1.4

tion of 1.5 and 2.5 mg/kg cisplatin prior to UN did not appear to compromise the UN-induced increase in ERPF (Fig. 3b).

Renal platinum concentration

The mean concentration of platinum measured in the left kidney removed by UN at 24 weeks after the administration of cisplatin was 77.5±9.1 ng/g kidney per mg/kg cisplatin; there was no significant difference in the platinum content of this kidney between the different groups when the platinum data were normalized in this way. The platinum concentration in the remaining kidney had decreased significantly (P <0.001) to a mean value of 18.8±4.9 ng/g kidney per mg/kg cisplatin by the time of its removal 24 weeks later. These findings indicate a loss of renal platinum with time. However, due to the marked hypertrophy resulting from UN, the weight of these kidneys was significantly greater (P <0.001) than that of kidneys that were removed at UN (Table 1). The renal weights of the cisplatin-treated pigs were not significantly different from those observed in the control animals (401.8±32.6 g and 445.7±41 g, respectively). To take account of this marked increase in organ weight, we estimated the amount of platinum present in the whole kidney (Table 1). The results confirmed the loss of platinum from the kidney with time, i.e. the mean platinum content of kidneys removed at 24 and 48 weeks after cisplatin infusion was 18.2±2.5 and 7±1.4 μg/kidney, respectively.

Morphological findings at 24 weeks after cisplatin administration

The most consistent change noted at this time was the presence of narrow, interconnecting rays of interstitial fibrosis in the deep cortex and medulla that occasionally contained small numbers of mononuclear inflammatory cells. Although these areas extended from the cortico-medullary junction into the medulla as far as the renal pelvis in some kidneys, they were usually limited to the inner one-half to one-third of the renal cortex. Associated tubules were sometimes moderately compressed; however, neither severe epithelial degeneration nor necrosis was observed at this time. Glomeruli associated with these areas of fibrosis often exhibited thickened Bowman's capsules and occasionally contained shrunken sclerotic capillary tufts. Although the remaining glomeruli usually appeared to be free of lesions, in one pig we found a widespread increase in the mesangial matrix in glomeruli at all cortical levels, a change that was accompanied by the presence of numerous sclerotic glomeruli. The renal changes observed at this time were generally most severe in the kidneys of pigs that had received a dose of >2 mg/kg cisplatin, with only mild changes being observed in the 1.5-mg/kg treatment group.

Morphological findings at 24 weeks after UN, or 48 weeks after cisplatin administration

In contrast to the changes noted in cisplatin-treated kidneys removed at 24 weeks after drug administration, kidneys removed at 48 weeks were strikingly free of morphological changes despite the finding that renal mass had been reduced by 50%. The characteristic peritubular fibrosis noted in the medulla and deep cortex at the former time point either became essentially absent or consisted of only small focal areas of non-inflammatory fibrosis at the end of the later period. Other than the presence of occasional small, scarred tufts, glomeruli exhibited little evidence of residual mesangial scarring or capsular thickening.

Discussion

The present findings clearly demonstrate that the administration of single doses of cisplatin in the range of 1.5–2.5 mg/kg to pigs had no significant effect on either GFR or ERPF for periods of up to 24 weeks after drug infusion. However, UN performed at this time subsequently led to a significant reduction in the ability of the remaining kidney to exhibit a compensatory increase in GFR and, to a lesser degree, in ERPF. This cisplatin-induced reduction in the compensatory increase in GFR was seen at all dose levels, but it failed to show a clearly defined dose-related effect. In contrast, a reduction in the compensatory increase in ERPF was observed only following a dose of 2 mg/kg cisplatin. In general, these results confirm previous findings in which the administration of cisplatin at 4 weeks prior to UN induced a significant reduction in renal reserve [29]. However, there are some interesting discrepancies in that in the previous study, the reduction in renal reserve was dose-related and was seen for both GFR and ERPF. Despite these differences, the present findings confirm that initial treatment with cisplatin severely compromises the ability of the kidney to respond to a subsequent insult. Even an extension of the

interval between cisplatin infusion and UN from 4 to 24 weeks failed to prevent the cisplatin-induced reduction in renal reserve.

There are few clinical studies with which the present findings can be compared. Hrushesky et al. [20] reported that the administration of cisplatin to individuals with a single functioning kidney, i.e. UN patients or subjects presenting with complete unilateral ureteral obstruction resulting in a non-functioning kidney, did not result in increased nephrotoxicity as compared with that observed in patients with two functioning kidneys. However, cisplatin was given to these patients after a functioning kidney had been lost, whereas in the present study it was given prior to UN.

Histological evaluation of kidneys removed by UN at 24 weeks after the infusion of cisplatin revealed evidence of chronic morphological alterations. These consisted of narrow, interconnecting rays of interstitial fibrosis in the deep cortex and medulla along with compression of associated tubules. There was little evidence of severe epithelial degeneration or necrosis at this time. It is noteworthy that the glomeruli associated with these fibrotic regions also exhibited damage in the form of thickening of the Bowman's capsule, which was occasionally associated with sclerotic glomeruli. These chronic cisplatin-induced lesions are similar to those previously observed in the pig as well as to those reported in other experimental animals [6, 37] and man [13, 32].

However, there would appear to be possible species-related differences in some aspects of these chronic morphological changes induced by cisplatin. Evaluation of chronic alterations in renal morphology in rats has consistently revealed the presence of grossly dilated proximal tubules, predominantly S_3 in origin. With time these form microcysts in the outer stripe of the outer medulla. These progress, forming large cysts at around 6 months after cisplatin treatment [6, 10, 22]. The production of these cysts is so characteristic that cisplatin has been used to produce an in vitro model of polycystic kidney disease [2]. Cyst formation has also been observed in mice at ca. 14 months after cisplatin administration [11]. Such changes have never been reported clinically [13, 32] and indeed do not seem to occur in the pig kidney for up to 48 weeks after cisplatin administration. This indicates possible differences in particular target-cell specificity in cisplatin nephropathy between unipapillate and multipapillate kidneys and provides further evidence of the clinical applicability of studies on porcine cisplatin nephropathy.

Despite the presence of these cisplatin-induced lesions in kidneys that had been treated 24 weeks earlier, it should be noted that there was little evidence of any significant functional impairment. The presence of "normal" levels of renal function for up to 24 weeks after cisplatin administration would therefore appear to reflect hyperfiltration by a reduced number of relatively undamaged nephrons rather than a genuinely undamaged or repaired organ. Such a conclusion is supported by the identification of obsolescent sclerotic glomeruli in the kidneys removed at UN.

These findings of a significant reduction in renal functional reserve in terms of the GFR following UN in all of the cisplatin-treated pigs suggests a deterioration of the cisplatin-induced lesions identified at the time of UN. However, evaluation of these kidneys at 24 weeks after UN, i.e. at 48 weeks after cisplatin infusion, revealed a general resolution of the lesions identified earlier. The characteristic peritubular fibrosis observed in the medulla and deep cortex at UN had either resolved or been reduced to focal areas of non-inflammatory fibrosis. Moreover, although some sclerotic glomeruli were present, glomeruli generally exhibited little evidence of residual damage such as mesangial scarring or thickening of the Bowman's capsule.

However, despite the apparent resolution of the lesions following UN and the absence of significant diffuse glomerular injury, UN did result in a significant reduction in renal reserve expressed in terms of GFR. Pigs treated with doses of 1.5–2.5 mg/kg cisplatin showed an increase in GFR that was only ca. 50%–70% of that observed in the age-matched UN controls. Could this chronic nephrotoxicity reflect the continued presence of platinum in the kidney? Previous studies of renal platinum content following cisplatin administration in rats [6, 9] showed a significant elevation of platinum levels for up to 4 weeks after treatment, but by 2 months these values had become similar to those seen in the controls [9]. However, these studies were limited by the relative insensitivity of the analytical techniques used.

The recent development of ICP-MS has enabled the detection of platinum concentrations of <1 ng/g tissue. Use of this highly sensitive technique has enabled the detection of platinum in the kidney of rats for up to 12 weeks after drug administration [35]. In the present study, platinum was detected at levels of 77.5 ± 9.1 ng/g kidney per mg/kg cisplatin at 24 weeks after drug infusion, and although the platinum content had decreased significantly by the time of its remeasurement 24 weeks later, the mean values remained 18.8 ± 4.9 ng/g kidney per mg/kg cisplatin. Thus, platinum remained detectable in the kidney for nearly 1 year after the initial administration of cisplatin. The continued long-term presence of platinum might at least in part explain the chronic nephrotoxic action of cisplatin. Unfortunately, no information is available regarding the chemical form of this renal platinum. Moreover, the precise pathophysiological mechanism(s) involved in the cisplatin-induced reduction in porcine renal reserve remain(s) ill-defined.

There is some evidence suggesting that cisplatin may adversely affect the proliferative capacity of renal tissue. The injection of a single dose of 8 mg/kg cisplatin in mice reduced the capacity of the kidney to mount an effective compensatory response to folic acid-induced renal tubular necrosis for up to 45 days after cisplatin injection [21]. Furthermore, a cisplatin-induced reduction in the "proliferative reserve" of normal tissues, including the kidney, has been reported for up to 120 days after the initial treatment [5]. More recently, the administration of cisplatin to mice has been reported to impair the renal regenerative response to treatment with the nephrotoxin uranyl nitrate at 14 days after cisplatin [11]. In addition, studies on the proliferative response of rat kidneys treated with cisplatin have demonstrated increased proliferation throughout the 21-day experimental period, which was interpreted as indi-

cating incomplete tissue repair [22]. Thus, the chronic nephrotoxic effects of cisplatin reported herein may reflect manifestations of transient or long-lasting reductions in the proliferative reserves of the renal cell-renewal system. The elucidation of the role of such a cisplatin-induced reduction in renal proliferative capacity requires more detailed investigations, in particular cell kinetics studies to identify cell turnover in this system.

It should be noted that despite the administration of only a single dose of cisplatin (<150 mg/m^2) in these studies, a significant reduction in renal reserve was noted. It would seem likely that if large cumulative doses similar to those used clinically had been given, then an even more marked reduction in renal reserve would have occurred. These observations may be of particular importance in clinical situations involving UN, particularly in the treatment of paediatric primary renal tumours such as Wilms' tumour and mesoblastic nephroma using both UN and cisplatin-containing chemotherapy [36].

Despite the present inability to define accurately the mechanism(s) responsible for the cisplatin-induced reduction in renal reserve, these findings reinforce the need for caution in considering the further treatment of patients who have previously received cisplatin along with a second potentially nephrotoxic agent. As discussed earlier, the finding of "normal" renal function for up to 24 weeks after cisplatin treatment need not imply a normal undamaged or repaired organ but may rather indicate hyperfiltration by the remaining undamaged nephrons. Thus, measurements of "normal" renal function alone are insufficient to identify patients at particular risk. In addition, the apparent resolution of the cisplatin-induced lesions in the kidney following UN, associated with a concomitant reduction in renal reserve, indicates that morphological evaluation alone may also be inappropriate. It has been suggested that renal insufficiency may be expressed in terms of a reduced renal functional reserve (RFR), i.e. the increase in GFR observed following a protein load [4, 30, 38]. Therefore, measurement of the RFR might enable the identification of patients who, if subjected to subsequent nephrotoxins, might exhibit clinical renal injury. However, the question as to whether or not patients displaying a reduced nephron population indeed exhibit a reduced RFR remains controversial [3, 39]. In view of this controversy, it would be interesting to ascertain whether or not the cisplatin-induced reduction in renal haemodynamics observed following UN indeed results in a detectable reduction in the RFR.

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