Cancer Chemother Pharmacol (1990) 27: 211–218

Cancer Chemotherapy and Pharmacology

Cisplatin-induced reductions in renal functional reserve uncovered by unilateral nephrectomy: an experimental study in the pig*

Mike E. C. Robbins[1], Darryl Campling[1], Elizabeth Whitehouse[1], John W. Hopewell[1], and Adam Michalowski[2]

[1] Cancer Research Campaign, Normal Tissue Radiobiology Research Group, Research Institute (University of Oxford), Churchill Hospital, Oxford, UK.
[2] Medical Research Council Cyclotron Unit, Hammersmith Hospital, DuCane Road, London, UK.

Received 11 November 1990/Accepted 26 June 1990

Summary. Groups of mature Large White female pigs, approximately 10 months of age, received single intravenous infusions of 1.5, 2 or 2.5 mg/kg body weight (equivalent to ~90, ~120 and ~150 mg/m^2) cisplatin. Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured before and at 4 weeks after cisplatin infusion by renography using [^{99m}Tc]-DTPA (diethylenetriamminepentaacetic acid and iodohippurate sodium I 131, respectively. The left kidney of each cisplatin-treated animal plus that of four age-matched control pigs was then removed surgically, and GFR and ERPF were measured in the remaining kidney at 4 weekly intervals for up to 24 weeks after unilateral nephrectomy (UN). The pigs treated with cisplatin exhibited no consistent change in either GFR or ERPF at 4 weeks after treatment. A histological evaluation of kidneys from animals treated with $\geqq 2$ mg/kg cisplatin that had been removed at UN revealed both tubular and glomerular lesions. The latter consisted of cell proliferation on the parietal surface of the urinary space; damage to the S_1 portion of the proximal convolution was also noted. Following UN there was a pronounced dose-dependent reduction in the functional status of the remaining kidney such that the increase in GFR and ERPF in pigs initially receiving 2.5 mg/kg cisplatin was <50% of that seen in age-matched UN controls. Moreover, the glomerular lesions observed at 4 weeks after cisplatin infusion had apparently progressed to glomerular hyalinisation by 24 weeks after UN. Thus, prior treatment with cisplatin may cause a permanent reduction in renal functional reserve that may be clinically "silent" until exposure to an additional nephrotoxic insult.

* This study was supported by the Cancer Research Campaign

Offprint requests to: Mike Robbins, Cancer Research Campaign, Normal Tissue Radiobiology Research Group, Research Institute (University of Oxford), Churchill Hospital, Oxford OX3 7LJ, U.K.

Introduction

cis-Diamminedichloroplatinum(II), or cisplatin (CDDP), is one of the most important chemotherapeutic agents to have emerged in the last decade. Cisplatin has become the drug of choice in the treatment of a number of solid tumours, such as those of the head and neck, bladder, cervix, lung and oesophagus [30], and, in particular, testicular [11] and ovarian cancers [38]. Unfortunately, cisplatin is also one of the most toxic anticancer drugs, causing severe emesis, neuropathy, anaemia and deafness. Its dose-limiting toxicity is nephrotoxicity [24], which was predicted from initial animal studies.

Despite the use of optimal methods for cisplatin administration, such as concomitant active hydration or the use of sodium chloride as the vehicle [6, 26], approximately 30% of patients treated manifest nephrotoxicity as defined by a creatinine clearance of <50 ml/min.

Initial clinical and experimental studies concentrated on the acute nephrotoxicity of cisplatin. Patients treated with the drug exhibited an increase in serum creatinine within 6–7 days of treatment [18], followed by a return to pre-treatment values within approximately 3 weeks. Similar results have been reported following the administration of cisplatin to rats [4, 37]. However, more recent findings have suggested that this drug causes a permanent reduction in glomerular filtration rate (GFR) [9, 13, 17], which may indeed be progressive [16, 19].

Since cisplatin may produce a permanent reduction in GFR, it is likely that patients initially given the drug may be at risk if they are subsequently subjected to another nephrotoxic modality. Although this has been mentioned in the literature, there appear to be few data available on this potentially deleterious consequence of cisplatin treatment.

The present study addresses this question by investigating the effect of a second renal insult, i.e. unilateral nephrectomy (UN), on the functional status of the kidney in pigs previously treated with a single dose of cisplatin. The pig and man are unique among mammals in possessing a multipyrimidal multipapillate kidney [32]. In addi-

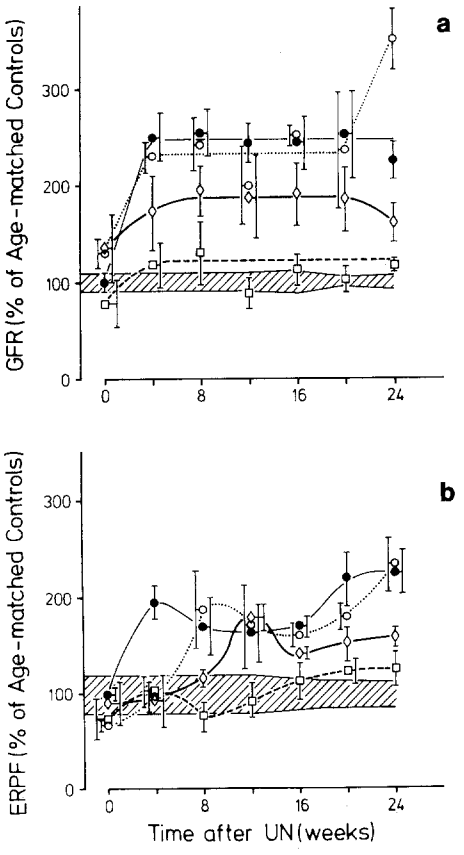

Fig. 1 a, b. Time-related changes in right kidney **a** GFR and **b** ERPF of mature pigs following UN performed 4 weeks after the infusion of cisplatin. (○), 1.5 mg/kg; (◇), 2 mg/kg; (□), 2.5 mg/kg; ●——●, the changes in right-kidney function in age-matched control animals after UN. The *hatched area* represents the 95% confidence limits on GFR and ERPF values for individual kidneys in intact, age-matched control animals. *Error bars*, ±SE

tion, there are marked similarities in renal function and biochemistry between the pig and man [23, 26]. Cisplatin produces functional changes in the pig kidney that are similar to those found in other species [5, 25, 29]. The similarities between the pig and the human kidney would suggest that findings of cisplatin nephrotoxicity in the pig may be of direct clinical relevance.

Materials and methods

A total of 16 mature Large White female pigs, approximately 10 months of age, were used in this study. Of these, 12 received single intravenous infusions of 1.5, 2 or 2.5 mg/kg body weight (i.e. ~90, ~120 and ~150 mg/m^2) cisplatin in groups of 4, 5 and 3 pigs, respectively. All procedures were carried out with the animals under anaesthesia maintained using a mixture of 2%–3% halothane, ~30% nitrous oxide and ~70% oxygen. Prior to the infusion of cisplatin each pig was hydrated via an ear vein with 2 l saline at a rate of 1.3 l/h. Cisplatin was then infused in 1 l saline to which an anti-emetic agent (Maxolon, 1.5 mg/kg body weight) had been added. Additional saline (1 l) was subsequently infused.

Prior to and approximately 4 weeks after cisplatin administration, individual kidney GFR and effective renal plasma flow (ERPF) were assessed by renography using [^{99} mTc]-DTPA (diethylenetriamminepentaaceticacid) and iodohippurate sodium I 131, respectively. Three matched NaI crystal scintillation detectors (diameter, 5 cm) were used; one was positioned over each kidney and the other, over the heart. The detectors were connected to a multichannel analyser (Nuclear Data ND 66) with windows set to record activity in the photopeak regions for technetium Tc 99 m and iodine 131. The analyser was operated in the multiscaling mode, with counts for each tracer from the three detectors being integrated over 20-s intervals for the duration of each study.

The tracers used were human serum albumin (HSA) labelled with technetium Tc 99 m (BYK-Mallinckrodt) and with iodine 131 (CIS, UK), [^{99} mTc]-DTPA (BYK-Mallinckrodt) and iodohippurate sodium I 131 (Amersham International). The studies were carried out using the following standard protocol:

1. HSA tagged with technetium Tc 99 m (250–500 μCi, depending on the size of the pig) was injected into an ear vein via an indwelling butterfly cannula. A 5-min interval enabled the tracer to equilibrate within the blood; radioactivity was then recorded for 2 min over each detector. This data was used to determine the radiolabel subtraction factor for each kidney [28].

2. HSA labelled with iodine 131 (25–40 μCi) was then injected into the same ear vein. After 5 min, radioactivity was recorded over each detector for 2 min to determine the radiolabel subtraction factor. A blood sample (2 ml) was then drawn from the contralateral ear vein via an indwelling butterfly cannula.

3. A solution containing [^{99} mTc]-DTPA (7–10 mCi) and iodohippurate sodium I 131 (300 μCi) was injected via an ear vein and the time-related changes in radioactivity over the kidneys and the heart were recorded for up to 40 min).

4. Blood samples were taken at 5-, 10-, 15-, 30-, 40-, 60- and 120-min intervals after injection of the isotope cocktail. Aliquots (1 ml) of plasma were counted in a well-type NaI detector connected to a multichannel analyser (Nokia, LP 4840). The activity of both nuclides was determined and the GFR and ERPF were estimated from the plasma disappearance curves of [^{99} mTc]-DTPA and iodohippurate sodium I 131, respectively [27].

The relative contribution of each kidney to the total function was assessed by determining the uptake function of each tracer in each kidney [28]. Measurements of individual kidney GFR and ERPF values were also carried out in four age-matched control animals.

At 4 weeks after cisplatin infusion, the left kidney of each treated animal as well as that of the four age-matched, untreated control pigs was removed surgically. After UN, each pig received analgesics (1 mg Temgesic; Reckitt and Coleman) intramuscularly twice daily for 4 days. All animals recovered well from the surgical procedure. GFR and ERPF were subsequently assessed in the remaining kidney at 4 week intervals for periods of up to 24 weeks after UN; the animals were then killed. The surgically removed left kidney and the right kidney, which was removed at post-mortem examination, were fixed in a solution of 1% acetic acid in 10% formol saline. Several renal tissue samples were then dehydrated and embedded in paraffin wax. A histological evaluation was carried out on 5 μm-thick sections stained with either periodic acid-Schiff's reagent (PAS) or periodic acid-methenamine silver.

Results

Early haemodynamic changes

A comparison of individual kidney GFR and ERPF values measured 4 weeks after cisplatin administration with their respective pre-treatment values revealed considerable variability in terms of response, such that no consistent or dose-related change in renal haemodynamics was evident.

Effect of UN on renal haemodynamics

At 4 weeks after the administration of cisplatin, the left kidney of each pig was surgically removed, and GFR and ERPF values in the remaining kidney were determined sequentially for periods of up to 24 weeks after UN. The left kidney was also removed in four age-matched control

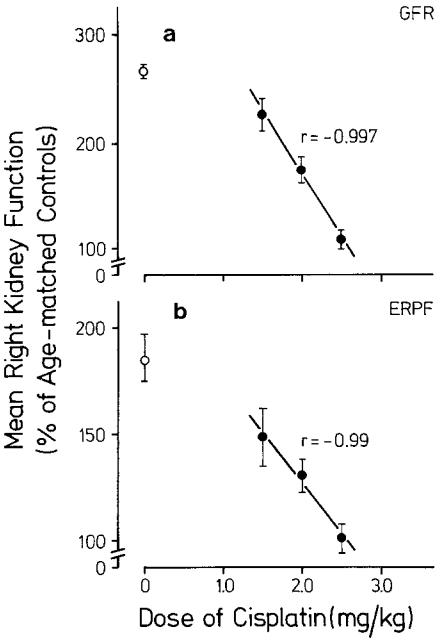

Fig. 2 a, b. Dose-related reductions in the mean **a** GFR and **b** ERPF assessed over the period 4–24 weeks after UN. Animals received a single dose of cisplatin 4 weeks prior to UN. *Error bars*, ±SE

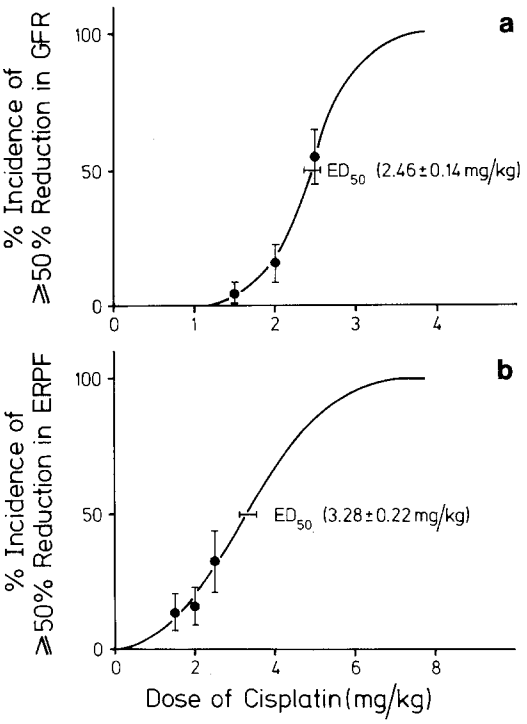

Fig. 3 a, b. Dose-related changes in the percentage of kidneys exhibiting a ≧50% reduction in **a** GFR and **b** ERPF over the period 4–24 weeks after UN. Animals received single doses of cisplatin 4 weeks prior to UN. *Error bars*, ±SE

animals. The time-related changes in the GFR and ERPF of these animals are shown in Fig. 1. Within 4 weeks of UN, the GFR in the remaining kidney of the age-matched control animals had increased markedly, reaching levels approximately 2.5 times greater than those found in individual kidneys of intact, age-matched control pigs. The GFR remained at this value throughout the period of investigation (Fig. 1 a).

A similar pattern of change was evident in the remaining kidney of pigs that had previously been treated with 1.5 mg/kg cisplatin. There appeared to be an increase in the GFR of these animals at between 20 and 24 weeks after cisplatin treatment. However, this increase was not statistically significant ($P >> 0.10$). Pigs previously treated with higher doses of cisplatin exhibited a dose-related reduction in the extent of the compensatory increase in GFR following UN. In pigs treated with 2 mg/kg cisplatin, the GFR increased within 4–8 weeks of UN to values 1.8 times those observed in individual kidneys of intact, age-matched control pigs. This increase, however, was not statistically significant as compared with the pre-UN values for this group of animals. By 24 weeks after UN the GFR appeared to be declining, although values did not significantly decrease below those seen at between 4 and 20 weeks after UN ($P >> 0.10$). In pigs treated with 2.5 mg/kg cisplatin, there appeared to be a slight increase in GFR following UN, but this was not statistically significant; GFR values in these kidneys were only marginally greater than those found in individual control kidneys.

The changes in ERPF following UN in the age-matched animals were qualitatively similar to those seen in GFR, although the absolute increase was smaller (Fig. 1 b). Within 4 weeks of UN, the ERPF in the remaining kidney had increased to a level nearly 1.8 times greater than that seen in individual kidneys of intact, age-matched controls. At between 8 and 16 weeks after UN, the ERPF values remained close to these levels; this was followed by a further increase at between 16 and 24 weeks after UN, when the mean ERPF was ~2.3 times greater than that observed in individual age-matched control kidneys. As shown for GFR, prior infusion with cisplatin produced a dose-related reduction in the magnitude of the increase in ERPF following UN, although the response was more variable.

It is noteworthy that increasing cisplatin dose was associated with an increase in the time required post-UN for ERPF values in the remaining kidney to increase above those measured in individual age-matched control kidneys. Thus, after doses of 1.5, 2 and 2.5 mg/kg cisplatin, the respective periods were 8, 12 and 20 weeks post-UN. The increase in ERPF seen in pigs infused with 1.5 mg/kg cisplatin was similar to that seen in the remaining kidney of age-matched control pigs after UN. However, at doses of ≧2 mg/kg cisplatin the increase in ERPF in the remaining kidney was less than that seen in controls after UN. Indeed, in pigs receiving 2.5 mg/kg cisplatin, ERPF values were not significantly different from pre-UN values until 16–20 weeks after UN, and they remained well below those seen in both the other cisplatin-treated animals and the age-matched control pigs that underwent UN.

The dose dependence of these cisplatin-induced reductions in the compensatory increase in renal haemodynamics following UN was demonstrated by examining the mean values measured for both GFR and ERPF, at 4–24 weeks post-UN expressed relative to those found for individual kidneys of intact, age-matched controls (Fig. 2). Values for both mean GFR and mean ERPF were well correlated with the cisplatin dose ($r = -0.99$, $P < 0.001$).

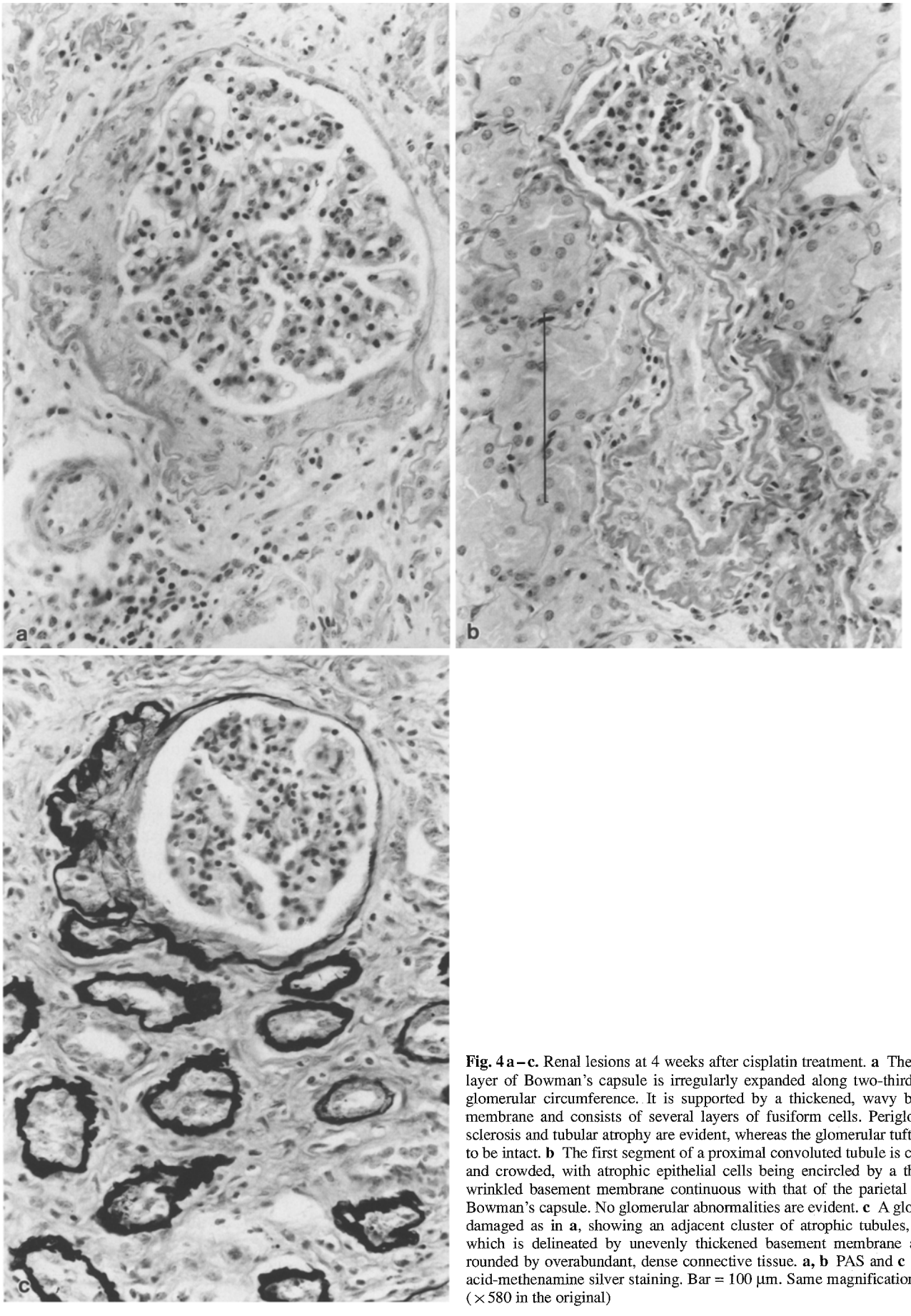

Fig. 4 a–c. Renal lesions at 4 weeks after cisplatin treatment. **a** The parietal layer of Bowman's capsule is irregularly expanded along two-thirds of the glomerular circumference. It is supported by a thickened, wavy basement membrane and consists of several layers of fusiform cells. Periglomerular sclerosis and tubular atrophy are evident, whereas the glomerular tuft appears to be intact. **b** The first segment of a proximal convoluted tubule is collapsed and crowded, with atrophic epithelial cells being encircled by a thick and wrinkled basement membrane continuous with that of the parietal layer of Bowman's capsule. No glomerular abnormalities are evident. **c** A glomerulus damaged as in **a**, showing an adjacent cluster of atrophic tubules, each of which is delineated by unevenly thickened basement membrane and surrounded by overabundant, dense connective tissue. **a, b** PAS and **c** periodic acid-methenamine silver staining. Bar = 100 μm. Same magnification in **a–c** (×580 in the original)

Dose-effect curves were also constructed by assessing, at each dose level, the percentage of renal function tests at 4–24 weeks after UN in which the remaining kidney exhibited a $\geqq 50\%$ reduction in GFR and ERPF as compared with values seen in individual kidneys of age-matched controls. The dose-response curves for the impairment in GFR and ERPF were fitted by probit analysis [12]. All data related to the reduction in ERPF were below the ED_{50} value. For fitting of a dose-effect curve, it was assumed that the curve for ERPF was parallel with that for GFR. The data are illustrated in Fig. 3. The doses associated with the 50% level of effect (i.e. the ED_{50} values) were 2.46 ± 0.14 and 3.28 ± 0.22 mg/kg for GFR and ERPF, respectively. These ED_{50} values were significantly different, indicating that measurements of GFR were a more sensitive indicator of cisplatin-induced damage.

Morphological findings at 4 weeks after cisplatin infusion

At cisplatin doses of 2 and 2.5 mg/kg, the surgically removed kidneys showed both glomerular and tubular lesions (Fig. 4). In the affected glomeruli, the tufts appeared to be largely intact, whereas the normally inconspicuous cells of Bowman's capsule were focally replaced by multilayered cell deposits. The abnormal cells had ellipsoidal, pale-staining nuclei and poorly defined cell outlines and were interspersed with fine PAS-positive fibres. The cells tended to localise towards the tubular pole of the glomerulus and only seldom occupied the entire surface of the capsule. Bowman's space was preserved in most of the affected glomeruli, with adhesions of the glomerular capillary loops being rare. The basement membrane encircling these new formations was thickened, wrinkled and often bulging. In pigs treated with 2.5 mg/kg cisplatin, these lesions affected 13% of glomeruli; they occurred less frequently in animals receiving 2 mg/kg and were only occasionally seen in those treated with 1.5 mg/kg.

In the cortex, there were also clusters of tubules that were markedly reduced in size, often consisting of solid groups of small epithelial cells surrounded by a thick and folded basement membrane. These atrophic tubules were separated by excessive amounts of intervening, dense connective tissue that was variably infiltrated by mononuclear cells, including plasma cells. The tubular lesions could be demonstrated to include the initial portion of the proximal convolution (Fig. 4b). Both the glomerular and the tubular lesions were more widespread and severe in the deep cortex than in the subcapsular region.

The long loops of Henle were also affected, as judged by the thickening and wrinkling of the basement membrane of some small and thin-walled tubules in the inner medulla. These changes were accompanied by focal increases in density and a chronic inflammatory infiltration of the medullary stroma.

Morphological findings at 24 weeks after UN

All of the single kidneys left in situ showed glomerular enlargement and a uniform dilatation of Bowman's spaces and tubular lumina. In addition, the kidneys of pigs that had received 2 or 2.5 mg/kg cisplatin 28 weeks previously showed focal glomerular and tubular lesions (Fig. 5). The spectrum of glomerular changes ranged from segmental sclerosis of the tufts, through shrinkage with widespread hyalinisation, to total replacement by solid, amorphous hyaline deposits in which a few spindle-shaped nuclei were occasionally embedded. The more advanced glomerular lesions were associated with periglomerular fibrosis and usually scanty chronic inflammatory infiltration. In pigs treated with 2.5 mg/kg cisplatin, these changes affected between 6% and 27% of glomeruli. In animals receiving 2 mg/kg, the frequency of these lesions tended to be lower, and only occasionally were lesions of this type found in those treated with 1.5 mg/kg cisplatin. The renal cortex of pigs exhibiting these lesions showed irregular scarring, with the dense connective tissue incorporating scattered atrophic tubules that were either encircled by a thin basement membrane or apparently lacked one. Mononuclear cell infiltration was usually sparse. The juxtamedullary layer of the cortex tended to be more severely affected than the superficial regions. The medullary stroma showed less well-defined areas of expansion and an increase in density, with a variable admixture of lymphocytes and occasional tubular atrophy.

Discussion

The present results indicate that pigs given a range of single doses of cisplatin exhibit no consistent change in either GFR or ERPF at 4 weeks after drug administration. In contrast, the surgical removal of the left kidney produced a pronounced dose-dependent reduction in the compensatory functional response of the remaining kidney. Thus, treatment with cisplatin may produce a permanent reduction in renal functional reserve that may be clinically "silent" until the instigation of a second nephrotoxic insult.

Earlier investigations of the nephrotoxicity associated with cisplatin appeared to indicate that the drug produces an acute but reversible reduction in renal function [18]. Maximal impairment was observed within several days of drug administration, followed by apparently full recovery. Hayes et al. [18] reported that patients treated with a single dose of cisplatin (3–5 mg/kg) showed a transient increase in serum creatinine levels for up to approximately 7 days after infusion; this was followed by a return to pre-treatment levels. Similar findings have been reported in dogs [7] and rats [4, 37]. These early studies, however, were limited because the investigators used relatively insensitive parameters such as serum creatinine and blood urea nitrogen to measure decrements in renal function [22].

More extensive studies using radionuclide techniques in rodents to measure GFR and ERPF have confirmed that cisplatin indeed produces an acute reduction in renal haemodynamics [20, 33]. These results have also suggested that cisplatin causes a permanent reduction in GFR [10, 20, 33]. Similar findings have been reported clinically [9, 13, 17]; indeed, in some cases the reduction in GFR appears to have been progressive [16, 19].

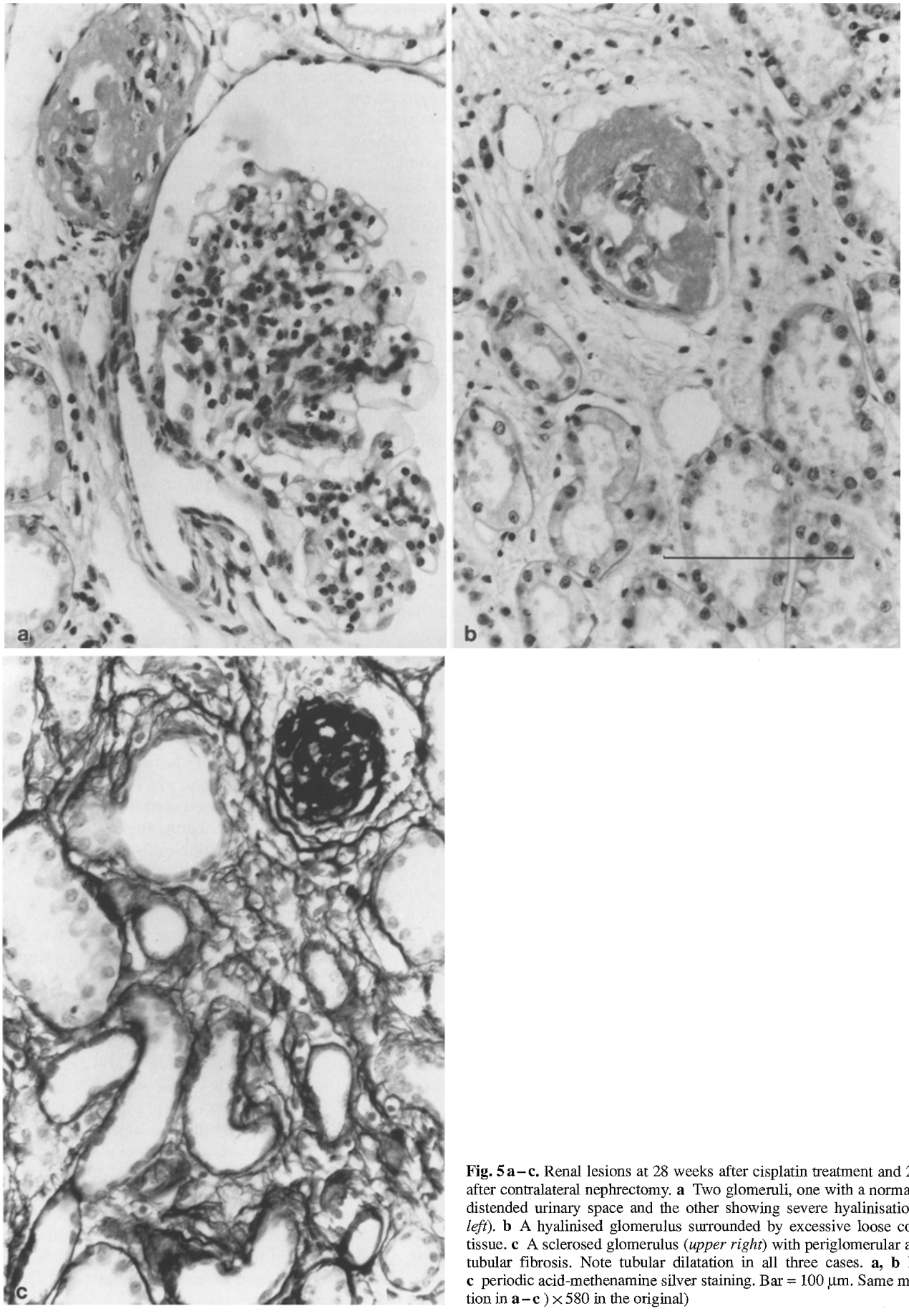

Fig. 5 a–c. Renal lesions at 28 weeks after cisplatin treatment and 24 weeks after contralateral nephrectomy. **a** Two glomeruli, one with a normal tuft but distended urinary space and the other showing severe hyalinisation (*upper left*). **b** A hyalinised glomerulus surrounded by excessive loose connective tissue. **c** A sclerosed glomerulus (*upper right*) with periglomerular and intertubular fibrosis. Note tubular dilatation in all three cases. **a, b** PAS and **c** periodic acid-methenamine silver staining. Bar = 100 μm. Same magnification in **a–c**) × 580 in the original)

The pathophysiology of this reduction in GFR remains ill-defined. It is well established that cisplatin produces an acute, mainly proximal, tubular functional impairment within hours of its administration [7]. Groth et al. [16] attributed the chronic reduction in GFR observed in patients following cisplatin treatment to increased hydraulic pressure within the damaged tubules.

Histological studies in the rat have shown that cisplatin-induced damage is largely confined to the proximal convoluted tubules, in particular the S_3 segment located in the outer stripe of the outer medulla [1, 4]. In contrast, clinical observations showed that the distal tubules and collecting ducts were more affected than the proximal convolutions [9, 15]. More recently, a histopathological study of kidneys from patients who died 3–60 days after the completion of cisplatin treatment was reported [34]. The results revealed sporadic lesions of degeneration, necrosis and regenerative changes in the pars convoluta and pars recta of the proximal tubule, distal tubule and the collecting duct. In all of these cases the glomeruli did not appear to be involved. These findings would seem to indicate that the reduction in GFR results from the primary action of cisplatin on the renal tubules.

In contrast to these studies, the present findings indicated pronounced glomerular changes following cisplatin infusion. These lesions consisted of cell proliferation on the parietal surface of the urinary space. In addition, the tubules were damaged, including the S_1 segment of the proximal convoluted tubule. It is not known to what extent these structural lesions were responsible for the small reductions in GFR observed 4 weeks after cisplatin administration. However, the recent findings of Daugaard et al. [8] in cisplatin-treated patients, all of whom developed proteinuria, are noteworthy. During cisplatin infusion, low-molecular-weight proteins such as beta-2-microglobulin were excreted, indicating tubular damage. After cisplatin treatment, however, increased excretion of high-molecular-weight proteins (albumin and IgG) was seen, demonstrating glomerular damage. The present findings of glomerular lesions 4 weeks after cisplatin infusion may explain the reduction in GFR observed after treatment with this drug.

Although it is unlikely that the initial reduction in renal function observed clinically is by itself life-threatening, the apparently progressive nature of this damage would suggest that further treatment associated with nephrotoxicity could be counter-indicated [9, 13]. This possibility was investigated in the present study by a UN that was performed 4 weeks after cisplatin infusion. Although all remaining kidneys exhibited a compensatory increase in renal function after UN, this increase was reduced dose-dependently as compared with that seen in pigs that had not received cisplatin. Thus, the increase in GFR and ERPF observed after UN in animals initially treated with 2.5 mg/kg cisplatin was <50% of that seen in age-matched controls following UN. This dose-dependent reduction in renal functional reserve was noted even in animals that had "normal" levels of renal function prior to UN. This finding would indicate that the "normal" renal function seen after cisplatin administration reflects hyperfiltration by a reduced number of undamaged nephrons rather than a genuinely undamaged organ. Histological examination at 24 weeks after UN appeared to suggest that the glomerular lesions seen 4 weeks after cisplatin treatment were irreversible and had by then progressed to glomerular hyalinisation and glomerulosclerosis, with the preferential involvement of juxtamedullary nephrons.

Is it possible that this late glomerulosclerosis was a consequence of the UN performed 4 weeks after cisplatin treatment? Renal ablation produces progressive glomerulosclerosis in the rat, and recent findings indicate that this may also occur in man [21, 31]. A reduction in the total amount of renal tissue causes a compensatory hyperperfusion and hyperfiltration by the remaining nephrons that maintains the overall GFR close to normal levels. The increase in glomerular perfusion results in glomerular sclerosis, leading to a positive feedback stimulus for compensatory adaptation in less severely affected glomeruli, which, in turn, contributes to their eventual destruction [3]. In the pig, UN might result in a similar nephropathy. However, there was no evidence of glomerular damage in the kidneys of age-matched control pigs after UN. Moreover, different glomerular lesions had been seen in cisplatin-treated pigs at UN, at which time their incidence was nearly the same as that of subsequent glomerulosclerosis. It is not possible to conclude from the current findings that the chronic glomerular lesions seen 28 weeks after cisplatin infusion were a *direct* result of cisplatin treatment alone, since no data are available on the long-term effects of cisplatin in the otherwise untreated pig. Despite this reservation, however, the present results indicate that cisplatin played a major role in the development of glomerulosclerosis subsequent to UN, possibly through a direct mode of action.

The findings reported herein add weight to earlier warnings concerning the susceptibility of patients treated with cisplatin to further renal damage. Before such patients are subjected to additional nephrotoxic treatment, their renal functional reserve, defined as the increment in GFR induced by an adequate stimulus [14], should be accurately determined by the administration of an oral protein load [2] or vasodilatory drugs [35] to indicate the possible subsequent development of renal failure.

Acknowledgements. The authors are indebted to Mr. F. Dickinson and Mr. N. Hubbard for their expertise in pig anaesthesia and husbandry. The authors also thank Mr. F. Paice of the MRC Cyclotron Unit for his assistance with the microphotography.

References

1. Aggarwal SK, Whitehouse MW, Ramachandran C (1980) Ultrastructural effects of cisplatin. In: Prestayako AW, Cooke ST, Carter SK (eds) Cisplatin. Academic Press, New York, pp 79
2. Bosch JP, Lew S, Glabman S, Lauer A (1986) Renal haemodynamic changes in humans. Response to protein loading in normal and diseased kidneys. Am J Med 81: 809
3. Brenner BM, Meyer TW, Hostetter TH (1982) Dietary protein intake and the progressive nature of kidney disease. N Engl J Med 307: 652
4. Chopra S, Kaufman JS, Jones TW, Hong WK, Gehr MK, Kamburger RJ, Flamenbaum W, Trump BF (1982) *cis*-Diamminedichloroplatinum-induced acute renal failure in the rat. Kidney Int 21: 54

5. Cupak M, Vorlicek J (1982) Isotope nephrography in the study of cisplatin nephrotoxicity in pigs. Vet Med (Praha) 27: 541
6. Cvitkovic E, Spaulding J, Bethune V, Martin J, Whitmore WF (1977) Improvement of *cis*-dichlorodiammineplatinum (NSC-119875): therapeutic index in an animal model. Cancer 39: 1357
7. Daugaard G, Abildgaard U, Holstein Rathlou NH, Leyssac PP, Amtorp O, Dikhoff T (1986) Acute effect of cisplatin on renal haemodynamics and tubular function in dog kidneys. Renal Physiol 9: 308
8. Daugaard G, Rossing N, Rorth M (1988) Effects of cisplatin on different measures of glomerular function in the human kidney, with special emphasis on high-dose treatment. Cancer Chemother Pharmacol 21: 163
9. Dentino M, Luft FC, Yum MW, Williams SD, Einhorn LH (1978) Long term effect of *cis*-diamminedichloride platinum (cDDP) on renal function and structure in man. Cancer 41: 1274
10. Dobyan DC (1985) Long-term consequences of *cis*-platinum-induced renal injury: a structural and functional study. Anat Rec 212: 239
11. Einhorn LH, Donohue J (1977) *cis*-Diamminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. Ann Intern Med 87: 293
12. Finney DJ (1964) Probit analysis, 2nd edn. Cambridge University Press, London
13. Fjeldborg P, Sorensen J, Helkjaer PE (1986) The long-term effect of cisplatin on renal function. Cancer 58: 2214
14. Friedlander G, Blanchet F, Amiel C (1989) Renal functional reserve. Toxicol Lett 46: 227
15. Gonzalez-Vitale JC, Hayes DM, Cvitkovic E, Sternberg S (1977) The renal pathology in clinical trials of *cis*-platinum(II) diamminedichloride. Cancer 39: 1362
16. Groth S, Wielsen H, Sorensen JB, Christensen AB, Pedersen AG, Rorth M (1986) Acute and long-term nephrotoxicity of cisplatin in man. Cancer Chemother Pharmacol 17: 191
17. Hamilton CR, Bliss JM, Horwich A (1989) The late effects of *cis*-platinum on renal function. Eur J Cancer Clin Oncol 25: 185
18. Hayes DM, Cvitkovic E, Golberg RB, Scheiner E, Helson L, Krakoff IH (1977) High dose *cis*-platinum diammine chloride. Cancer 39: 1372
19. Jaffe N, Keifer R, Robertson R, Cangir A, Wang A (1987) Renal toxicity with cumulative doses of *cis*-diamminedichloroplatinum-II in pediatric patients with osteosarcoma. Cancer 59: 1577
20. Jongejan HTM, Provoost AP, Woolf ED, Molenaar JC (1986) Nephrotoxicity of cisplatin comparing young and adult rats. Pediatr Res 20: 9
21. Kiprov DD, Colvin RB, McCluskey RT (1982) Focal and segmental glomerulosclerosis and proteinuria associated with unilateral renal agenesis. Lab Invest 46: 275
22. Levey AS, Perrone RD, Madias NE (1982) Serum creatinine and renal function. Ann Rev Med 39: 465
23. Livio M, Chiabrando C, Macconi D, Benigni A, Zimei M, Pietro MT de, Remuzzi G (1988) Metabolism of arachodonic acid in isolated glomeruli from pig kidney. Biochim Biophys Acta 961: 110
24. Madias NE, Harrington JT (1978) Platinum nephrotoxicity. Am J Med 68: 307
25. Neuhybel P, Beranek L, Zert Z, Vorlicek J, Kelinwachter V, Kiss F (1982) Testing the toxicity of *cis*-diamminedichloroplatinum complex in pigs in view of pathomorphology. Vet Med (Praha) 27: 275
26. Ozols RF, Coden BJ, Jacob J, Wesley MN, Ostchaga Y, Young RC (1984) High dose cisplatin in hypertonic saline. Ann Intern Med 100: 19
27. Robbins MEC (1984) Single injection techniques in determining age-related changes in porcine renal function. Int J Appl Radiat Isot 35: 85
28. Robbins MEC, Soper M, Gunn Y (1984) Techniques for the quantitative measurement of individual kidney function in the pig. Int J Appl Radiat Isot 35: 853
29. Robbins MEC, Robinson M, Rezvani M, Golding SJ, Hopewell JW (1988) The response of the pig kidney to the combined effects of cisplatin and unilateral renal irradiation. Radiother Oncol 11: 271
30. Rozencweig M, Von Hoff DD, Slavik M, Muggia FM (1977) *cis*-Diamminedichloroplatinum(II): a new anticancer drug. Ann Intern Med 86: 803
31. Solomon LR, Mallick NP, Lawler W (1985) Progressive renal failure in a remnant kidney. Br Med J 291: 1610
32. Sperber I (1944) Studies on the mammalian kidney. Zool Bidr Fr 22: 249
33. Stewart F, Bohlken S, Begg A, Bartelink H (1986) Renal damage in mice after treatment with cisplatinum alone or in combination with X-irradiation. Int J Radiat Oncol Biol Phys 12: 927
34. Tanaka H, Ishikawa W, Teshima S, Shimuzi E (1986) Histopathological study of human cisplatin nephropathy. Toxicol Pathol 14: 247
35. Ter Wee PM, Geerlings W, Rosman JB, Sluiter WJ, Geest S van der, Donker AJM (1985) Testing renal reserve filtration capacity with an amino acid solution. Nephron 41: 193
36. Terris JM (1986) Swine as a model in renal physiology and nephrology: an overview. In: Tumbleson ME (ed) Swine in biomedical research. Plenum Press, New York, p 1673
37. Ward JM, Fauvie KA (1976) The nephrotoxic effects of *cis*-diamminedichloroplatinum(II) (NSC-119875) in male F344 rats. Toxicol Appl Pharmacol 38: 535
38. Wiltshaw E, Kroner T (1976) Phase II study of *cis*-dichlorodiammineplatinum(II) (NSC-119875) in advanced adenocarcinoma of the ovary. Cancer Treat Rep 60: 55