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Ultrastructural Alterations and DNA Synthesis of Renal Cell Nuclei following Cisplatin or Carboplatin Injection in Rats

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Abstract—To clarify the difference in nephrotoxicity between cisplatin and carboplatin, ultrastructural alterations and DNA synthesis of renal cell nuclei were studied in Sprague-Dawley rats which had received intravenously either cisplatin or carboplatin at an equitoxic dose. Twelve hours after cisplatin injection, nucleolar segregation accompanied by aggregated nuclear heterochromatin was observed in the third segment of the proximal tubules. Seventy-two hours after cisplatin injection, nuclear damage was more widespread while regenerative cells were also observed. Nuclear damage was not observed in the carboplatin-treated rats. Nuclear DNA synthesis of renal cells was suppressed at 8, 12 and 24 h and was accelerated at 72 h after cisplatin injection. Carboplatin did not suppress nuclear DNA synthesis at any time. The results indicate that cisplatin, but not carboplatin, can affect the renal cell nuclei. Cisplatin-induced nephrotoxicity is related to its effects on renal cell nuclei.

Cisplatin is an effective antitumour agent which is widely used in anticancer regimens. However, the major dose-limiting toxicity is renal tubular damage resulting in renal insufficiency or acute renal failure (Chopra et al 1982; Weiner & Jacobs 1983; Jones et al 1985). Numerous analogues have been synthesized in a search for alternative active compounds with reduced toxicity (Daley-Yates & McBrien 1985; Boven et al 1985; Smith & Brock 1988). Carboplatin (*cis*-diammine-1, 1-cyclobutane dicarboxylato platinum II) is a new platinum-containing analogue which has good antitumour activity and reduced nephrotoxicity (Calvert et al 1982; Curt et al 1983; Daley-Yates & McBrien 1985; Boven et al 1985; Smith & Brock 1988). Cisplatin-induced acute renal failure has been extensively studied. Histological alterations are localized to the third segment (S_3) of the proximal tubules in the outer stripe of the outer medulla (Chopra et al 1982; Weiner & Jacobs 1983; Jones et al 1985). The early changes in the S_3 portion are characterized by nucleolar segregation and ribosome dispersion (Jones et al 1985). Moreover, it is generally accepted that nuclear DNA is the target responsible for the cytotoxic action of platinum compounds (Calvert et al 1982; Roberts et al 1986; Hanušovská & Ujházy 1987).

Based on these previous reports, the present study was performed to determine the differences in the effect on renal cell nuclei between cisplatin and carboplatin.

Materials and Methods

Drugs and animals

Cisplatin (Nippon Kayaku, Tokyo, Japan) was freshly dissolved in saline and carboplatin (Bristol Myers Japan, Tokyo, Japan) was dissolved in 5% glucose before use. Male Sprague-Dawley rats, 200–300 g, were housed with free access to water and rat chow throughout the experiment.

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Morphological studies

The rats received cisplatin intravenously at a dose of 8·5 mg kg^{-1} or carboplatin at a dose of 100 mg kg^{-1} under light ether anaesthesia. These doses were approximately equal to the LD50 determined at Nippon Kayaku Laboratory and Bristol Myers Laboratory. In these toxicity studies, animals were observed up to 14 days following the dose; deaths occurred 5–7 days after cisplatin and 5–8 days after carboplatin. The LD50 was considered the maximum dose that could be used in our studies. Control animals were injected with 2 mL 0·9% NaCl (saline). The treated rats were killed at 12, 24 and 72 h after the injection. The kidneys were removed after vascular perfusion with 2% formaldehyde, 1% glutaraldehyde and were placed in fixative until further processing for electron microscopy. Electron microscopic observations were performed to evaluate the cell damage in the proximal tubules.

Effects on nuclear DNA synthesis

The effects of cisplatin and carboplatin on nuclear DNA synthesis of renal cells were studied according to the method of Lynch et al (1970) as follows. The rats (n = 5) were intravenously injected with cisplatin at a dose of 8·5 mg kg^{-1} or carboplatin at a dose of 100 mg kg^{-1}, and were then killed at 8, 12, 24 or 72 h after the injection in order to prepare the fraction of the renal nuclei. Both kidneys were removed after perfusion with a buffer solution containing 0·34 M sucrose, 3·3 mM $CaCl_2$ and 10 mM Tris (pH 7·4). The renal cortex and outer medullary tissues were separated macroscopically and were immediately homogenized with a Waring blender and a loose Dounce homogenizer containing 9 parts vol of buffer solution (described above) at 0°C. The homogenate of the renal tissue was passed through four sheets of gauze, and the filtrate was centrifuged at 700 *g* for 10 min. The pellet

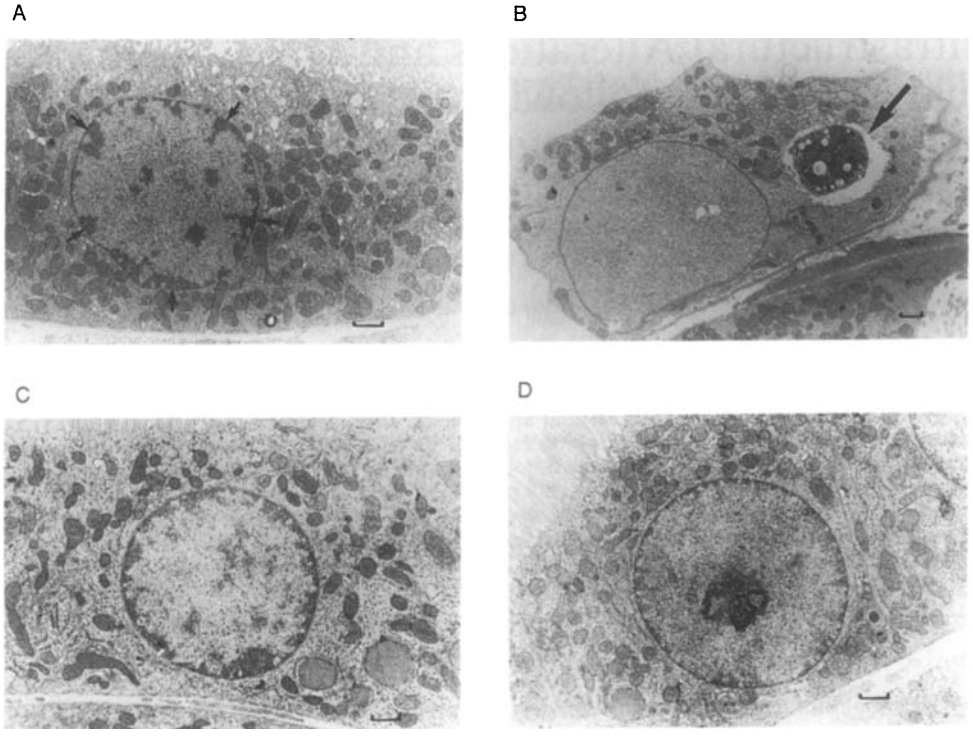

FIG. 1. A. Electron micrograph of an S_3 cell, 12 h after cisplatin injection (8·5 mg kg^{-1}). The aggregated heterochromatin (arrow) is prominent. B. Electron micrograph of an S_3 cell, 72 h after cisplatin injection (8·5 mg kg^{-1}). Clear nucleus and autophagocytic vacuole (arrow) can be observed in the regenerative cell. C. Electron micrograph of an S_3 cell in the control rat. D. Electron micrograph of an S_3 cell, 72 h after carboplatin injection at a dose of 100 mg kg^{-1}. Nuclear damage cannot be observed. Bar indicates 1 μm × 10 000.

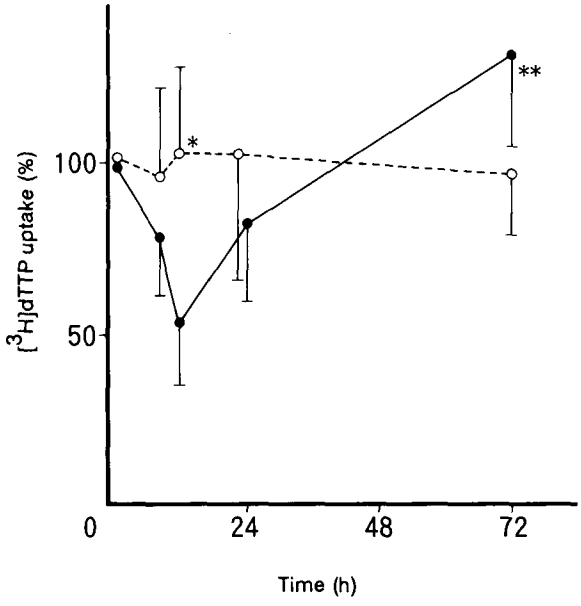

FIG. 2. [^{3}H]dTTP uptake in isolated renal nuclei after either cisplatin (● 8·5 mg kg^{-1}, n = 5) or carboplatin (○ 100 mg kg^{-1}, n = 5) injection. Data are expressed as mean ± s.d. Asterisks indicate significant differences between cisplatin and carboplatin with $^*P < 0.05$ and $^{**}P < 0.01$.

containing renal nuclei was resuspended in 20 mL of 2 M sucrose, 1 mM $CaCl_2$ and 10 mM Tris buffer (pH 7·4). The suspension was laid over 20 mL of 2·2 M sucrose, 10 mM $MgCl_2$ and 10 mM Tris buffer (pH 7·4) and was then centrifuged at 68 420 *g* for 1 h. Finally, the nuclear fraction was suspended in 1 mL of 0·3 M sucrose (pH 7·4). Isolated nuclei were incubated with 0·016 mM [^{3}H]dTTP ([^{3}H]deoxythymidine triphosphate) in reaction mixtures (0·5 mL) containing 0·1 M Tris-HCl (pH 7·4), 4 mM KCl, 16 mM $MgCl_2$, 4 mM 2-mercaptoethanol, 2 mM ATP, 0·08 mM dGTP, 0·08 mM dCTP and 0·08 mM dATP at 37°C for 30 min. Reactions were halted with 1 mL of 1 M NaOH and nuclear DNA was precipitated with 5 mL of ice-cold 10% trichloroacetic acid. The DNA was then washed three times in 1 mL of 1 M NaOH. The radioactivity was measured in the liquid scintillation mixture to determine [^{3}H]dTTP uptake by nuclei. The DNA content in the nuclei was measured by diphenylamine according to the method of Bruton (1955). The results were expressed as a percent of [^{3}H]dTTP uptake by control nuclei obtained from untreated rats.

Statistical differences were evaluated by Student's *t*-test.

Results

Morphological studies

The characteristic ultrastructural change was nucleolar segregation accompanied by aggregated nuclear heterochromatin in the S_3 cells, 12 h after cisplatin injection (Fig. 1A). Twenty-four hours after cisplatin injection, nucleolar segregation and aggregated heterochromatin were more prominent throughout S_3 cells and dispersed cytoplasmic polysome and large aggregates of smooth endoplasmic reticulum were observed. Seventy-two hours after cisplatin injection, tubu-

lar necroses involving the S_3 portion in the outer strip of the outer medulla were more widespread. Regenerative cells which had a clear nucleus and autophagocytic vacuole were also observed (Fig. 1B). On the other hand, morphology of S_3 cells at 12, 24 and 72 h after carboplatin injection was the same as the control group (Fig. 1C, D). Morphological damage of nuclei was not observed in the carboplatin-treated rats at any time after the injection.

Effects on nuclear DNA synthesis

The [^{3}H]dTTP uptake by nuclei isolated from cisplatin treated rats at 8, 12, 24 and 72 h after the injection were $76{\cdot}1 \pm 22{\cdot}1$, $52{\cdot}7 \pm 23{\cdot}5$, $84{\cdot}4 \pm 28{\cdot}4$ and $142{\cdot}5 \pm 27{\cdot}1$% of the [^{3}H]dTTP uptake by control nuclei, respectively. Nuclear DNA synthesis of renal cells was suppressed markedly at 12 h but was accelerated at 72 h after cisplatin injection. The [^{3}H]dTTP uptake by nuclei isolated from carboplatin treated rats at 8, 12, 24 and 72 h after the injection were $88{\cdot}6 \pm 30{\cdot}8$, $104{\cdot}6 \pm 26{\cdot}8$, $102{\cdot}0 \pm 40{\cdot}7$ and $92{\cdot}8 \pm 15{\cdot}8$% of the [^{3}H]dTTP uptake by control nuclei, respectively. Carboplatin did not inhibit nuclear DNA synthesis after the injection. There were significant differences in DNA synthesis between cisplatin and carboplatin at 12 and 72 h after the injection. These results are shown in Fig. 2.

Discussion

Cisplatin produces severe nephrotoxicity (Chopra et al 1982; Weiner & Jacobs 1983; Jones et al 1985) but carboplatin produces little or no kidney damage (Calvert et al 1982; Curt et al 1983; Siddik et al 1986). It is generally accepted that DNA is the target responsible for the cytotoxic and antitumour action of platinum compounds (Roberts et al 1986; Hanušovská & Ujházy 1987). Whether this possible mechanism of antitumour activity is associated with nephrotoxicity is not known. There is little information concerning the in-vivo effects of cisplatin and carboplatin on the nuclear function of renal cells. To clarify the differential nephrotoxicity between cisplatin and carboplatin, we studied the effects of both drugs on renal cell nuclei.

In our study, the most common and characteristic ultrastructural change in S_3 cells 12 h after cisplatin injection was nucleolar segregation accompanied by aggregated heterochromatin, as previously reported (Chopra et al 1982; Jones et al 1985). However, carboplatin did not produce any morphological damage of nuclei at any time. Moreover, our study showed that nuclear DNA synthesis of renal cells was markedly suppressed 12 h after cisplatin injection, but was not suppressed after carboplatin injection. These early morphological and biochemical findings suggest that the nucleus is the first organella affected by cisplatin in the renal cells. However, it is unknown whether cisplatin affects a nucleus directly or indirectly. The change in structure and nuclear DNA syr .hesis may be epiphenomena of another mediating injurio ıs process induced by cisplatin. On the other hand, nuclear DNA synthesis was accelerated 72 h after cisplatin injection. This may have been due to the presence of the regeneration of renal tubular cells (Lynch et al 1970), and which were observed by electron microscopy 72 h after cisplatin injection. These results clearly indicate that the morphological alterations of nuclei correlate with the nuclear biochemical function after each drug injection.

In conclusion, cisplatin, but not carboplatin, can affect nuclei in the S_3 cells. The nephrotoxicity induced by cisplatin may be related to the effects on renal cell nuclei, but a cause and effect relationship has not been established.

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