ABSTRACT

The nephrotoxic potential of cis-diamminedichloroplatinum (II) (CDDP) in rabbits, as well as its effect on cell viability, cellular synthetic activity, and specific enzyme activities in rabbit renal proximal tubule cells, was investigated. Male New Zealand White rabbits were given a single i.v. dose of either 2.5 or 5.0 mg/kg CDDP via the ear vein and sacrificed 5 days later. No drug-induced changes were observed in the kidneys of rabbits given 2.5 mg/kg CDDP. However, histopathological examination of kidneys from rabbits administered 5.0 mg/kg CDDP revealed marked tubular degeneration and necrosis, with the majority of lesions being situated in the outer zone of the cortex. This is in contrast to the effect of CDDP in the kidney of the rat where the necrosis is reported to be predominantly localized to the pars recta of the proximal tubule in the outer stripe of the medulla. The results from the in vitro experiments indicated that the viability of cells after a 6-h exposure to CDDP at concentrations up to 100 μM was greater than 95%. However, a dose-dependent decrease in cell viability was obtained after 24 h exposure with a TD₅₀ (50% viability) of approximately 90 μM. In addition, the results after 24 h exposure to CDDP also indicated that Na⁺, K⁺-ATPase, a basolateral membrane marker enzyme, and alkaline phosphatase, a brush-border marker enzyme, were inhibited by 35–40% and 20%, respectively. No effect on succinic dehydrogenase, a mitochondrial marker enzyme, was obtained. Inhibition of all three marker enzymes was minimal at 6 h posttreatment. On the other hand, inhibition of DNA, RNA, and protein syntheses was evident as early as 6 h posttreatment with DNA (48–77%) and RNA (36–77%) syntheses being inhibited to a greater extent than protein synthesis (14–33%). These results demonstrate that inhibition of renal synthetic activity by CDDP, rather than its effect on enzyme activity, precedes the onset of cell lethality and may therefore be an important event in the initiation of CDDP-induced nephrotoxicity.

INTRODUCTION

CDDP, a platinum-containing coordination complex, is an effective antitumor agent utilized in the treatment of a wide variety of human malignancies (1). However, its usefulness in chemotherapy is compromised by its acute nephrotoxic potential which remains one of the most important dose-limiting side effects (2–5) of CDDP.

The mechanism of antitumor action of CDDP has been the focus of numerous studies (6–10). The antitumor activity of CDDP has been causally related to its interaction with DNA and to the inhibition of DNA replication through formation of inter- and intrastrand cross-links between platinum and the N² position of guanine (11–16). On the other hand, the mechanism of CDDP-induced nephrotoxicity still remains relatively unresolved (17). A number of in vitro studies have postulated that the nephrotoxicity of CDDP may be due to its ability to inactivate cellular enzymes (18–20), mitochondrial function (21, 22), and specific renal membrane transport processes (23–25) as well as its ability to affect cellular membranes through reactions with protein-bound sulfhydryl groups (26).

The purpose of these studies is to examine the mechanism of CDDP-induced nephrotoxicity using primary cultures of normal kidney epithelial cells and to determine whether cellular interactions by these cells with CDDP are unique in comparison to those reported for the other cell types (11–16). To accomplish this, we selected the rabbit as our experimental model system. The site of injury in the kidney following CDDP administration in the rabbit was determined prior to the in vitro studies since a review of the literature revealed that CDDP nephrotoxicity in rabbits has not been described. Subsequently, primary cultures of cells from the target area of the kidney were established in a hormonally defined serum-free medium as described by Chung et al. (27). These cells were utilized in our present studies to determine whether the toxicity of CDDP to normal kidney cells could be correlated with either the effect of CDDP on specific enzyme activities or to an inhibition of DNA, RNA, and protein synthesis.

MATERIALS AND METHODS

Chemicals. Both unformulated and formulated CDDP (Platinol), were obtained from the Product Development Division of Bristol-Myers Co., Syracuse, NY. [methyl-3H]Thymidine (50–80 Ci/mm) was purchased from New England Nuclear Research Products (Boston, MA) while 1-[4,5-3H]Uricine (1 Ci/mm) and [5-3H]Uridine (29 Ci/mm) were obtained from Amersham (Arlington Heights, IL). Medium 199, SFFD medium, phosphate-buffered saline, and soybean trypsin inhibitor were all obtained from Gibco (Grand Island, NY). Collagenase, Type IV, was purchased from Cooper Biomedical (Malvern, PA). Other supplements to the culture medium, insulin, human transferrin, hydrocortisone, and selenium were obtained from Sigma Chemical Co. (St. Louis, MO).

Nephrotoxicity Study of CDDP in Rabbits. Male New Zealand White rabbits (2–4 kg body weight), obtained from Hazelton Research Products (Denver, PA), were individually housed in stainless steel cages and received food and water ad libitum except as necessary to obtain BUN determinations. These rabbits were weighed and randomly divided into 3 treatment groups. Group 1 was the control group and received 0.9% Sodium Chloride for Injection (Travenol Laboratories, Deerfield, IL). Groups 2 and 3 received 2.5 or 5.0 mg/kg CDDP (Platinol), respectively, administered through the ear vein. Platinol was reconstituted in Sterile Water for Injection (Travenol Laboratories) at a concentration of 1.0 mg/ml. Blood samples were drawn for BUN determinations from each rabbit on Day 1 (prior to CDDP treatment) and 5 days posttreatment. BUN measurements were determined using a Centrifichem 600 Analyzer (Baker Instruments, Allentown, PA). All rabbits were killed 5 days after treatment. Both kidneys were removed and a representative section from each kidney was obtained and fixed in 10% neutral buffered formalin. After fixation, the sections were trimmed, embedded in paraffin, sectioned at 3–5 μm, and stained with hematoxylin and eosin and periodic acid-Schiff stain. The severity and extent of tubular necrosis and degeneration were determined by light microscopy and scored as follows: 0, absence of lesion; 1, lesion represented in fewer than 25% of the tubular sections of the cortex; 2, lesions
present in 26–50% of tubules; 3, lesions present in 51–75% of tubules; 4, lesions present in 76–100% of tubules.

Preparation of Kidney Proximal Tubule Cells. Primary cultures of rabbit kidney epithelial cells were established from proximal tubules, isolated according to the method of Chung et al. (27). Essentially, the kidney was removed from the rabbit with the renal artery and vein intact. Under sterile conditions, the renal artery was cannulated with a blunt tipped 19-gauge needle and the kidney perfused sequentially with sterile phosphate-buffered saline, medium 199, and medium 199 containing 0.5% (w/v) iron oxide, collagenase (0.25 mg/ml), and 0.0025% soybean trypsin inhibitor. During perfusion, the iron oxide is trapped by the glomeruli and is intended to facilitate the separation of glomeruli from tubules in a step described below. The kidney was decapsulated and sliced longitudinally and the cortical region was carefully dissected away from the medulla and the papillary region. The pieces of cortex were then minced and homogenized (3–4 strokes) in a loose-fitting Dounce glass-on-glass homogenizer. The resulting homogenate was poured over two sterile nylon screens with mesh sizes of 235 µm (top) and 85 µm (bottom), respectively. Tubular and glomerular structures which were retained on the bottom screen (85 µm) were collected and resuspended in 40 ml SFFD medium in a conical centrifuge tube containing a sterile magnetic stir bar. The purpose of the magnetic stir bar was to attract the iron oxide-containing glomeruli thus allowing the separation and purification of tubules from glomerular structures. This purification step was repeated 2–3 times with a new magnetic bar each time to provide a final cell preparation that is enriched with proximal tubular structures. The resulting cell preparation was then treated with collagenase (0.25 mg/ml) and 0.0025% soybean trypsin inhibitor for 2 min. These cells were then washed twice by centrifugation with SFFD medium. The cells were plated in T-25 flasks (Corning, NY) in serum-free SFFD medium supplemented with insulin (5 µg/ml), human transferrin (5 µg/ml), hydrocortisone (18 mg/ml), and sodium (0.173 µg/ml). These cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. The medium was changed at 24 h after plating and every 2–3 days thereafter.

Determination of Cell Viability. In vitro studies of CDDP were carried out with 6-day cell cultures. Previous studies on the growth of these cell cultures have shown that the logarithmic phase of growth is between 3 and 8 days with a doubling time of approximately 48 h (Fig. 1). Confluence occurred after 9–10 days in culture. For determination of cell viability, CDDP was dissolved at a concentration of 0.5 mg/ml in medium 199 and determined at 6 and 24 h posttreatment using the trypan blue dye exclusion method and expressed as the percentage of viable relative to controls (% of V.C.).

\[
\% \text{ of V.C.} = \frac{\text{number of live cells in test}}{\text{number of live cells in control}} \times 100
\]

Measurement of DNA, RNA, and Protein Synthesis. The effect of CDDP on cellular synthetic activity was determined in 6-day cultures of proximal tubule cells. DNA, RNA, and protein syntheses were measured by the incorporation of \[^{3}H\]thymidine (0.2 µCi/ml), \[^{3}H\]uridine (0.5 µCi/ml), and \[^{3}H\]leucine (0.5 µCi/ml), respectively. In these experiments, both radioactive precursor and CDDP were added to the cells at the beginning of the experiment. Incorporation of \(^{3}H\) label into a TCA-precipitable fraction was determined at various times up to 24 h. For cultures exposed to \[^{3}H\]thymidine and \[^{3}H\]uridine, a further 1-h postincubation with 1 mM cold thymidine or uridine was included. Cells were then washed twice with warm medium and lysed with 1% sodium dodecyl sulfate in 0.15 M sodium chloride-0.015 M sodium acetate buffer (pH 7.0). The cell lysates were then precipitated with ice-cold 10% TCA onto Whatman GF/C glass microfiber filters. After being washed with 70% ethanol, 100% ethanol, and acetone, the filters were dried and counted in 10 ml Instagel (Packard Instrument Co., Downers Grove, IL) with a Beckman 6800 liquid scintillation system.

Measurement of Cellular Enzyme Activity. The activities of AP, a brush border enzyme, SD, a mitochondrial enzyme, and ATPase, a basolateral enzyme, were monitored following exposure of proximal tubule cell cultures to CDDP. Cells were harvested using a rubber policeman into 400 µl of buffer (pH 7.0) consisting of 25 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid and 100 mM mannitol and lysed by sonication (Vibra-Cell; SMI, Danbury, CT). Undiluted cell lysates were used for assaying AP activity (28) and SD activity (29). ATPase activity was assayed (30) after dilution of cell lysates to a protein concentration of 1 mg/ml with 0.1% Tris-sodium deoxycholate followed by freeze-thawing twice in dry ice-methanol. Protein concentrations were obtained according to the method of Lowry et al. (31).

RESULTS

Nephrotoxicity Study of CDDP in Rabbits. The results of BUN determinations and microscopic renal examinations are summarized in Table 1. The mean BUN concentration of rabbits treated with 5.0 mg/kg of CDDP was 13 times greater than their mean predose BUN value and the mean BUN of the control rabbits. The mean BUN of the low-dose group, 2.5 mg/kg, was only slightly elevated above both predose and control group values, but was well within the normal range for male New Zealand White rabbits established in our laboratory.

Grossly, kidneys of rabbits administered 5.0 mg/kg of CDDP were tan to whitish tan and were slightly swollen. The kidneys of rabbits treated with 2.5 mg/kg of CDDP appeared normal. All 3 rabbits treated with a single dose of 5.0 mg/kg of CDDP exhibited drug-related moderate to marked diffuse acute renal tubular degeneration and necrosis (Fig. 2). Tubular degenera-

![Fig. 1. Growth curve of rabbit kidney proximal tubule cells in primary culture.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose of cisplatin (mg/kg)</th>
<th>BUN (mg/dl)</th>
<th>Histopathological nephrotoxicity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>16.3 ± 1.1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>16.7 ± 1.5</td>
<td>215.0 ± 2.3</td>
</tr>
<tr>
<td>3</td>
<td>5.0</td>
<td>16.3 ± 1.1</td>
<td>215.0 ± 72.7</td>
</tr>
</tbody>
</table>

TABLE 1 Summary of BUN values and microscopic examination results

- Three rabbits per treatment group.
- Platino! was prepared in Sterile Water for Injection at a concentration of 1.0 mg/ml and administered as a single i.v. dose through the ear vein. Control rabbits received 5.0 ml/kg of 0.9% sodium chloride solution.
- Mean ± SD.
- Student's t test was used to analyze the following comparisons and these differences were shown to be significant: Group 2 versus 1, P = 0.006; Group 3 versus 1, P = 0.009.
CDDP EFFECTS IN VIVO AND IN VITRO

Necrosis and necrosis were most severe in the outer zone of the cortex. Necrosis was most severe in the convoluted portion of proximal tubules, while in the straight portion of the proximal tubules necrosis was slight and degeneration was slight to mild. Necrotic tubules were nearly or totally devoid of epithelial cells and contained clumps of lytic, sloughed epithelial cells and masses of eosinophilic, granular, partially mineralized, necrotic cellular debris. In less severely affected tubules, degenerate proximal tubular epithelial cells were characterized by near total loss of brush borders and cell swelling with increased cytoplasmic granularity and pallor. Many proximal tubules, particularly the straight portions, were mildly dilated and filled with pink to deeply eosinophilic (hyaline) protein casts (Fig. 3). The epithelium of distal tubules (convoluted and straight portions) and collecting ducts appeared to have been spared the nephrotoxic effects of CDDP except for mild occasional dilatation and the presence of protein casts. Active regeneration, as indicated by an increase in mitotic activity in tubular epithelium, was not a feature of the renal lesion 5 days after CDDP treatment. No drug-induced glomerular changes were observed.

Of the 3 rabbits administered 2.5 mg/kg of CDDP, only 1 showed a very slight focal tubular degeneration and dilatation of a few straight proximal tubules (P3) in the outer zone of the medulla.

**In Vitro** Toxicity Studies. The viability of 6-day proximal tubule cell cultures exposed to CDDP is shown in Fig. 4. Even at 30 μg/ml (100 μM) CDDP, the results indicated that the percentage of viability after 6 h of treatment ranged from 95 to 100% of control cultures. On the other hand, after 24 h exposure to CDDP, a dose-dependent decrease in cell viability was obtained with a TD50 (50% viability) of approximately 28 μg/ml (90 μM).
The number of viable cells was determined by the trypan blue exclusion method using duplicate cell cultures which were simultaneously exposed to CDDP under identical conditions.

In another case (40). In the most recent description of human CDDP nephrotoxicity in 12 patients appropriate model for CDDP nephrotoxicity in man. Gonzalez-Vitale et al. (4) reported the site of nephrotoxicity in 12 patients to be in the distal tubule and collecting duct. Two other reports described the major sites of injury as the proximal and distal tubules in 3 cases (3) and in the proximal convoluted tubules in another case (40). In the most recent description of human CDDP nephrotoxicity (41), the pars convoluta and pars recta were used as early as 6 h posttreatment. The significance of this observation is that the effect of CDDP on cellular synthetic activity is an early event and occurred prior to any observed changes in trypan blue exclusion.

Since viability was also assayed at 6 and 24 h, the extent of 3H incorporation was further normalized with respect to the number of viable cells. The effect of CDDP after 6 and 24 h was then calculated as disintegrations per minute/10^6 viable cells and the inhibition of DNA, RNA, and protein synthesis at these time points expressed as percentage of inhibition of control values. These results are summarized in Table 2 and confirmed the data shown in Fig. 5 in that inhibition of DNA and RNA synthesis by CDDP at all dose levels was greater than its effect on protein synthesis. As expected, almost complete inhibition of DNA and RNA activity was obtained after 24 h exposure to CDDP. However, the CDDP inhibition of protein synthesis still remained less pronounced when compared to either DNA or RNA synthesis.

Table 3 summarizes the effect of CDDP on the activities of the 3 enzymes assayed after 6 or 24 h exposure. Unlike its effect on cellular synthetic activity, the results indicated that CDDP at 6 h posttreatment did not have any effect on the enzyme activities even at the highest dose used. After 24 h exposure, AP activity was inhibited by approximately 20% while ATPase activity was decreased by 35–40%. Inhibition of these 2 enzymes by CDDP was, however, not dose dependent and similar inhibitory levels were obtained with doses between 10 and 30 μg/ml.

**DISCUSSION**

In this paper we examined by light microscopy kidneys of rabbits which were administered a nephrotoxic dose of CDDP. This in vivo study was carried out because to our knowledge, the morphology of CDDP-induced nephrotoxicity in rabbits has not been reported. Thus, light microscopic examination of kidneys from CDDP-treated rabbits showed that acute necrosis of proximal convoluted tubules was the primary lesion and that the primary site of this lesion was in the outer cortex (Fig. 2). This site differs from the primary site of CDDP nephrotoxicity in the rat. In the animal model in which CDDP nephrotoxicity has been most thoroughly characterized and in which most studies of the mechanism of nephrotoxicity have been conducted, tubular damage occurs primarily in the pars recta (P3) of the proximal tubule in the outer stripe of the medulla (32–37), and to a lesser extent, in the proximal convoluted tubule (18, 38, 39) and the distal tubule (32).

Morphological studies of CDDP nephrotoxicity in the human are few and at variance with one another, making it unclear as to whether or not the rabbit or, for that matter, the rat is an appropriate model for CDDP nephrotoxicity in man. Gonzalez-Vitale et al. (4) reported the site of nephrotoxicity in 12 patients to be in the distal tubule and collecting duct. Two other reports described the major sites of injury as the proximal and distal tubules in 3 cases (3) and in the proximal convoluted tubules in another case (40). In the most recent description of human CDDP nephrotoxicity (41), the pars convoluta and pars recta were used as early as 6 h posttreatment. The significance of this observation is that the effect of CDDP on cellular synthetic activity is an early event and occurred prior to any observed changes in trypan blue exclusion.

Table 2 Inhibition of cellular synthetic activity by cisplatin in rabbit kidney primary proximal tubule cultures

<table>
<thead>
<tr>
<th>Dose (μg/ml)</th>
<th>DNA synthesis</th>
<th>RNA synthesis</th>
<th>Protein synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>24 h</td>
<td>6 h</td>
</tr>
<tr>
<td>5</td>
<td>48.5 ± 2.1</td>
<td>88.3 ± 0.6</td>
<td>36.8 ± 0.8</td>
</tr>
<tr>
<td>10</td>
<td>64.8 ± 0.4</td>
<td>91.3 ± 1.2</td>
<td>51.4 ± 4.7</td>
</tr>
<tr>
<td>20</td>
<td>77.0 ± 3.3</td>
<td>95.2 ± 0.2</td>
<td>72.2 ± 4.2</td>
</tr>
<tr>
<td>30</td>
<td>68.5 ± 2.8</td>
<td>96.2 ± 0.2</td>
<td>77.1 ± 7.9</td>
</tr>
</tbody>
</table>

* Obtained by normalizing the extent of 3H incorporation to the number of viable cells after 6 and 24 h exposure to each concentration of CDDP.

\[
\text{% of inhibition of control} = \frac{\text{dpm/10}^6 \text{ cells (control)} - \text{dpm/10}^6 \text{ cells (CDDP)}}{\text{dpm/10}^6 \text{ cells (control)}} \times 100\% 
\]

The number of viable cells was determined by the trypan blue exclusion method using duplicate cell cultures which were simultaneously exposed to CDDP under identical conditions.

![Fig. 5. Effect of cisplatin on DNA (A), RNA (B), and protein (C) synthesis in rabbit kidney proximal tubule cells. Both cisplatin and the appropriate 3H-labeled precursor were applied to the cells at time zero. These results are from one complete set of experiments and are representative of two separate experiments carried out in duplicate.](image-url)
of the proximal tubule, distal tubule, and collecting duct were observed to be the major sites of injury. However, despite the lack of agreement, it would appear that CDDP nephrotoxicity of the rat with its characteristic necrosis of the P3 segments distributed along the outer stripe of the medulla may not be typical of the CDDP lesion in man, and that the rabbit may be a better model.

Based on the results of our in vivo studies, primary cultures of proximal tubule cells were therefore established from the cortical region of the rabbit kidney. Viability studies with these cultures (Fig. 4) following exposure to increasing concentrations of CDDP indicated that although cell viability was greater than 95% after 6 h, a dose-dependent decrease in viability was obtained after 24 h, an observation that could be important to the interpretation of data concerning the effect of CDDP on cellular synthetic and enzyme activities in relation to its mode of action. Thus, whereas viability of renal cells was not altered after 6 h of CDDP treatment, continuous treatment with doses as low as 16 μM CDDP (5 μg/ml) dramatically inhibited DNA, RNA, and protein syntheses as early as 6 h after exposure (Fig. 5). The delayed and decreased effect on protein synthesis is probably due to an initial effect of CDDP on RNA, in particular on mRNA production, which would be expected to result in a reduction of protein synthesis. These results are in agreement with studies which have demonstrated that CDDP can inhibit DNA replication in human amnion AV3 (11), HeLa (7), and Chinese hamster ovary cells (11, 16) in culture, thus indicating that suppression of cellular synthetic activity is not unique to kidney cells. The most significant observation, however, is that the inhibitory effect of CDDP on cellular synthetic activity (Fig. 5; Table 2) occurred as early as 6 h posttreatment at a time when the cells were viable (Fig. 4). This suggests that inhibition of cellular synthetic activity may be important in the pathogenesis of CDDP-induced cellular toxicity since it occurs prior to overt cellular injury and necrosis. The role of inhibition of DNA replication in the initiation of cellular toxicity is further supported by preliminary in vitro data obtained with two platinoil analogues possessing low nephrotoxic potential in vivo, JM-8 (carboplatin) and JM-9 (iproplatin) (data not shown). At nontoxic doses (0–30 μg/ml), these two compounds had no effect on DNA synthesis. However, at concentrations (800 μg/ml for JM-8 and 200 μg/ml for JM-9) which reduced cell viabilities to 50% of control by 24 h, an inhibition of DNA synthesis was observed as early as 6 h similar to that obtained with CDDP. Similarly, transplatin, another nonnephrotoxic platinate compound, is also reported to be much less effective when compared to CDDP at inhibiting DNA replication (9–11). Interestingly, this apparent early effect on cellular synthetic activity also coincides with that of the morphological nuclear changes described by Jones et al. (38) in vivo in rats treated with CDDP. In these studies, electron microscopic analysis indicated that nucleolar segregation, ribosome dispersion, and formation of aggregates of smooth endoplasmic recticulum throughout the P3 portion of the proximal tubules were evident as early as 6 h after CDDP administration. Thus, our in vitro data and the data of Jones et al. (38) demonstrate the involvement of a nuclear macromolecule in CDDP-induced renal tubular toxicity similar to the mechanism currently proposed for CDDP tumoricidal activity.

A number of studies have postulated as a possible mechanism of toxicity the effect of CDDP on specific cellular enzyme activity (18–20). Toward this end, we selected the marker enzymes SD, AP, and ATPase in order to investigate the effects of CDDP on mitochondrial function, brush border membrane, and basolateral membrane functions of proximal tubule cells, respectively, and to relate these effects with cell toxicity. Of particular interest was the effect of CDDP on ATPase activity. Inhibition of ATPase activity in vitro by CDDP has been reported by Guarino et al. (42) using isolated flounder tubules, Daley-Yates and McBrien (19) using rat kidney homogenates, and Nechay and Neldon (20) with human kidney homogenates. In these studies, however, it was reported that to achieve at least 50% inhibition of ATPase activity, exceedingly high concentrations of CDDP and/or long incubation periods were required. More recently, Uozomi and Litterst (43) reported that concentrations of CDDP lower than 100 μM (30 μg/ml) could not inhibit more than 50% of purified ATPase activity after a 4-h incubation. Similarly, we observed no inhibition of ATPase activity in proximal tubule cultures after a 6-h incubation at CDDP concentrations up to 100 μM (Table 2). Only after 24 h incubation with CDDP when cell viability was reduced to 50% was ATPase activity actually inhibited. Using histochemical methods, Jones et al. (18) also observed that changes in ATPase activity in rat kidneys did not occur until 48 h following CDDP administration. These results in conjunction with data presented in this paper therefore demonstrate that the effect on ATPase activity by CDDP is not an early event and suggest that inhibition of this enzyme may not be critical for induction of CDDP-induced cytotoxicity.

As with ATPase, CDDP did not affect the activities of SD and AP, respectively, at 6 h posttreatment. In fact, SD activity was not inhibited even after 24 h treatment, confirming the histochemical data of Jones et al. (18). Thus, even though CDDP is known to effect mitochondrial function (21, 22), these results do not support CDDP effect on mitochondria as central to its mechanism of action. In the case of the brush border marker enzyme AP, inhibition of this enzyme activity was also not an early event and occurred only after 24 h incubation, again in agreement with the data of Jones et al. (18) who reported that this enzyme was not affected until 48 h posttreatment of rats with CDDP.

In summary, we have demonstrated that administration of CDDP to rabbits results in renal damage to proximal tubules located in the cortex of the kidney. This is in contrast to results obtained in rats where the majority of CDDP-induced lesions are predominantly located in the corticomedullary region of the kidney. This difference in the site of lesions between the rabbit and the rat suggests that perhaps the rabbit may be a more

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**Table 3. Effect of cisplatin on specific enzyme activities in rabbit kidney proximal tubule cultures**

<table>
<thead>
<tr>
<th>Dose (μg/ml)</th>
<th>Alkaline phosphatase</th>
<th>Succinic dehydrogenase</th>
<th>NA*, K*-ATPase</th>
</tr>
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<tr>
<td>10</td>
<td>91.0 ± 7.3</td>
<td>106.5 ± 1.2</td>
<td>97.6 ± 6.8</td>
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<td>20</td>
<td>89.5 ± 8.5</td>
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<td>30</td>
<td>92.5 ± 6.1</td>
<td>105.0 ± 4.9</td>
<td>93.5 ± 5.3</td>
</tr>
</tbody>
</table>

*a* AP activity was measured by following the rate of hydrolysis of p-nitrophenyl phosphate at 420 nm (28).

*b* SD activity was measured by following the oxidation of succinate at 600 nm (29).

*NA*, K*-ATPase activity was measured by following the oxidation of NADH at 340 nm in the presence and absence of 1 mM ouabain (30).

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appropriate model for studies to simulate CDDP nephrotoxicity in man. Exposure of primary cultures of rabbit kidney proximal tubule cells to CDDP in vitro resulted in an apparent inhibition of AP and ATPase activity. However, these effects were not early events and were not apparent until 24 h posttreatment, suggesting that the observed inhibition of enzyme activities may be the result of CDDP-induced cellular injury rather than the cause of the injury. On the other hand, inhibition of cellular synthetic activity by CDDP occurring as early as 6 h posttreatment precedes the onset of renal cellular toxicity and therefore could be an important contributing factor to the initiation of CDDP-induced nephrotoxicity. In addition, these results also suggest, because of the primary effect of CDDP on nuclear macromolecules, that the mechanism of CDDP-induced nephrotoxicity and tumoricidal activity may be similar. However, it should be noted that factors such as changes in cellular ion concentrations and calcium homeostasis have also been postulated to be important factors in the pathogenesis of cell injury (44, 45). The contribution and significance of these factors should also be evaluated in order to obtain a more complete picture regarding the mechanism of nephrotoxicity due to CDDP.

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REFERENCES


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