Zinc protects renal function during cadmium intoxication in the rat


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Cadmium (Cd²⁺) is one of the most common toxic metals in our environment. The major sources of exposure to Cd²⁺ in the general population are contaminated food and water, tobacco and industrial smoke, and dust. (18). Cd²⁺ accumulates in the body and has a very long biological half-life (10–30 yr) in humans (15, 18).

It is known that chronic exposure to Cd²⁺ can induce severe nephropathy in humans (18) and animals (4, 9). This nephrotoxicity causes reabsorptive and secretory dysfunction of the renal tubule. The main signs include proteinuria, ion losses, glucosuria, aminoaciduria, and polyuria (1, 2, 21, 24, 29, 31). Experimental chronic intoxication with Cd²⁺ has been performed at various doses over several weeks (9, 30, 48) and causes a Fanconi-like syndrome with predominant tubular dysfunction that develops into renal failure (47, 48). However, to our knowledge no detailed study of renal function during chronic Cd²⁺ intoxication has been carried out using clearance methods. Thus a documented time course for the appearance of the deleterious effects of Cd²⁺ exposure has not been described and would be useful in understanding the toxic mechanisms involved.

Several mechanisms have been proposed to explain the toxic effect of Cd²⁺ on renal cells. Cd²⁺ may cause nephrotoxicity by generating free radicals (20, 42) and by inducing necrosis and apoptosis (13). Interestingly, a protective effect of zinc (Zn²⁺) has been reported in vitro against the cellular toxicity due to Cd²⁺. Zn²⁺ protection is probably due to an action on oxidative stress and apoptosis (8, 17, 23, 43). In addition, exposure of renal cultured cells (LLC-PK1 line) to Cd²⁺ caused a decrease in transepithelial electrical resistance and in the number of domes, suggesting alterations of the tight junctions (41).

For the reasons outlined above, we decided to study the renal effects of Cd²⁺ during chronic exposure to Cd²⁺ and the putative protective effects of zinc.

MATERIALS AND METHODS

Clearance Experiments

These experiments were performed to analyze the effect of chronic intoxication with Cd²⁺ on whole kidney function and were carried out in female Wistar rats weighing 180–220 g. The animals were fed a standard laboratory diet. They had free access to water until the beginning of the experiments and were starved for 18 h before surgery. Anesthesia was induced by intraperitoneal (ip) injection of pentobarbital sodium (Nembutal, 5 mg/100 g body wt) and maintained by additional 1-mg doses when necessary. The animals were placed on a heated table to maintain their body temperature between 37 and 38°C. A tracheotomy was performed leaving the thyroid gland untouched. One catheter (Clay Adams, PE-20) was inserted into the right jugular vein for infusion and another (Clay Adams, PE-10) in the left ureter for urine collection. A third catheter (Clay Adams, PE-50) was inserted into the right femoral artery for blood sampling and arterial blood pressure recording (Research BP Transducer, Harvard Apparatus). Clearance experiments were carried out in rats infused with 0.9% NaCl solution at a rate of 20 µl/min. [³H]methoxy-inulin (TRA.324 Specific radioactivity, 120 µCi/ml/mg inulin, 0.53 Ci/mmol; Amersham Pharmacia Biotech) was used to estimate the glomerular filtration rate (GFR). Urine samples were collected serially during 20-min periods, and blood samples were taken halfway through each urine collection. In all experiments, a loading dose of [³H]inulin (4 µCi) was given, followed by a continuous infusion of 0.4 µCi/min for the duration of each experiment. Urine collection began 1 h after the administration of the [³H]inulin priming dose.

At the end of the experiment, the rats were killed by an overdose of Nembutal, and the liver and kidneys were removed for measurement of cadmium content. The use of animals was in accordance with the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals.

Chronic Intoxication with Cd²⁺ and Protection by Zn²⁺

In the Cd²⁺-treated groups, rats were intoxicated by daily ip injection for 5 days with 500 µg Cd²⁺·kg⁻¹·day⁻¹. There was a...
recovery period of 15 days, without further Cd$^{2+}$ exposure. Clearance experiments were performed on days 0 (control group), 1, 3, 5, 7, 10, and 20. To study the protective effect of Zn$^{2+}$, Wistar rats were injected daily ip with a solution containing both 500 μg Cd$^{2+}\cdot$kg$^{-1}\cdot$day$^{-1}$ and 500 μg Zn$^{2+}\cdot$kg$^{-1}\cdot$day$^{-1}$ for 5 days. Clearance experiments were performed on day 10 and day 20. Experiments were also performed in a control group and a treated group with 500 μg Zn$^{2+}\cdot$kg$^{-1}\cdot$day$^{-1}$ for 5 days. Each group comprised five rats.

Rat body weights were recorded daily to determine the effect of Cd$^{2+}$ on the general health of the animals.

**Analytic Procedures**

$^3$H radioactivity was measured by liquid scintillation counting (Packard) in plasma and urine samples. Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Cl$^-$, and P$^i$ concentrations were determined by ion exchange chromatography (ASS50/BioLC, Dionex), and Cd$^{2+}$ was measured by atomic absorption spectrometry using a Zeeman furnace system (Solaar 969, Thermo Optek).

**Cd$^{2+}$ Measurement in Tissue**

The technique used to measure Cd$^{2+}$ was described earlier by Playle et al. (40). Liver and kidney samples were taken, weighed, and digested in a solution of 1 N nitric acid (TraceMetal Grade HNO$_3$; Fisher Scientific) at a temperature of 80°C for 4 h. At the end of this time, the supernatant was taken, and its volume was measured to calculate Cd$^{2+}$ concentration. The supernatant was then diluted 1:1,000 with deionized water, and Cd$^{2+}$ content was determined by atomic absorption spectrometry using a Zeeman furnace system (Solaar 969, Thermo Optek).

**Apoptosis Measurement in Tissue**

Rats were intoxicated with one ip injection of either NaCl as a control, 500 Cd$^{2+}$/kg, both 500 μg Cd$^{2+}$/kg and 500 μg Zn$^{2+}$/kg, or only 500 μg Zn$^{2+}$/kg. Twenty-four hours after the injection, the kidneys were perfused with 1× PBS and then dissected from euthanized animals. The kidney cortexes were then removed and homogenized immediately in lysis buffer (Euromedex). Protein was quantified using the Bradford assay (Bio-Rad) using BSA as a standard. Apoptosis measurement in cortex samples was monitored by measuring the color generated due to hydrolysis of a chromogenic caspase-3 substrate (Apopain, Euromedex) added to the assay medium. Color was measured using a plate reader.

**Rat Frozen Kidney Tissue Sections**

Kidneys of experimental rats were removed at the end of each experiment and washed with PBS. Cubes (0.5 cm/side) were cut and immediately immersed in 2 min in 2-methylbutane (263–1, Aldrich), which was previously cooled in liquid nitrogen. The cubes were then transferred for 10 min to liquid nitrogen. Eight-micrometer sections which were previously cooled in liquid nitrogen. The cubes were then immediately immersed for 2 min in 2-methylbutane (263–1, Aldrich), and Cd$^{2+}$/H$^9$262 was digested in a solution of 1 N nitric acid (TraceMetal Grade HNO$_3$; Playle et al. (40). Liver and kidney samples were taken, weighed, and digested in a solution of 1 N nitric acid (TraceMetal Grade HNO$_3$; Playle et al. (40). Liver and kidney samples were taken, weighed, and digested in a solution of 1 N nitric acid (TraceMetal Grade HNO$_3$; Playle et al. (40). Liver and kidney samples were taken, weighed, and digested in a solution of 1 N nitric acid (TraceMetal Grade HNO$_3$; Playle et al. (40).

**Immunofluorescence.** The frozen sections were incubated overnight with one of the following rabbit polyclonal antibodies: claudin-2 (51–6,100, dilution 1 μg/ml, Zymed); claudin 3 (34–1,700, dilution 5 μg/ml, Zymed); and claudin-5 (34–1,600, dilution 20 μg/ml, Zymed). The sections were washed three times with PBS and incubated for 1 h with a FITC-conjugated goat anti-rabbit IgG (65–6,111, 6 μg/ml, Zymed) or with anti-mouse IgG TRITC conjugate developed in goat (T-5393, 7.5 μg/ml, Sigma). After three more washes with PBS, the sections were transferred to glass coverslips and mounted with the antifade reagent Fluogard (170–3140, Bio-Rad). The fluorescence of the sections was examined using a confocal microscope (Leica DMR2). The images collected had an optical thickness of 1 μm. The image shown represents a projection of the sections made for each slide.

**Statistical Analyses**

Student’s t-test and ANOVA were used to analyze differences in quantitative variables between control and experimental groups. Re-
RESULTS

Chronic Cd\(^{2+}\) Intoxication

Renal function. Data of the effect of a daily dose of 500 μg Cd\(^{2+}\)/kg for 5 days are shown in Figs. 1 and 2.

 Urinary flow decreased on day 5 and then had an overshoot on days 10 and 20 (Fig. 1A). The urinary-to-plasma inulin ratio (U/P) remained unchanged until day 7 and then decreased significantly (Fig. 1B). GFR was not significantly modified from day 1 to day 7. It decreased from 1.01 ± 0.04 (n = 3) in controls to 0.72 ± 0.04 (n = 3) on day 10 and to 0.77 ± 0.07 ml·min\(^{-1}\)·kidney\(^{-1}\) (n = 3) on day 20 in Cd\(^{2+}\)-treated animals.

 Fractional excretion and plasma concentration of Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^-\) and PO\(_4\) for each experimental group are shown in Fig. 2.

 Regarding phosphate handling, its fractional excretion did not change from day 1 to day 7, but significantly increased on...
day 10 and remained higher than control until day 20. Plasma phosphate concentration was increased from day 5 to day 10 and then decreased below control at day 20.

Cl\textsuperscript{-}, Na\textsuperscript{+}, and Ca\textsuperscript{2+} handling followed a similar pattern: at day 10, the fractional excretions of Cl\textsuperscript{-}, Na\textsuperscript{+}, and Ca\textsuperscript{2+} increased, and by day 20 they were higher than control. Plasma concentrations of Cl\textsuperscript{-}, Na\textsuperscript{+}, and Ca\textsuperscript{2+} showed different changes over time. Cl\textsuperscript{-} concentration in plasma was constant until days 10 and 20, when it became higher than control. Plasma Na\textsuperscript{+} concentration was not changed. Plasma Ca\textsuperscript{2+} concentration started to decrease at day 5 and thereafter remained low until day 20.

Interestingly, the excretory pattern for renal K\textsuperscript{+} and Mg\textsuperscript{2+} was similar. From day 1 to day 7, fractional excretions of K\textsuperscript{+} and Mg\textsuperscript{2+} decreased until days 10 and 20, when they became higher than control. Plasma concentration of K\textsuperscript{+} increased significantly from the first day of intoxication and decreased with time but stayed still higher than control. Mg\textsuperscript{2+} concentration in plasma was constant until day 5, when it became to decrease significantly.

It is important to emphasize that the fractional excretions of each ion increased significantly on days 10 and 20 and that these changes occurred 5 days after the end of Cd\textsuperscript{2+} intoxication, indicating a delayed deleterious effect of Cd\textsuperscript{2+} on renal function. All changes suggested the induction of a Fanconi-like syndrome by Cd\textsuperscript{2+}.

Zn\textsuperscript{2+} protection. Experiments were also performed to investigate the effect of Zn\textsuperscript{2+} on Cd\textsuperscript{2+} nephrotoxicity. Urine flow rate, inulin U/P, GFR, plasma ion concentrations, and fractional excretion of ions were measured at days 10 and 20 after exposure to Cd\textsuperscript{2+} alone, to Zn\textsuperscript{2+} alone, and to exposure to both Cd\textsuperscript{2+} and Zn\textsuperscript{2+}. The results are shown in Figs. 3 and 4. Chronic treatment by zinc (500 µg·kg\textsuperscript{-1}·day\textsuperscript{-1}) alone did not modify renal function or plasma ion concentrations, indicating that at this concentration Zn\textsuperscript{2+} did not cause any detectable nephrotoxic effect (Figs. 3 and 4). When the intoxication by Cd\textsuperscript{2+} was performed in the presence of an equivalent amount of Zn\textsuperscript{2+}, the effects observed on urine flow rate, inulin U/P, and GFR were significantly less that the effects observed with Cd\textsuperscript{2+} alone. Thus at day 10, the decrease in inulin U/P, the increase in urinary flow rate, and the decrease in GFR induced by Cd\textsuperscript{2+} alone were partially attenuated by Zn\textsuperscript{2+}. At day 20, the presence of Zn\textsuperscript{2+} completely prevented the effect of Cd\textsuperscript{2+}, because no modification of inulin U/P, urine flow rate, or GFR was observed compared with the values obtained in the absence of Cd\textsuperscript{2+} or in the presence of Zn\textsuperscript{2+} alone.

Figure 4 shows the plasma concentrations and fractional excretions of Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Cl\textsuperscript{-}, and PO\textsubscript{4}\textsuperscript{3-} of rats given Cd\textsuperscript{2+} and/or Zn\textsuperscript{2+}. In Cd\textsuperscript{2+}-intoxicated animals, the presence of Zn\textsuperscript{2+} also prevented the changes in plasma concentrations and fractional excretions of Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, Cl\textsuperscript{-}, and PO\textsubscript{4}\textsuperscript{3-} induced by Cd\textsuperscript{2+}. Interestingly, the effect of Zn\textsuperscript{2+} was observed from day 10 and persisted until day 20.

Our data indicate that in the presence of Zn\textsuperscript{2+}, the renal function in Cd\textsuperscript{2+}-intoxicated rats remains normal and therefore Zn\textsuperscript{2+} showed a protective effect against the nephrotoxicity due to Cd\textsuperscript{2+}.

Cd\textsuperscript{2+} accumulation. Figure 5A illustrates the time course of Cd\textsuperscript{2+} accumulation in the kidney and liver during chronic intoxication. In these organs, Cd\textsuperscript{2+} content gradually increased after each ip injection of Cd\textsuperscript{2+} from day 1 to day 5 and reached...
Fig. 4. Effect of Zn²⁺ administration on plasma concentrations and fractional excretions of Ca²⁺, Mg²⁺, Na⁺, K⁺, Cl⁻, and PO₄²⁻ during Cd²⁺ intoxication. Experiments were performed with ip injection of 500 μg Cd²⁺·kg⁻¹·day⁻¹ for 5 days in the absence or the presence of 500 μg Zn²⁺·kg⁻¹·day⁻¹ followed by a recovery period of 15 days. Values are means ± SE calculated for each clearance period. *P < 0.02, **P < 0.01, ***P < 0.001: different from control.
in body weight in the Cd2+-treated group, compared with control or Zn2+-treated groups. In the Cd2+-intoxicated group, there was no significant difference between animals exposed to Cd2+ alone for 5 days or with 500 μg Zn2+⋅kg−1⋅day−1. Cd2+ content was determined as a function of time, and values are means ± SE calculated for each experimental group. *P < 0.02 different from control.

Body weight gain. The gain in body weight of rats during chronic exposure to Cd2+, Cd2+ + Zn2+, and in control conditions is shown in Fig. 5B. Cd2+ ip injection reduced body weight gain. At the end of the experiment (day 20), the increase in body weight in the Cd2+-intoxicated group was reduced by 23% compared with control. In the Cd2+ + Zn2+-intoxicated group, there was no significant difference between animals exposed to Cd2+ + Zn2+ and control animals.

Apoptosis. Figure 6 shows results of caspase 3 activity, as a marker of apoptosis, measured by the degradation of a caspase 3 substrate. Data show an increase in the caspase 3 enzymatic reaction compatible with an increase in apoptosis in Cd2+-treated compared with control or Zn2+-treated animal. In Cd2+-intoxicated animals, the simultaneous injection of Zn2+ has prevented the increase in cell apoptosis.

Intercellular junctions. Immunofluorescence of renal cryosections detected claudins 2 and 5 of the tight junction complex. We analyzed the tubular distribution of claudin-2 in kidney slices from control rats, Cd2+-treated rats, and Cd2+ + Zn2+-treated rats (Fig. 7). As expected, in control, claudin-2 was only detectable in proximal tubules (Fig. 7, A and B), showing a clear “chicken fence” pattern at the cell borders. In Fig. 7B, a merged image shows that two tubules do not depict fluorescence as would be expected for these segments. In the presence of Cd2+, after 5 days of ip injection (Fig. 7C), claudin-2 was mainly detectable in the cytoplasm and barely at the cell border, indicating an alteration in its cellular expression and trafficking to the cell plasma membrane. When a linear fluorescence pattern was present at the cell borders, it was disrupted. These abnormalities were observed in most of the tubules that showed fluorescence for claudin-2. In rats treated with Cd2+ and Zn2+, renal sections obtained after 5 days of treatment and 5 days of recovery (Fig. 7D) showed partial preservation of the pattern of claudin-2, albeit with evident damage. After 10 days, 5 days of intoxication, and 5 days of recovery (Fig. 7E), the expression of claudin-2 was sparse and only a few proximal tubules expressed claudin-2 with a cytoplasmic pattern, indicating progression of the toxic effects of Cd2+ on proximal cells. Figure 7, D and F, shows the claudin-2 pattern in rats intoxicated with Cd2+ + Zn2+ at day 5 and day 10, respectively. In these two cases, the cell border pattern of claudin-2 was preserved, indicating a protective effect of Zn2+. Sections obtained from a rat after 5 days of Cd2+ treatment and 15 days of recovery show that the alterations observed in animals at days 5 and 10 were less severe but still evident (Fig. 7G). In contrast to the observations in Fig. 7G, sections from animals treated with Cd2+ + Zn2+ obtained after 15 days of recovery showed an almost complete recovery of the pattern for claudin-2 at the cell borders (Fig. 7H). These findings indicate that treatment with
Zn$^{2+}$ partially protected tubules from the damage caused by Cd$^{2+}$ and accelerated recovery.

Figure 8 shows immunofluorescence for claudin-5. Claudin-5 had been demonstrated to be specific for endothelial cells. Normally, in control rats (Fig. 8A), claudin-5 stains the endothelia of glomeruli with a dotted pattern, whereas in renal vessels a linear cell border pattern is observed. In the presence of Cd$^{2+}$ on day 5, vascular expression of claudin-5 was altered, and it was possible to see the disrupted elastin band, which appeared as strong autofluorescence in vessel walls (Fig. 8B). This change was not seen in the presence of Zn$^{2+}$ (Fig. 8C). After the presence of Cd$^{2+}$ for 5 days and after 5 days of recovery, glomeruli and a small blood vessels (Fig. 8D) showed thicker fluorescence than control, indicating vascular damage and disruption of the claudin-5 pattern. Surprisingly, with Zn$^{2+}$ (Fig. 8E), glomerular capillaries were still thicker than in control, suggesting that the protective effect of Zn$^{2+}$ observed in the tubules was not present in endothelial cells.

In control animals, claudin-3 fluorescence was located at the distal segments of the nephron (Fig. 9). In contrast to claudin-5, in glomerular capillaries only very scant dotted fluorescence was detectable (Fig. 9B). As for claudin-2, the epithelial pattern of claudin-3 was disrupted in Cd$^{2+}$-treated animals, both after 5 days of treatment with Cd$^{2+}$ alone (Fig. 9C) and after 5 days of Cd$^{2+}$ treatment and 5 days of recovery (Fig. 9E). There was partial rearrangement of claudin-3 at the cell borders in the animals treated 5 days with Cd$^{2+}$ and then killed.
Fig. 8. Immunofluorescence of claudin-5 in kidney of control and intoxicated rat. Claudin-5 is located in renal vessels and glomeruli. A: frozen section from a control untreated Wistar rat. A linear fluorescent pattern is observed in the vessel (*). In the glomerulus, a fine staining is present in all the capillaries and has a dotted pattern. B: in a section from a rat treated for 5 days with Cd$^{2+}$ (500 µg·kg$^{-1}$·day$^{-1}$ ip), the linear distribution is lost, with an irregular and dotted pattern (arrow). There is observed fluorescence corresponding to the band of elastin (arrowhead). This structure is fragmented, and some parts of the subendothelium are lost. C: section from a rat that received Cd$^{2+}$ and Zn$^{2+}$ simultaneously for 5 days (500 µg·kg$^{-1}$·day$^{-1}$ ip of each). There is dotted labeling in the endothelium (arrow), and the structure of elastin is preserved (arrowhead). D: section from a rat that received Cd$^{2+}$ for 5 days. Histological evaluation was performed on day 10. Glomerular capillaries are thicker (arrowheads) than in control (A), and the fluorescence distribution is more evident in the lower half of the glomerulus. Fluorescence is also present in small vessels and is also thick (arrows). E: renal section at day 10 from a rat treated simultaneously with Cd$^{2+}$ and Zn$^{2+}$ as in C. Claudin-5 fluorescence is homogeneously distributed in the glomerulus. Capillaries are also thicker (arrowheads) than in control. Bar = 40 µm.

15 days later (Fig. 9F). Treatment with Zn$^{2+}$ partially protected against those alterations (Fig. 9, D, F, and H).

DISCUSSION

In recent years, a large number of studies have examined the renal effects of Cd$^{2+}$ intoxication. These investigations have clearly demonstrated that Cd$^{2+}$ toxicity depends on the dose, the route of administration, and duration of exposure (31). However, several crucial points remain unclear. The aim of our study was to determine the time course of renal damage during chronic intoxication and to determine whether the kidney is able to recover normal function after intoxication. Moreover, we assessed the possible therapeutic benefit of Zn$^{2+}$ on the nephrotoxic effect of Cd$^{2+}$. This approach has not been previously assayed.

First, to better understand the chronic effect of Cd$^{2+}$ on renal function, we chose to intoxicate Wistar rats with 0.5 mg Cd$^{2+}$/kg by daily ip injection for 5 days. According to Liu et al. (31), Dudley et al. (14), and Goyer et al. (19), such a dose corresponds to low-dose chronic intoxication. The present study showed that during intoxication and at the beginning of the recovery period, GFR was not affected, although significant changes in renal handling of the measured ions occurred between days 1 and 7: fractional excretions of K$^+$, Ca$^{2+}$, and Mg$^{2+}$ were decreased, suggesting that, in the early stage of chronic intoxication, Cd$^{2+}$ acts on ion transports without affecting renal tissue. Evidence of renal damage was only apparent at day 10, during the recovery period, and 5 days after the end of the exposure to Cd$^{2+}$; GFR was decreased, and fractional excretions of all ions were increased, indicating both glomerular and proximal tubular damages. This delayed toxic effect might be explained by the long biological half-life of Cd$^{2+}$. Indeed, each new daily dose was cumulative, because this heavy metal is poorly eliminated by the kidney. These results are in accordance with the data of Aoyagi et al. (3), who reported that the concentration in the kidney reached 120 µg Cd$^{2+}$/g after 8 wk of daily subcutaneous injections with 0.6 mg Cd$^{2+}$/kg body wt and that this treatment was nephrotoxic. These authors also described severe damage to the tubular structure due to necrotic and apoptotic cell death, which could also explain the deleterious renal effects we observed.

Another phenomenon could be responsible for tubular dysfunction and renal tissue damage observed after day 10; tubular transport occurs through a transcellular route and paracellular pathways. Thus the proximal tubulopathy might be due to a defect in tight junction organization. The tight junction constitutes the main barrier in epithelia to the passive movement of electrolytes and macromolecules through the paracellular pathway. It also functions as a barrier that maintains a polarized distribution of lipids and proteins between the apical and basolateral surfaces of epithelial cells. The organization of tight junctions is stabilized by the claudins, a family of transmembrane proteins.

In our study, claudin-5 was stained in renal vessels and glomeruli. In control kidneys, claudin-5 displayed a linear staining pattern in small vessels and was also thick in the lower half of the glomerulus. In intoxicated kidneys, claudin-5 distribution was more evident in the lower half of the glomerulus. Moreover, the fluorescence was present in small vessels and was also thick. These results are in accordance with the data of Aoyagi et al. (3), who reported that the concentration in the kidney reached 120 µg Cd$^{2+}$/g after 8 wk of daily subcutaneous injections with 0.6 mg Cd$^{2+}$/kg body wt and that this treatment was nephrotoxic. These authors also described severe damage to the tubular structure due to necrotic and apoptotic cell death, which could also explain the deleterious renal effects we observed.

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basolateral plasma membrane (10, 28). Claudins are constitutive junction proteins of epithelia. Reyes et al. (42) showed that in the kidney claudin-2 is predominantly located at the leaky portions of the nephron (proximal segments), whereas claudin-5 is present in the renal vasculature. Immunofluorescence experiments demonstrated that the expression of claudin-2 and claudin-5 was strongly modified after exposure to Cd\(^{2+}\): proximal epithelium and renal endothelium showed disorganization of the pattern of expression of proteins of the tight junction in rat kidney. To our knowledge, these alterations of claudin-2 and claudin-5 induced by Cd\(^{2+}\) have not been previously reported. In agreement with our findings, Prozialeck et al. (41) reported that, in rats intoxicated with 0.6 mg Cd\(^{2+}\)/kg, cadherin-dependent cell-cell junctions in the proximal tubule were disrupted. Cadherin and catenin constitute the zonula adherens, which is present in all epithelia and located just below the tight junction complex in the proximal segment. Our results confirmed that proteins of the functional complexes like claudins, cadherins, and catenins are early targets of Cd\(^{2+}\) toxicity. Moreover, it must be pointed out that we observed alterations in glomerular claudin-5, whereas Prozialeck et al. did not show such modifications. These early changes in endothelial tight junctions could announce more damage to the glomerulus, leading later to severe renal failure.

The cryosections also showed dilatation of the luminal compartment of many tubules and severe damage to glomeruli and vascular endothelium. Concomitantly, this change in renal structures induced severe losses of all ions. These observations confirm the data of Brzoska et al. (9), who described that chronic exposure to Cd\(^{2+}\) induced injuries affecting the main
reabsorptive parts (proximal convoluted tubules and straight tubules) and the filtering part (glomeruli) of the nephron. These authors also demonstrated an increase in urinary excretion of enzymes that are markers of cytotoxicity, such as N-acetyl-β-F-glucosaminidase, isoenzyme B, and alkaline phosphatase. Finally, as previously reported, exposure to Cd²⁺ causes a Fanconi-like wasting of many filtered solutes (49).

Another goal of our study was to explore a way of protecting renal function against Cd²⁺ intoxication. Because Zn²⁺ had been demonstrated in vitro to decrease cellular damage induced by Cd²⁺ (12, 31, 47), it seemed reasonable to test this issue in vivo. The most noteworthy findings of the present study are that the effect of cotreatment with Zn²⁺ during Cd²⁺ administration completely prevented the changes in renal function produced by the toxic metal. Among the possible mechanisms, it might occur that Zn²⁺ reduced the renal uptake of Cd²⁺ by competition for a common transporter. Although it as been demonstrated that both metals can share the DMT1 transporter in epithelial cells, this explanation seems unlikely because we showed that Cd²⁺ accumulation in the kidney was not decreased by Zn²⁺. Perhaps a better explanation for this protection is that Zn²⁺ plays a role in preventing apoptosis and necrosis. Kondoh et al. (25) have demonstrated that Cd²⁺ induces cytochrome c release from mitochondria, leading to apoptosis via the activation of the caspase 3 and 9 cascade (25, 27, 32). The work of Perry et al. (39), who demonstrated that Zn²⁺ inhibited caspase-3, suggests that Zn²⁺ protection against Cd²⁺ could be due to an inhibition of caspase-3 and apoptosis. This is in agreement with our results showing an increase in apoptosis in a kidney cortex sample from a Cd²⁺-treated animal, measured by the activity of caspase 3, and prevented by Zn²⁺ treatment. Moreover, it should also be noticed that we have recently shown an increase in apoptosis in a proximal cell line incubated in the presence of Cd²⁺ and the prevention of necrosis phenomena when cells were incubated in the presence of Zn²⁺ (5).

In rabbit and mouse kidney, claudin-2 is expressed mainly in the proximal tubule, whereas claudin-3 is in the distal segments of the nephron and claudin-5 in the endothelium (26, 42). In the rat, we found that claudin-2, claudin-3, and claudin-5 are also expressed in proximal segments, distal segments, and renal vessels, respectively. As for claudin-2, expression of claudin-3 was altered in Cd²⁺-treated animals and Zn²⁺ afforded partial protection against those alterations. These data suggest that in chronic Cd²⁺ contamination, Cd²⁺ has an effect in the proximal as well as in distal tubule, leading to a pronounced renal defect. The protective effect of Zn²⁺ on renal function could be used as the basis of preventive treatment for potential Cd²⁺ intoxication.

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