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Morphological and Histochemical Evidence of the Protective Effect of Procainamide Hydrochloride on Tissue Damage Induced by Repeated Administration of Low Doses of Cisplatin

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Abstract. *Background: The class I antiarrhythmic drug procainamide hydrochloride might protect against acute cisplatin-induced nephrotoxicity and hepatotoxicity in mice and rats. In this report, the protective activity of procainamide hydrochloride against renal and hepatic tissue damage induced by repeated administration of low doses of cisplatin was analyzed morphologically and histochemically. Materials and Methods: Light microscopy observations were performed on liver, renal and heart samples obtained from female Wistar rats treated twice a week for 10 weeks with 1 mg/kg cisplatin (cumulative dose: 20 mg/kg), with or without 100 mg/kg procainamide hydrochloride (cumulative dose: 2 g). Samples were then submitted to histochemical stainings [i.e. H & E, periodic acid Schiff (PAS) and Sudan Black]. Results: Light microscopy analysis revealed that the coadministration of cisplatin and procainamide hydrochloride significantly reduced tissue alterations both in the kidneys and liver, while in the heart, neither cisplatin nor the combination of cisplatin and procainamide hydrochloride caused any evident tissue damage. Conclusion: The morphological and histochemical data confirm that procainamide hydrochloride is able to protect not only from acute cisplatin-induced toxicities, but also from tissue alterations induced in the liver and kidneys by the administration of repeated low doses of cisplatin.*

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In our previous work, we demonstrated that procainamide hydrochloride, a class I antiarrythmic drug, was able to reduce some of the acute toxic effects of cisplatin, in particular the dose-limiting nephrotoxicity and the mild and transient hepatotoxicity (1-3). In general, the risk of these and other cisplatin-induced toxic effects is strictly linked to the intensity of the single or cumulative doses of cisplatin administered (4, 5). On the other hand, only a few papers, in particular concerning ovarian cancer (6), have demonstrated that dose intensification may represent a good option to obtain a survival benefit, but probably at the cost of the high incidence and morbidity of acute and cumulative toxicities.

Different strategies have been used in order to reduce the side-effects of cisplatin, which include nephro-, oto- and neurotoxicity (7-11). Hydration to modulate the elimination of the drug, the administration of diuretics to increase renal clearance, the use of antidotes to detoxify cisplatin and, finally, the modulation of schedules of administration to give similar cumulative doses but which are split into less dangerous subdoses, thus decreasing the systemic concentration of the drug, are examples of such strategies. Both the modulation of schedules of administration and hydration are, at present, clinically widely used. However, in spite of these protective measures, the toxicity of cisplatin, in particular its nephrotoxicity, may still occur and become more severe after repeated courses of the platinum drug.

The mechanisms of cisplatin toxicity are various and well-studied. In the case of the dose-limiting nephrotoxicity, important roles are played by oxidative stress, DNA damage and lipid peroxidation (12, 13). Other possible mechanisms involving mitochondrial functions, adenosine triphosphate activity and intracellular calcium homeostasis may also play significant roles (14, 15). For other forms of toxicity, due to

their less frequent appearance, the study of mechanisms is less advanced. This is the case, for example, for hepatotoxicity where the mechanisms are less understood, although they seem to be linked, at least in part, to apoptosis and to the functions of metallothioneins (16).

In this experimental study, 1 mg/kg cisplatin was given twice a week for 10 weeks to rats. Under these conditions, it is possible to administer cumulative doses of cisplatin as high as 20 mg/kg to rats without causing death. In spite of the administration of such high doses, the nephro- and hepatotoxic effects of cisplatin are less evident than in conditions of acute administration, where a single dose of 7-8 mg/kg cisplatin can cause irreversible damage to the kidneys. Nevertheless, the animals treated under our experimental conditions presented evident tissue damage on completion of the treatment.

Our aim was to evaluate, from a morphological and histochemical point of view, the possible reduction of toxic effects of cisplatin through the repeated coadministration of procainamide hydrochloride, which we have already demonstrated to be an optimal protector against acute cisplatin-induced nephro- and hepatotoxicity.

Materials and Methods

Animal protocols. Female Wistar rats (Harlan Italy, S. Pietro al Natisone, Italy), weighing 200-220 g, were used. The animals were allowed a 7-day rest before the experiments, housed 3 per cage, maintained at 22˚C with a 12-h light/dark cycle and fed on a standard diet and water *ad libitum*. Four groups of 3 animals each were then treated twice a week for 10 weeks: the first with cisplatin alone (Sigma, 1 mg/kg in normal saline; cumulative dose, 20 mg/kg); the second with cisplatin plus procainamide hydrochloride (100 mg/kg; cumulative dose, 2 g/kg); the third group of rats was treated with procainamide hydrochloride diluted in distilled water; the last (control) group of rats was treated with normal saline. In all cases the drugs were administered *i.p.* separately in a relative volume of 400 μl per rat of 200 g body weight.

Ten weeks later, at the end of treatment, the animals were euthanized in a CO_2-satured chamber and the liver, kidneys and heart excised and processed for morphological examination. All the procedures involving animals were performed in accordance with the current national regulations regarding the protection of animals used for scientific purpose and the research protocols were reviewed and approved by the I.A.C.U.C.

Light microscopy. Samples of liver, kidney and heart were obtained from the treated rats and processed for morphological analysis under light microscopy.

The specimens were fixed in 2% paraformaldheyde in 0.1 M phosphate buffer, pH 7.4, for 24 h, processed routinely, embedded in Paraplast wax and sectioned at 6 μm. The sections underwent hematoxylin-eosin (H&E) staining for structural evaluation. Some sections were analyzed by histochemical light microscopy with periodic acid Shiff (PAS) to detect glycogen and glycoproteins content, with and without prior digestion with 0.2% α-amylase. Liver sections were also submitted to Sudan Black staining to detect the lipid content. The sections were then evaluated qualitatively.

Results

Light microscopy analysis revealed that cisplatin treatment alone induced some alterations of both the liver and kidney parenchyma when compared to both control and procainamide hydrochloride-treated rats.

In the liver, cisplatin induced some vacuolation within the cytoplasm of the hepatocytes that appeared pale after H&E staining. In addition, in these specimens numerous vessels and sinusoids were filled with erythrocytes (Figure 1A). Compared to both control and procainamide hydrochloride-treated samples, the liver of cisplatin-treated animals exhibited a considerable reduction of PAS-positive hepatocytes (Figures 2A, 2B). After the treatment with both procainamide hydrochloride alone and cisplatin plus procainamide hydrochloride, the liver parenchyma was almost comparable with that of the controls (Figure 1B). Moreover, PAS reactivity was intense in large areas of the liver parenchyma of animals treated with the combination (Figure 2C).

In control rats the hepatocytes showed light to moderate Sudan Black staining. After application of the different treatment modalities, the liver parenchyma showed no relevant differences in terms of Sudan Black staining when compared to the controls (data not shown).

In the kidneys the structure (H&E staining) and the PAS reactivity of sections from control and procainamide hydrochloride-treated animals were similar, showing normal morphology (Figure 3A and 4A). Prominent alterations were observed in the kidney parenchyma of rats treated with cisplatin after both H&E and PAS stainings. In these samples, cisplatin caused some tubular dilation and epithelial cell damage, mainly in the mid cortex area. In particular, numerous proximal tubules displayed disruption of the brush border, whereas some distal tubules showed pale cytoplasm, probably due to mitochondrial vacuolization. In some tubules flocculent material was observed. Different glomeruli showed a dilated Bowman's space. A number of vessels intermingled with tubules appeared dilated. In addition, focal cell infiltrates were often observed close to vessels and among tubules (Figure 3B).

In the sections stained with the PAS reaction, both the tubular and glomerular basement membranes appeared very intensively stained. The reaction was still observed after α-amylase digestion; the staining also evidenced numerous proximal tubules showing a damaged brush border (Figure 4B).

After the simultaneous administration of procainamide hydrochloride plus cisplatin, the kidney parenchyma showed many fewer damaged tubules (Figure 3C), however a moderate to intense PAS reactivity of the basement membrane was still observed in some of them (Figure 4C). Some vessels still appeared dilated; occasionally cell infiltrates were observed nearby the vessels.

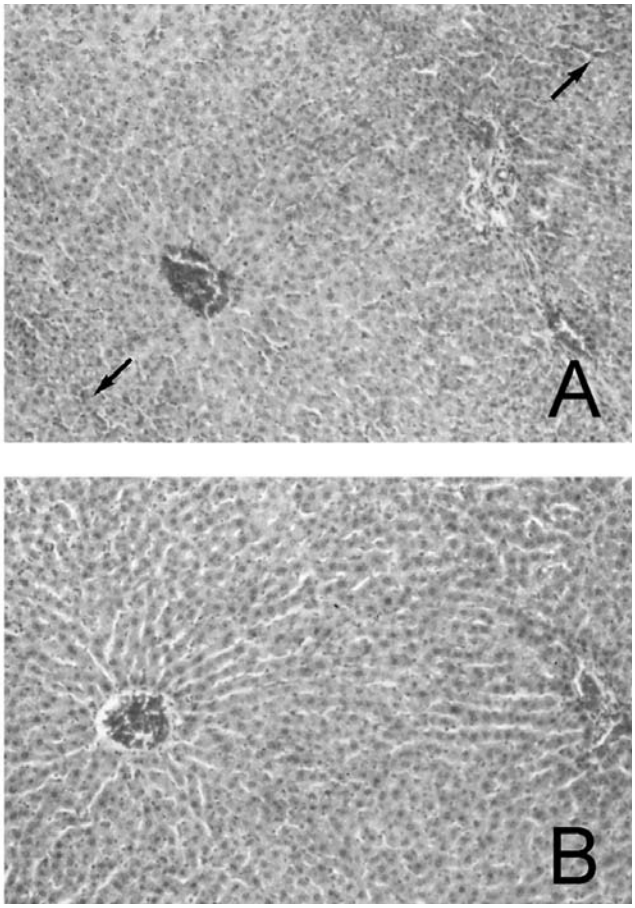

Figure 1. *H&E staining. A. Rats treated with cisplatin alone. The cytoplasm of most hepatocytes appears pale; several sinusoids are filled with erythrocytes (arrows). B. Rats treated with the combination of cisplatin and procainamide hydrochloride. Normal hepatic laminae are observed within the lobules. (x 100).*

No structural alterations in the heart of any of the treated rats was observed, compared to controls.

Discussion

Our previous papers have demonstrated that the class I antiarrhythmic drug procainamide may act as an efficient chemoprotector against acute cisplatin-induced nephro- and hepatotoxicity (1-3). Usually the clinical protocol applied to protect patients from cisplatin-induced toxicity is based on the infusion of isotonic or hypertonic saline before and after the administration of cisplatin, which is in turn dissolved in normal saline to stabilize the reactive chloride ligand. The stabilization of the cisplatin molecule reduces the formation of aquated species that are more nephrotoxic and less effective than the parent compound (17-19). Moreover, in order to further reduce its toxic effects, which become

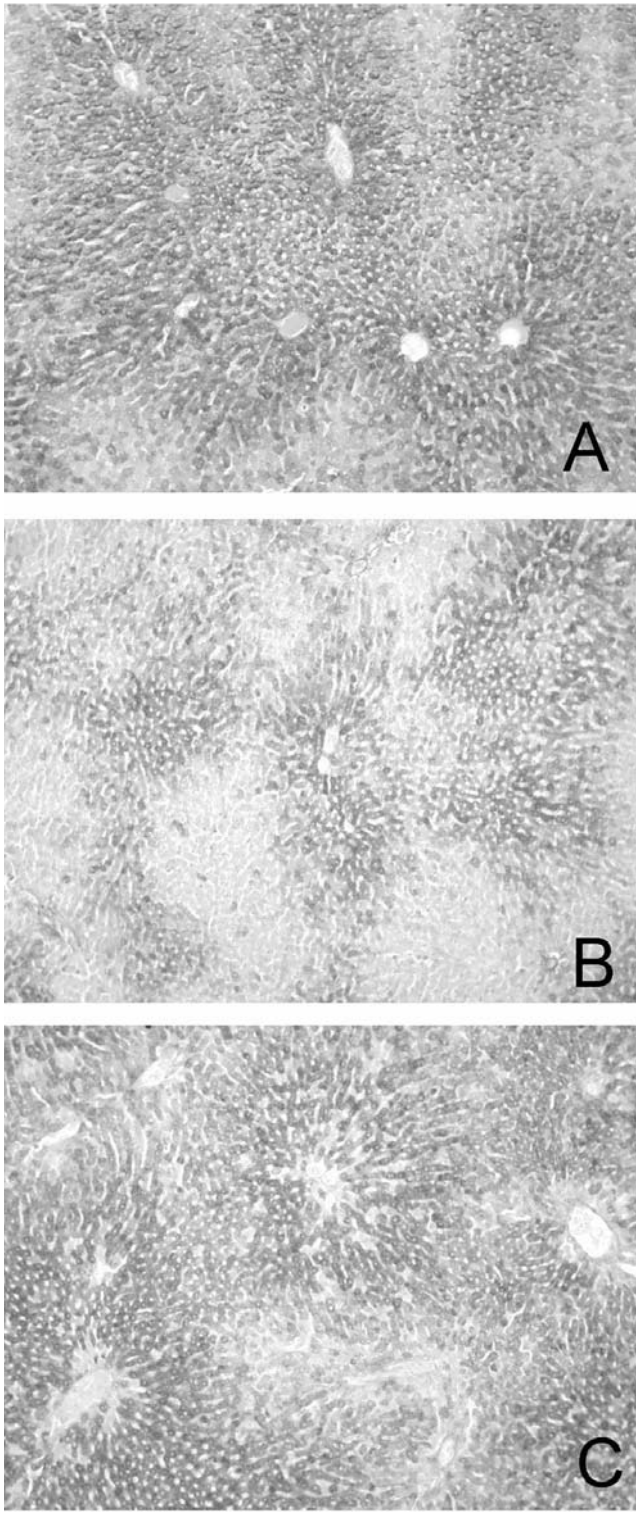

Figure 2. *PAS reaction. A. Rats treated with procainamide hydrochloride alone. Intense reactivity due to glycogen content is observed in the hepatic laminae. B. Rats treated with cisplatin. A drastic reduction of PAS-positive areas is noticed in these samples. C. Rats treated with cisplatin-procainamide hydrochloride. Most hepatocytes constituting the hepatic lobules exhibit intense PAS-positivity. (x 100).*

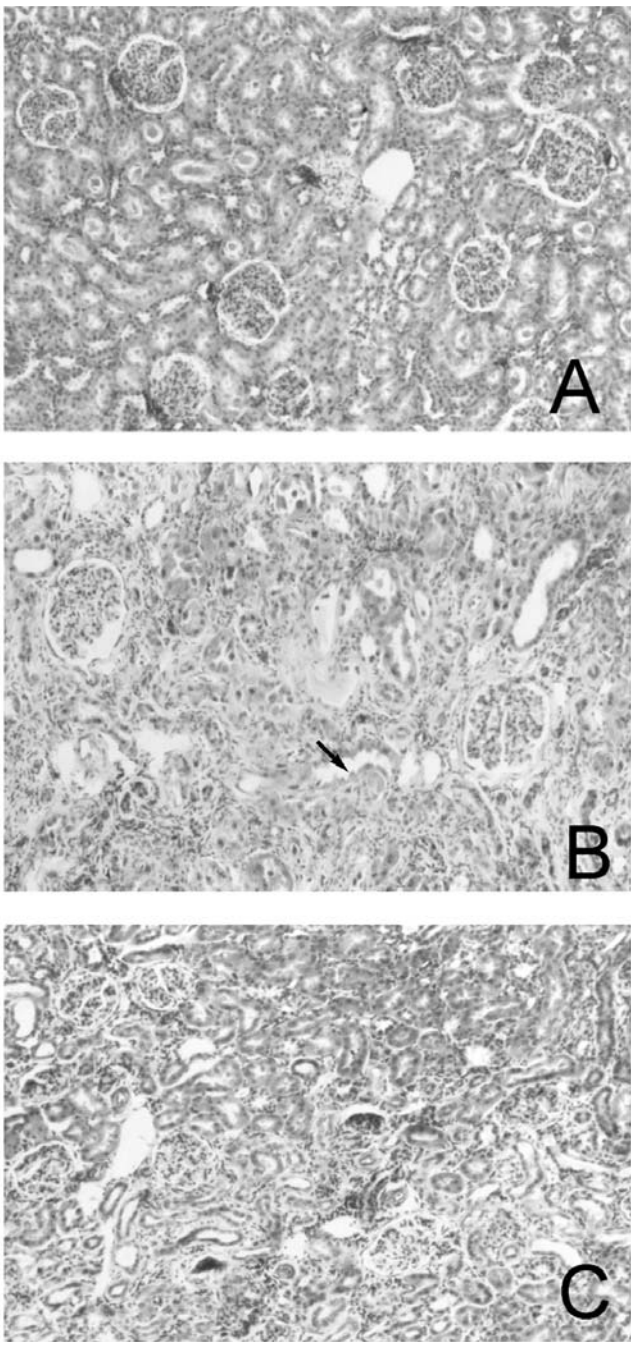

Figure 3. *H&E staining. A. Rats treated with procainamide alone. The morphology of the renal tissue appears normal. B. Rats treated with cisplatin. The epithelium constituting numerous tubules, and especially proximal tubules, shows prominent alterations (arrow). Some glomeruli show a dilated Bowman's space. C. Rats treated with cisplatin-procainamide hydrochloride. In general, the renal parenchyma shows an almost normal structure, although some tubules still present a partially damaged structure.*

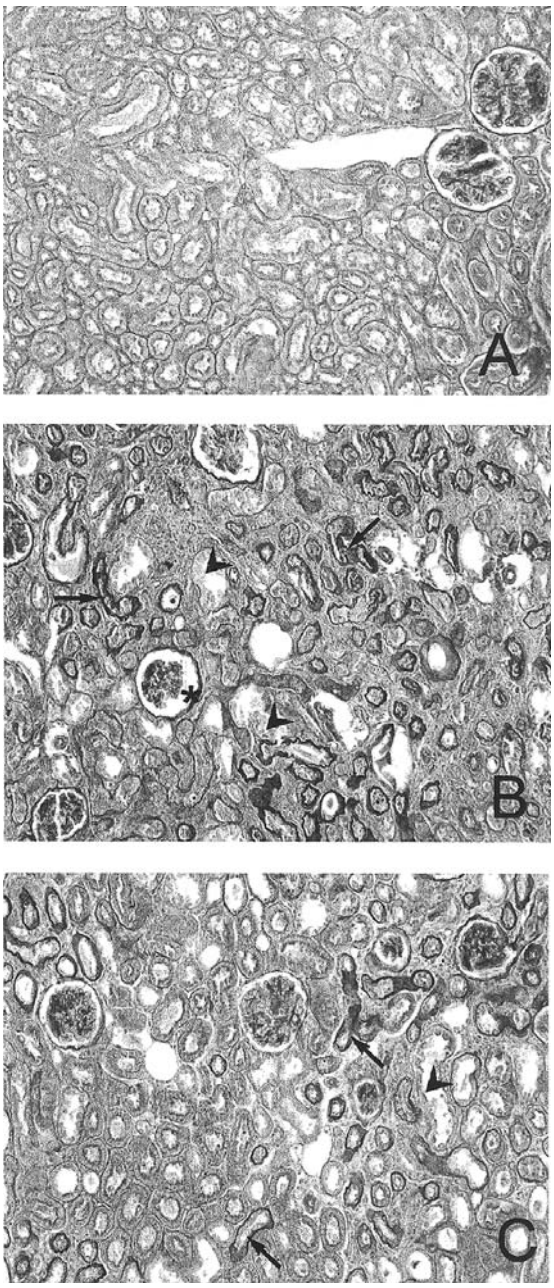

Figure 4. *PAS reaction. A. Rats treated with procainamide hydrochloride alone. The renal tubules and the glomeruli show normal morphology; The PAS-positive basement membrane appears very thin in both structures. B. Rats treated with cisplatin. The epithelium of numerous tubules appears disrupted (arrowheads) and some glomeruli show a dilated Bowman's space (asterisk). A thick basement membrane lines a great number of tubules, intensely stained by PAS reaction (arrows). C. Rats treated with cisplatin-procainamide hydrochloride. Restricted areas of the kidney parenchyma show some tubules with altered epithelium (arrowheads) and thick basement membrane (arrows) (x 100).*

particularly evident for cumulative doses higher than 100 mg/m^2, cisplatin is generally split into subdoses of 20 mg/m^2 and given for 5 consecutive days, thus decreasing the daily systemic drug concentration (20). Unfortunately, in spite of these protective strategies, the use of cisplatin is still followed by a significant percentage of grade 3-4 toxic

events or irreversible and cumulative toxicities, such as renal and neurotoxicity, which remain, together with ototoxicity, the most important dose-limiting adverse effects (21, 22).

Our previous data showed that procainamide hydrochloride may protect mice and rats from the dose-limiting nephrotoxicity and the more transient and mild hepatotoxicity.

The aim of this study was to demonstrate, from a strict histological and histochemical point of view, that procainamide hydrochloride may protect not only against acute cisplatin-induced toxicities, but also that it might represent a good option for the protection of tissues sensitive to the cumulative damaging action of repeated low doses of cisplatin. This is particularly relevant since such toxicities tend to limit subsequent treatment options and decrease the patient's quality of life. For our study, we chose to investigate the kidneys and the liver on the basis of our previous research demonstrating the protective effect of procainamide hydrochloride against the acute cisplatin-induced toxicity of these two organs. Moreover, the heart was also considered because it is the classic pharmacological target of the action of procainamide.

The morphological and histochemical analyses showed marked alterations in the kidney parenchyma of cisplatin-treated rats, whereas in the liver they were generally milder. In both organs, tissue alterations were mainly evidenced through the PAS reaction. In the liver, the far less numerous PAS-positive hepatocytes indicated a reduction in glycogen content, due to some metabolic impairment within the hepatocytes. On the contrary, dense parenchyma PAS positivity in the kidneys indicated a marked thickening of both the tubular and glomerular basement membranes, which may drastically influence the renal functions.

In rats treated with cisplatin alone, the parenchyma of the kidney and liver was clearly more affected by the treatment than respective samples obtained from animals given the combination.

It is important to underline that procainamide is characterized by a high incidence of adverse reactions when it is administered chronically. This is the case, for example, in Systemic Lupus Erythematosus-like syndrome, which may appear after months of continuous treatment (23, 24). In general, an oral dose of about 50 mg/kg should maintain the plasma concentration in the therapeutic range (6-8 μg/ml). Toxic effects on the electrical and mechanical performance of the heart become common and serious only when plasma concentrations exceed 12-16 μg/ml. However, the toxicological picture induced by the chronic administration of procainamide hydrochloride is one of the reasons why, in recent years, it has been substituted by other less toxic drugs for the treatment of arrhythmias. Nevertheless, on the basis of pharmacokinetic studies and given its chronic toxic effects, the administration of a cumulative dose of 2.5 g procainamide hydrochloride per 5 days per 6 cycles should not imply serious adverse effects in humans.

It is noteworthy that, together with significant protection, procainamide hydrochloride may also exert a valuable potentiation of the antitumour activity of cisplatin (1, 25), thus confirming the therapeutic value of its combination with cisplatin.

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