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Reduction of cisplatin hepatotoxicity by procainamide hydrochloride in rats

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Abstract

In preceding papers, we proposed that procainamide hydrochloride, a class I antiarrhythmic agent, was able to protect mice and rats from cisplatin-induced nephrotoxicity and that it could exert its action through accumulation in kidneys followed by coordination with cisplatin (or its hydrolysis metabolites) and formation of a less toxic platinum compound similar to the new platinum(II) triamine complex *cis*-diamminechloro-[2-(diethylamino)ethyl 4-amino-benzoate, N4]-chlorideplatinum(II) monohydrochloride monohydrate, obtained by the reaction of cisplatin with procaine hydrochloride. Hepatotoxicity is not considered as a dose-limiting toxicity for cisplatin, but liver toxicity can occur when the antineoplastic drug is administered at high doses. Here, we report that procainamide hydrochloride, at an i.p. dose of 100 mg/kg, reduces cisplatin-induced hepatotoxicity, as evidenced by the normalization of plasma activity of glutamic oxalacetic transaminase and γ-glutamyl transpeptidase, as well as by histological examination of the liver tissue. Twenty-four hours after i.p. treatment with the combination of 7.5 mg/kg cisplatin and 100 mg/kg procainamide, a significant increase of procainamide (+56%, $P<0.05$), total platinum (+31%, $P<0.05$), platinum–DNA adducts (+31%, $P<0.05$) and percent DNA–DNA interstrand cross-links (+69%, $P<0.02$) was found in liver tissue, as compared to animals treated with cisplatin alone. Moreover, in accordance with these findings, we also observed a slightly lower concentration and cumulative excretion of platinum in the feces. Since mitochondrial injury is considered a central event in the early stages of the nephrotoxic effect of cisplatin, the distribution of platinum in these subcellular organelles obtained from hepatocytes was determined after treatment with cisplatin with or without procainamide hydrochloride, together with platinum concentration in their cytosolic fraction. Our data show that the coadministration of procainamide hydrochloride produced a rearrangement of subcellular platinum distribution in hepatocytes with a slight decrease in mitochondria (−15%, $P<0.10$) and a slight increase in the cytosolic fraction (+40%, $P<0.10$) of platinum content, compared to the treatment with cisplatin alone. In analogy with our previous results in the kidney, confirmed here by our data in vitro, we suggest that the hepatoprotective activity of procainamide hydrochloride is linked to the formation of a less toxic platinum complex, which leads to inactivation of cisplatin itself and/or its highly toxic hydrolysis metabolites and to a different subcellular distribution of platinum.

Keywords: Cisplatin; Procainamide; Hepatotoxicity; Protection

1. Introduction

Cisplatin is one of the most active antineoplastic drugs that is particularly used for the treatment of ovarian, testicular and head and neck cancers (Van Basten et al., 1997; Thigpen et al., 1994). In spite of its significant antitumor activity, the clinical use of cisplatin is often limited by its undesirable side effects, nephrotoxicity and neurotoxicity being the most severe and dose-limiting ones (Mollman et al., 1988; Screnci and McKeage, 1999). Nevertheless, other less frequent toxic effects, as hepatotoxicity, which is frequently observed after administration of high doses of cisplatin, can alter the clinical situation of patients

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(Cersosimo, 1993; Cavalli et al., 1978; Pollera et al., 1987). Many efforts have been made to improve the therapeutic index of cisplatin using pharmacological strategies, such as the administration of chemoprotectors, intensive hydration and hypertonic saline (Skinner, 1995; Anand and Bashey, 1993; Pinzani et al., 1994). Unfortunately, some of the compounds used as chemoprotectors also inhibit the antitumor activity of cisplatin, while other therapeutic strategies were not completely efficient in reducing the dose-limiting nephrotoxicity (Aamdal et al., 1987; Jones et al., 1991; Abe et al., 1990; Pinzani et al., 1994).

In our previous studies (Esposito et al., 1996; Viale et al., 2000), we found evidence for a chemoprotective effect of procainamide hydrochloride against cisplatin-induced nephrotoxicity in mice and rats. This class I antiarrhythmic drug was able to reduce the weight loss and to protect mice against death induced by lethal doses of cisplatin. The combination therapy of procainamide hydrochloride and cisplatin resulted in a significant increase in survival, when drugs were administered either simultaneously i.p. or through different routes of administration (i.p. and i.v.). In rats, procainamide hydrochloride protected from nephrotoxicity when given simultaneously or from 1 h before to 1 h after cisplatin, as confirmed by plasma urea nitrogen and creatinine levels and microscopical analysis of kidney tissue. Moreover, procainamide hydrochloride decreased the urinary excretion of platinum and increased platinum concentration in the kidney of rats. Based on the capability of procainamide hydrochloride to react with cisplatin and its hydrolysis products, we proposed that the antiarrhythmic drug could accumulate in the kidney and coordinate with the anticancer drug forming a less toxic platinum coordination complex, which renders rats less susceptible to cisplatin-induced toxicity.

In the present paper, we studied the protective activity of procainamide hydrochloride on cisplatin-induced hepatotoxicity, in particular, on the level of cellular damage of liver parenchyma. This was determined by measuring plasma glutamic oxalacetic and glutamic pyruvate transaminases, γ-glutamyl transpeptidase and by histochemical analysis of liver tissue. The analysis in liver of total platinum, platinum–DNA adducts, percent interstrand cross-links, procainamide concentration and intracellular platinum distribution was also performed.

2. Materials and methods

2.1. Chemicals

Cisplatin and procainamide hydrochloride were purchased from Sigma (St. Louis, MO, USA). When the two drugs were administered to rats as single agents, cisplatin was dissolved in normal saline (0.9% NaCl), while the modulating agent was diluted in distilled water to prepare a 1.25% solution. Since dissolving procainamide hydrochloride in 0.9% NaCl increases the chloride anion concentration of the solution, when both drugs were administered together, they were diluted in appropriate NaCl solutions in order to obtain a final $[Cl^-]$ equivalent to that of normal saline. Each drug solution was prepared immediately before use. Methanol, acetonitrile and water of high performance liquid chromatography (HPLC) grade and Tris, acetic acid, methylene chloride, *N*-propionylprocainamide, triethylamine, zinc sulfate, potassium dihydrogen phosphate and phosphoric acid, all of analytical grade, were purchased from Sigma.

2.2. Animals

Experiments were performed using male Sprague–Dawley rats (Charles River Laboratories, Calco, Italy) with a body weight ranging from 300 to 400 g. Animals had free access to a commercial diet (4RF/25 Italiana Mangimi, Settimo Milanese, Italy) and tap water, and were kept in a temperature-controlled room. All experiments were performed in accordance with FELASA guidelines and approved by the institutional ethics committee.

2.3. Liver tissue damage

The influence of cisplatin and procainamide hydrochloride administration on liver toxicity was evaluated in normal rats ($n=4–6$) at maximal cisplatin-induced toxicity (day 5 post-treatment). Liver function was examined by measurement of the plasma activity of glutamic oxalacetic and glutamic pyruvate transaminases, as well as γ-glutamyl transpeptidase, using COBAS MIRA S Instrument (Roche Diagnostics, Rotkreuz, Switzerland).

2.4. Histopathology

Histopathologic changes in the liver of rats, sacrificed 5 days after injection of cisplatin either with or without procainamide hydrochloride, were examined. Different parts of liver were removed from rats ($n=2$) and the morphological studies were performed as described elsewhere (Viale et al., 2000). The tissue sections were examined by light microscopy at 400 × magnification.

2.5. Determination of procainamide in liver

The concentrations of procainamide hydrochloride in liver obtained from four animals were determined 24 h after i.p. injection of procainamide hydrochloride (100 mg/kg) administered alone or with cisplatin (7.5 mg/kg). Liver amples excised from rats were weighed and stored at −20 °C until analysis. Stock standard solutions of procainamide hydrochloride were prepared in water at a concentration of 200 μg/ml. A solution of *N*-propionylprocainamide (100 μg/ml) dissolved in water containing 5% ethanol was used as internal standard. Tissue samples (100–300 mg) were first homogenized in 250 μl of methanol and 500 μl Tris buffer (1

M, pH 8.5), and then allowed to stand on ice for 15 min before 1.25 ml of acetonitrile containing 100 μl of internal standard solution (100 μg/ml) were added. The mixtures were vortexed and allowed standing at room temperature for 15 min before the precipitated proteins were removed by centrifugation. Supernatants were evaporated to dryness under nitrogen stream, redissolved with 400 μl of HPLC-grade water and extracted and analyzed by HPLC method developed by Jamaly et al. (1988). Data were collected and analyzed by HP3365 series II ChemStation software (Hewlett Packard, Palo Alto, CA, USA). Retention times of procainamide and *N*-propionylprocainamide were 3.8 and 11.6 min, respectively. Recovery of procainamide hydrochloride from tissue was about 70% and the detection limit was 10 ng/ml.

2.6. Quantitation of total platinum, platinum–DNA binding and percent interstrand cross-links

The effect of procainamide hydrochloride on the accumulation of platinum in liver and feces as well as total DNA platination and DNA–DNA interstrand cross-link (ISCL) formation in liver was further examined. Twenty-four hours and five days after i.p. treatment with cisplatin (7.5 mg/kg) either alone or combined with procainamide hydrochloride (100 mg/kg), small pieces of liver from six to seven rats per group were collected. DNA was isolated from the tissue by the salting out technique (Gao et al., 1990). Briefly, tissue fragments (about 100 mg) were homogenized (Homogeniser Kinematica, Luzern, Switzerland) in 15-ml vials containing 3–4 ml of phosphate-buffered saline. Homogenized cells were then pelleted and washed twice in phosphate-buffered saline by centrifugation at 750 × *g* for 10 min. Six milliliters of a lysis solution (10 mM Tris/HCl, pH 8; 2 mM EDTA, 400 mM NaCl), 240 μl proteinase K (Boehringer Mannheim, Mannheim, Germany) and 800 μl 10% sodium dodecyl sulphate (Biorad Laboratories, Richmond, CA, USA) were added. The solution was mixed gently and left overnight in a water bath at 37 °C. After incubation, 2 ml of sodium acetate saturated solution was added and mixed vigorously for 15 s. Vials were then centrifuged at 2500 rpm for 30 min, supernatants recovered and DNA precipitated with one volume of isopropyl alcohol. Once isolated, DNA was dissolved overnight in Tris–EDTA solution (10 mM Tris/HCl, pH 8; 1 mM EDTA, pH 8) at 50 °C. DNA yield and purity were measured by absorbance at $\lambda = 260/280$ nm [the purity of DNA was on average 2.02 ± 0.07 (S.D.)]. DNA was then processed either to determine the total DNA platination or the percentage of ISCL. For the analysis of total platination, DNA was digested in 14 M nitric acid and the residue diluted in 10 mM nitric acid. Bound platinum was determined by flameless atomic absorption spectroscopy (AAS). The remaining part of the tissues was weighed and digested in 14 M nitric acid at 120 °C. The residue was diluted in 10 mM nitric acid and platinum content evaluated by AAS. Platinum content of feces was determined by AAS after digestion of feces in 14 M nitric acid at 120 °C and dilution of the residue in 5 M nitric acid.

The %ISCL in the kidney was evaluated in three to four rats per group by ethidium bromide fluorescence technique (Coluccia et al., 1995). DNA was resuspended in Tris–EDTA, and its yield and purity were estimated as before. Three milliliters of a solution containing ethidium bromide (0.5 mg/ml in 0.4 mM EDTA, 20 mM dipotassium phosphate, pH 11.8) was added to 0.2 ml (20 μg) aliquots of DNA extracted from cells. The fluorescence was measured before and after heating at 90 °C for 10 min (Perkin-Elmer LS-5B spectrofluorimeter; excitation wavelength, 525 nm; emission wavelength, 580 nm). The %ISCL was determined by the formula:

$$(f_t - f_n)/(1 - f_n) \times 100$$

where f_t and f_n represented the fluorescence after denaturation divided by the fluorescence before denaturation of treated (f_t) and control (f_n) samples.

2.7. Preparation of mitochondrial and cytosolic fractions

Liver tissue was homogenized in 4 mM NaCl solution (1 ml/g tissue) with a Potter homogenizer. The homogenate was centrifuged at 750 × *g* for 15 min at 4 °C. The resultant supernatant was centrifuged at 10 000 × *g* for 15 min at 4 °C, and the pellet resuspended in 4 mM NaCl solution and referred to as mitochondrial fraction. The supernatant obtained by the first step was further centrifuged at 105 000 × *g* for 1 h at 4 °C, and the supernatant so obtained considered as cytosolic fraction. The protein content of mitochondrial and cytosolic fractions were determined by the Bradford's method (Sigma). The analysis of platinum content was assayed as previously described and referred to the protein content of samples.

2.8. Interaction study between cisplatin and procainamide hydrochloride in the cytosol fraction of hepatocytes in vitro

To investigate the ability of procainamide hydrochloride to react with cisplatin in hepatic cytosol, a study was performed in vitro, following the time course of the reaction

Table 1
GPT, GOT and GGT plasma activities (IU/ml) of rats 5 days after i.p. administration of cisplatin (7.5 mg/kg), procainamide hydrochloride (100 mg/kg) or combined treatment

Treatment	GPT	GOT	GGT
Saline	50 ± 1[a]	143 ± 20	0.8 ± 0.8
Procainamide hydrochloride	63 ± 9	174 ± 26	1.3 ± 0.6
Cisplatin	83 ± 27	385 ± 105[b]	5.8 ± 1.3[b]
Cisplatin–procainamide hydrochloride	45 ± 5	184 ± 43[c]	2.2 ± 1.2[c]

[a] Mean ± S.E.M. ($n = 4–6$).

[b] $P < 0.05$, compared to controls, as calculated by ANOVA (GOT, $P = 0.028$; GGT, $P = 0.010$) followed by Newmann–Keuls test.

[c] $P < 0.05$, compared to cisplatin alone, as calculated by ANOVA followed by Newmann–Keuls test.

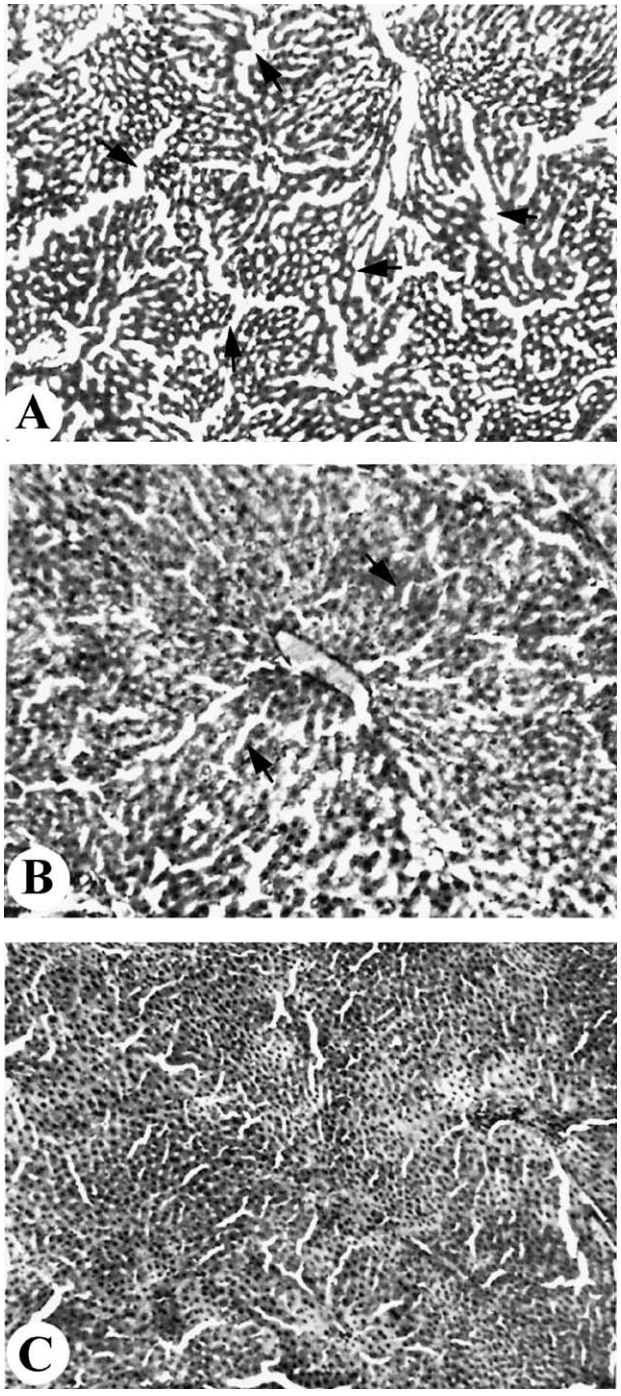

Fig. 1. Light micrographs of the liver from rats 5 days after treatment. In A, rats were treated with 7.5 mg/kg cisplatin. Parenchyma shows structural modifications of perilobular connective and hepatic lobules. Perilobular connective appears larger than normal and portal spaces are expanded. The lobules show an irregular shape and are crossed by trabeculae of smaller hepatocytes delimiting ipertrophic sinusoids (arrows). Central veins show a normal structure. Only about 20% of parenchyma appears without structural modifications. In B, rats were treated with 100 mg/kg procainamide hydrochloride. Only small alterations, characterized by expanded sinusoids with hepatic laminae regulary set (arrows), involved 10–15% parenchyma. In C, rats were administered cisplatin–procainamide hydrochloride. The normal structure of parenchyma increases to about 80–85%, presenting only some alterations of sinusoidal lumen. Original magnification, 400 ×.

as previously made in NS (Viale et al., 2000). Cisplatin (0.5 mg/ml) and procainamide hydrochloride (3.5 mg/ml) were incubated in hepatic cytosol by maintaining the reaction mixture for 24 h in a water bath at 37 °C. At definite time intervals (3, 4, 6 and 24 h), aliquots were withdrawn and analyzed by HPLC. Each sample (100 μl) was deproteinated with 5% zinc sulfate solution (100 μl) and acetonitrile (100 μl), and after centrifugation, the clear supernatant (10 μl) was injected into HPLC.

The HPLC system consisted of an HP1090 instrument equipped with a diode array detector (Hewlett Packard). The acquisition wavelength was 206 nm. The separation of cisplatin, procainamide hydrochloride and reaction compound in samples was achieved by reverse-phase chromatography using a chemically bonded stationary phase, LiChrospher 100 CN (Merck). The column was operating at room temperature. The mobile phase was phosphate buffer 0.01 M at pH 5.0; flow rate was 1.0 ml/min.

2.9. Statistical analysis

Data were analyzed by both one-way analysis of variance ANOVA followed by a multiple comparison procedure (Newmann–Keuls test) and unpaired Student's *t*-test. The level of significance was set at $P < 0.05$.

3. Results

3.1. Evidence of liver tissue damage

In our experiments, the high dose of cisplatin (7.5 mg/kg) administered to rats caused an evident liver damage characterized by a significant increase of glutamic oxalacetic trans-

Table 2
Platinum distribution, %ISCL and procainamide level in liver of rats 24 h after i.p. treatment with cisplatin (7.5 mg/kg), procainamide hydrochloride (100 mg/kg) or combined treatment

	Treatment[a]		
	Cisplatin	Procainamide hydrochloride	Cisplatin–procainamide hydrochloride
Total platinum (μg Pt/g tissue)	3.9 ± 0.4[b]	–	5.1 ± 0.3[c]
Platinum–DNA adducts (pg Pt/μg DNA)	23.6 ± 2.6	–	30.9 ± 1.9
%ISCL	2.6 ± 0.1	–	4.4 ± 0.4[d]
Procainamide (μg/g tissue)	–	144 ± 8	225 ± 33[e]

[a] Procainamide was given alone or in the same solution with cisplatin immediately after mixing.

[b] Mean ± S.E.M. ($n = 6–7$, for total platinum and platinum–DNA adducts; $n = 3–4$, for %ISCL and procainamide).

[c] $P < 0.05$ (Student's *t*-test), compared to cisplatin-treated group of rats.

[d] $P < 0.02$ (Student's *t*-test), compared to cisplatin-treated group of rats.

[e] $P < 0.02$ (Student's *t*-test), compared to procainamide hydrochloride-treated group of rats.

Table 3
Platinum accumulation in liver tissue 5 days after combined i.p. treatment with cisplatin (7.5 mg/kg) and procainamide hydrochloride (50, 100 and 200 mg/kg)

		Cisplatin plus procainamide hydrochloride at		
	Cisplatin	50 mg/kg	100 mg/kg	200 mg/kg
Total platinum (μg Pt/g tissue)	0.79 ± 0.08[a]	1.29 ± 0.16	1.84 ± 0.36	2.30 ± 0.43[b]

[a] Mean ± S.E.M. ($n = 4-5$).
[b] $P < 0.05$, compared to cisplatin alone, as calculated by ANOVA ($P < 0.05$) followed by Newmann–Keuls test.

aminase [385 ± 105 vs. 143 ± 20 IU/ml in controls] and γ-glutamyl transpeptidase [5.8 ± 1.3 vs. 0.8 ± 0.8 IU/ml in controls] plasma activities. A slight, but not significant, elevation of plasma glutamic pyruvate transaminase [83 ± 27 vs. 50 ± 1 IU/ml in controls] was also observed (Table 1). It is of note that the treatment with procainamide hydrochloride alone did not significantly alter these parameters, while its coadministration significantly reduced their increase due to cisplatin. This allows the normalization of these parameters to values not greatly different from those observed in procainamide hydrochloride-treated rats (glutamic oxalacetic transaminase,184 ± 43 vs.174 ± 26 IU/ml; γ-glutamyl transpeptidase, 2.2 ± 1.2 vs. 1.3 ± 0.6 IU/ml; glutamic pyruvate transaminase, 45 ± 5 vs. 63 ± 9 IU/ml) (Table 1). Similar results were also obtained by the histological analysis of liver tissue, as shown in Fig. 1 (see legend).

3.2. Determination of procainamide, total platinum, platinum–DNA binding and %ISCL

Twenty-four hours after treatment, we found in liver a situation similar to that observed in kidney (Viale et al., 2000), with a significant increase of total platinum content (+31%, $P < 0.05$), total DNA platination (+31%, $P < 0.05$) and %ISCL (+69%, $P < 0.02$) (Table 2). The effect of procainamide hydrochloride on liver platinum accumulation was also confirmed by the observation of a significant ($P < 0.05$) correlation between platinum content and the administered dose of procainamide hydrochloride (50, 100 and 200 mg/kg) 5 days after treatment (Table 3). In particular, when rats were administered cisplatin plus 100 mg/kg procainamide hydrochloride, we found twice the amount of platinum in liver tissue than when given cisplatin alone [0.79 ± 0.08 (S.E.M.) vs. 1.84 ± 0.36 (S.E.M.) μg/g tissue].

3.3. Determination of fecal platinum excretion

On the basis of the higher accumulation of platinum in liver tissue after coadministration of procainamide hydrochloride and cisplatin, we expected a reduction of the fecal excretion of platinum. The analysis of platinum content of feces of four rats treated with cisplatin, with or without procainamide hydrochloride, revealed a trend in the reduction of platinum concentration in animals coadministered with procainamide hydrochloride [40.7 ± 12.5 (S.E.M.) vs. 21.7 ± 3.9 (S.E.M.) μg Pt/g feces, $P < 0.10$], as well as a slight, but not significant, reduction of its percent cumulative excretion [7.9 ± 2.5% (S.E.M.) vs. 5.8 ± 2% (S.E.M.)].

3.4. Analysis of platinum in mitochondrial and cytosolic fractions

The analysis of platinum distribution in the subcellular fractions of hepatocytes showed that the coadministration of procainamide hydrochloride produced a trend towards a decrease of platinum content in mitochondria and an accu-

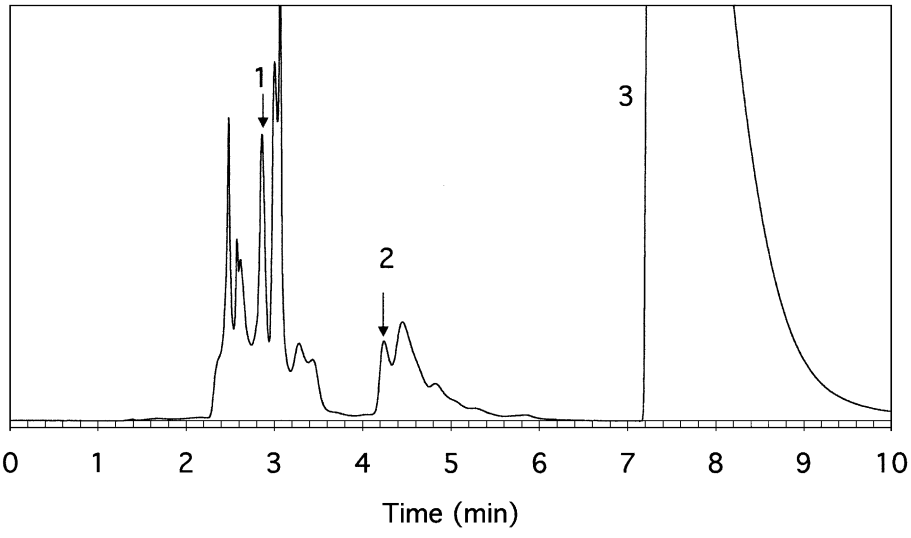

Fig. 2. Chromatogram from the reaction mixture of cisplatin and procainamide hydrochloride in hepatic cytosol after 3 h of incubation in vitro at 37 °C. Peak 1, cisplatin; peak 2, reaction product; peak 3, procainamide hydrochloride.

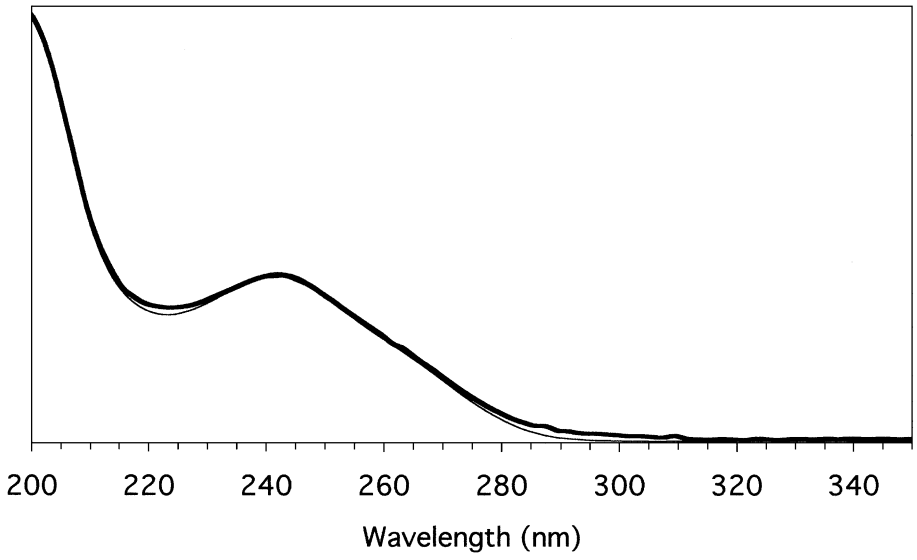

Fig. 3. UV spectra of the reaction product between cisplatin and procainamide hydrochloride: after 3 h of incubation in hepatic cytosol at 37 °C (thick line); after incubation in NS at the same temperature (thin line) (Viale et al., 2000).

mulation in the cytosolic fraction. In particular, in mitochondria, platinum concentration decreased from 25 ± 0.3 (S.E.M.) to 21.2 ± 1.3 (S.E.M.) ng Pt/mg protein ($P < 0.10$), while in the cytosolic fraction, the concentration of platinum increased from 8.6 ± 0.8 (S.E.M.) to 12 ± 1.1 (S.E.M.) ng Pt/mg protein ($P < 0.10$).

3.5. Interaction in vitro of cisplatin and procainamide hydrochloride

In Fig. 2, the chromatogram obtained from the aliquot of reaction mixture at 3 h is depicted. The mobile phase allowed a good separation of the compounds of interest, being effective in overcoming interferences by residual proteins in the sample. Peaks of cisplatin [retention time (r_t) = 2.8 min], procainamide (r_t = 7.3 min) and the hypothetical coordination complex (r_t = 4.2 min) were easily detectable. The latter was sufficiently resolved to give a UV spectrum (Fig. 3) substantially superimposable to that of the reaction product in NS previously described (Viale et al., 2000) and, in turn, very similar to that of the new platinum(II) triamine complex *cis*-diamminechloro-[2-(diethylamino)ethyl 4-amino-benzoate, N4]-chlorideplatinum(II) monohydrochloride monohydrate, obtained by synthesis from cisplatin and procaine hydrochloride (Cafaggi et al., 1992). The new platinum compound was still present, although reduced, at 24 h from starting of incubation.

4. Discussion

It is known that cisplatin is significantly taken up in human liver (Smith and Taylor, 1974); nevertheless, hepatotoxicity rarely occurs. For this reason, this toxic effect has not received much attention and only few articles deal with this aspect. Generally, liver toxicity of cisplatin is characterized by mild to moderate elevation of serum transaminases and, less frequently, by a mild elevation of serum alkaline phosphatase, lactate dehydrogenase, bilirubin and γ-glutamyl transpeptidase levels. Jaundice has rarely been described as consequence of elevated serum bilirubin (Cavalli et al., 1978). Some reports suggest that cisplatin-induced hepatotoxicity may be dose-related (Vermorken and Pinedo, 1982; Hesketh et al., 1990), reaching an incidence of 86% in patients treated with a single dose of 100 mg/m^2 cisplatin. Although hepatotoxicity has always been described of mild and of transient nature, at least when cisplatin is not administered at high doses, particular attention should be paid to the additional effects due to the coadministration of other more hepatotoxic agents. Little is known about the mechanism of cisplatin-induced liver damage, although apoptotic lesions seem to characterize the damaged liver parenchyma. Moreover, metallothioneins seem to play an important role in protecting against cisplatin-induced hepatotoxicity (Liu et al., 1998).

Our previous studies concerning the use of procainamide hydrochloride as a chemoprotector for cisplatin-induced nephrotoxicity suggested the possibility that this drug could also protect other organs against the toxic activity of this platinum compound. Our data about cisplatin-induced hepatotoxicity confirm that procainamide hydrochloride is able to counteract this toxic effect, as demonstrated by the normalization of parameters such as plasma glutamic oxalacetic transaminase and γ-glutamyl transpeptidase, as well as histological analysis of liver tissue.

In a previous paper (Viale et al., 2000), we described the significant increase of platinum content in kidneys of rats treated with cisplatin plus procainamide hydrochloride, compared to animals given cisplatin alone. This effect was also linked to a slight, although not significant, increase in

total DNA platination and formation of ISCL. When procainamide hydrochloride was administered together with cisplatin, we found in liver a similar, although not identical, situation characterized by a significant increase of platinum content (above all at day 5 after treatment), DNA platination and %ISCL. In general, it is possible to state that, in spite of the indicated small differences, both liver and kidney exhibit similar characteristics concerning platinum accumulation and binding to DNA. Moreover, after coadministration of cisplatin and procainamide hydrochloride, a significant elevation of the procainamide liver concentration was observed (Table 2), compared to procainamide hydrochloride-treated animals.

On the basis of the accumulation of platinum in liver, we expected that the feces of rats treated with cisplatin and procainamide hydrochloride contained less platinum than the feces from cisplatin-treated animals. Although we found only a trend for the decrease of platinum concentration, data obtained from the metabolic cages confirmed our hypothesis and that the antiarrhythmic drug determines the accumulation of platinum in liver without an increase of its toxic effects.

Many papers (Zhang and Lindup, 1993, 1996; Brady et al., 1990; Giurgiovich et al., 1997) report the important role of mitochondria as early event for the nephrotoxic activity of cisplatin. This drug causes mitochondrial lipid peroxidation, impairment of ATPase activity, depletion of glutathione, damage of mitochondrial DNA, disruption of intracellular Ca^{2+} homeostasis and reduction of state 3 renal mitochondrial respiration. The damage at the level of mitochondrion is the first step for the well-known nephrotoxic effect of cisplatin. Similarly to renal tubule cells, hepatocytes also contain a high number of mitochondria. For this reason, we studied the influence of procainamide hydrochloride on platinum distribution in these subcellular organelles and in the cytosolic fraction of liver cells. Our data suggest that the antiarrhythmic drug can alter the distribution of platinum not only on nuclear DNA but also in other subcellular structures. Whatever is the origin of platinum found in mitochondria and cytosol, it is important to observe that procainamide hydrochloride alters the subcellular distribution of platinum determining a decrease of potentially toxic platinum species in the most important target of cisplatin toxicity.

One of the key points of procainamide hydrochloride renal protection is linked to the presence of an organic cation transport system responsible for the urinary excretion of cisplatin or its metabolites. The mutual interference of cisplatin and procainamide at this level generates platinum (Viale et al., 2000) and procainamide (unpublished observation) accumulation in kidney. Similar transport systems for organic cations were also found in hepatocytes (Meijer, 1987; Meijer et al., 1990), where they are involved in the carrier-mediated uptake and secretion of drugs, toxic agents or endogenous substances in the bile. Similarly to the situation in the kidney, it is possible that mutual competition of cisplatin and/or its metabolites and procainamide for transport across the canalicular cell membrane occurs. This effect could in part explain the increased accumulation of platinum and procainamide in liver and the concomitant slight decrease of platinum excretion through the feces, in the first 24 h after treatment.

We already demonstrated (Viale et al., 2000) that procainamide hydrochloride is able to react in vitro with cisplatin and its toxic hydrolysis products forming a platinum compound similar to *cis*-diamminechloro-[2-(diethylamino)ethyl 4-amino-benzoate, N4]-chlorideplatinum(II) monohydrochloride monohydrate. This compound, whose acronym is DPR, is a new antiblastic monofunctional platinum triamine complex that, compared to the parent compound cisplatin, induces fewer nephrotoxic and neurotoxic effects (Cafaggi et al., 1992; Zhang et al., 1996; Mandys et al., 1998) and has a greater ability to accumulate into the cells and platinate DNA (Viale et al., 1995). Our data in vitro obtained in normal hepatic cytosol confirm the possibility that a DPR-like compound may be formed in the cytosolic fraction, thus suggesting, together with our other data on liver platinum accumulation and DNA binding, that this platinum compound may be produced in liver cells and actively take part in the hepatoprotective activity of procainamide hydrochloride.

Altogether, our data suggest that procainamide hydrochloride can reduce the toxic activity of cisplatin in liver, probably by a mechanism similar to that observed in kidney. This phenomenon is linked to the formation of a less toxic platinum compound, although the different distribution of platinum in the mitochondrial and cytosolic fractions suggests that the antiarrhythmic drug can also protect the liver by inducing a redistribution of potentially damaging platinum metabolites in subcellular organelles. Although hepatotoxicity is not considered a dose-limiting toxic effect of cisplatin, reduction of this side effect through coadministration of procainamide hydrochloride illustrates its potential for a more general control of cisplatin toxicity.

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