Background: Acute renal failure is a dose-limiting factor of cisplatin chemotherapy. Here, we show the protective effect of ozone oxidative preconditioning against cisplatin-induced renal dysfunction in rats. Ozone oxidative preconditioning is a prophylactic approach, which favors the antioxidant–pro-oxidant balance for preservation of the cell redox state by increasing antioxidant endogenous systems in various *in vivo* and *in vitro* experimental models.
Aims: To analyze the protective role of ozone oxidative preconditioning against cisplatin-induced nephrotoxicity.
Methods: Male Sprague–Dawley rats were pretreated with 15 intrarectal applications of ozone/oxygen mixture at 0.36, 0.72, 1.1, 1.8 and 2.5 mg/kg before cisplatin intraperitoneal injection (6 mg/kg). Serum and kidneys were extracted and analyzed 5 days after cisplatin treatment for determinations of the renal content of glutathione, thiobarbituric acid-reactive substances, renal concentration and enzymatic activities of catalase, superoxide dismutase and glutathione peroxidase.
Results: Ozone pretreatment prevented the increase in serum creatinine levels, the glutathione depletion and the inhibition of superoxide dismutase, catalase and glutathione peroxidase activities induced by cisplatin in the rat kidney. Also, the renal content of thiobarbituric acid-reactive substances was decreased by ozone therapy. These protective effects of ozone were dose dependent.
Conclusions: Intrarectal ozone therapy prevented effectively the renal antioxidant unbalance induced by cisplatin treatment.

Key words: Ozone oxidative preconditioning, Cisplatin-induced nephrotoxicity, Antioxidant defenses, Lipid peroxidation

Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats

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Introduction

Cisplatin (CDDP) is an effective chemotherapeutic agent commonly used in the treatment of a variety of solid-organs cancers, including those of the head, neck, testis, ovary and breast.[1] Unfortunately, 28–36% of patients receiving a dose of CDDP develop acute renal failure.[1] Therefore, its pathogenesis has been the focus of many investigations, which have shown that acute renal failure induced by CDDP is accompanied by the reduced renal blood flow associated with increased renal vascular resistance and histological damage to the proximal tubular cells.[2,3]

Additionally, evidence is available that the cellular events in CDDP-mediated nephrotoxicity, including apoptosis induction, decreased protein synthesis, membrane peroxidation, mitochondrial dysfunction and DNA injury, are a consequence of reactive oxygen species (ROS) generation, which produces oxidative renal damage.[4–9] Thus, administration of CDDP causes depletion of renal reduced glutathione (GSH) and an increase of lipid peroxidation accompanied with a decrease in the activity of enzymes that protect against lipid peroxidation in the kidney, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px).[4–9]

In agreement with this, administration of known antioxidant molecules like vitamin E, ascorbic acid, ebselen, lipoic acid, GSH and its esters, CAT and the SOD mimetic orgotein, among others, have shown nephroprotective effects in CDDP-treated rats.[10–16]

Recently it was reported that oxidative preconditioning with ozone prevents renal damage in rats submitted to warm ischemia,[17] suggesting that oxidative preconditioning with ozone may provide a prophylactic approach for minimizing renal damage before transplantation, which is also mediated by ROS production. Taking into account these findings, we decided to evaluate the role of ozone oxidative

preconditioning in CDDP-induced acute renal damage in rats and to determine its potential protective effects on some important constituents of the antioxidant system in the kidney.

Materials and methods

Chemicals

Serum creatinine (Cr) was measured spectrophotometrically with the Cr assay kits purchased from Biological Products Enterprise 'Carlos J Finlay' (Havana, Cuba). All reagents used in determinations of GSH, SOD, CAT, GSH-Px, thiobarbituric acid-reactive substances (TBARS) and cisplatin were purchased from Sigma Chemicals (St Louis, MO, USA). Other reagents of analytical grade were obtained from normal commercial sources.

Animals

Male Sprague–Dawley rats (200–250 g) were obtained from the National Center for Laboratory Animal Production (CENPALAB, Havana Cuba). The animals were housed under a 12 h light–dark cycle with room temperature maintained at 25°C, humidity at 60% and food and water available *ad libitum*. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the National Center for Scientific Research, Havana, Cuba.

Experimental design

Ozone (O_3) was generated by an OZOMED 01 equipment manufactured by the Ozone Research Center (Cuba). O_3 obtained from medical-grade oxygen (O_2) was used immediately. The O_3 concentration was measured using an ultraviolet spectrophotometer at 254 nm. An O_3/O_2 mixture was administered by rectal insufflations (i.r.) at doses of 0.36, 0.72, 1.1, 1.8 and 2.5 mg/kg. The volume of insufflated mixture was approximately 9 ml. Oxidative preconditioning was performed with 15 applications (one daily) of the O_3/O_2 mixture. CDDP was applied 1 day after the 15 applications of the O_3/O_2 mixture by an intraperitoneal injection (6 mg/kg). Five days after CDDP injection, animals were sacrificed by asphyxia in ether.

The animals were divided into 10 groups of seven rats each: (1) non-treated control rats, (2) rats treated with O_2, (3) rats treated with O_3/O_2 mixture (1.1 mg/kg), (4) rats treated with O_2+CDDP, (5) rats treated only with CDDP, (6) rats treated with O_3/O_2 mixture (10 μg of ozone/ml of mixture at a dose of 0.36 mg/kg)+CDDP, (7) rats treated with O_3/O_2 mixture (20 μg of ozone/ml of mixture at a dose of 0.72 mg/kg)+CDDP, (8) rats treated with O_3/O_2 mixture (30 μg of ozone/ml of mixture at a dose of 1.1 mg/kg)+CDDP, (9) rats treated with O_3/O_2 mixture (50 μg of ozone/ml of mixture at a dose of 1.8 mg/kg)+CDDP, and (10) rats treated with $O_3 O_2$ mixture (70 μg of ozone/ml of mixture at a dose of 2.5 mg/kg)+CDDP. Rats were killed 5 days after CDDP injection by an overdose of ether. The blood was collected and serum was separated by centrifugation for Cr analysis. The kidneys were dissected and immediately frozen at −20°C until analysis could be completed.

Kidney homogenates were obtained using a tissue homogenator (Ultraturrax T-25 Polytron) at 4°C. The homogenates were prepared by using a 100 mM KCl buffer (pH 7) containing 0.3 mM ethylenediamine tetraacetic acid (1:10 w/v) for GSH, TBARS, GSH-Px and SOD determinations (Buffer 1). The homogenates were spun down with a centrifuge at $600 \times g$ for 60 min at 4°C. The supernatants were taken for biochemical determinations.

Kidney homogenates for CAT enzymatic assay were carried out using 50 mM phosphate buffer (pH 7) containing 1% Triton X-100 (1:9 w/v) (Buffer 2). The homogenates were centrifuged at $600 \times g$ for 60 min at 4°C and the supernatants were used for the CAT assay.

Determination of GSH

GSH was determined by a slightly modified version of the method of Beutler *et al.*,[18] using a spectrophotometer. One milliliter of the kidney homogenate, as described earlier, was mixed with 1.5 ml of 5% metaphosphoric acid and centrifuged at $3000 \times g$ for 10 min at room temperature. Fifty hundred microliters of this acidic supernatant was mixed with 2 ml of 0.2 M phosphate buffer and 0.25 ml of 0.04% 5, 5′-dithio-bis(-2-nitrobenzoic acid). Absorbance of the yellow solution was measured at 412 nm within 10 min. A molar extinction coefficient of 13.6 M/cm that describes the formation of the thiolate anion by the reaction of sulfhydryl groups with 5,5′-dithio-bis-2-nitrobenzoic acid at 412 nm was used to quantify GSH.

Determination of SOD activity

Enzymatic activity of SOD was determined by a modified version of the method of Minami and Yoshikawa.[19] Fifty microliters of the kidney homogenate was mixed with 450 μl of cold deionized water, 125 μl of chloroform and 250 μl of ethanol. The mixture was centrifuged at $8000 \times g$ for 2 min at 4°C. Fifty hundred microliters of the extract was added to the reaction mixture containing 500 μl of 72.4 mM Tris cacodylate buffer with 3.5 mM diethy-

lene pentaacetic acid (pH 8.2), 100 µl of 16% Triton-X 100 and 250 µl of 0.9 mM nitro blue tetrazolium. The reaction mixture was incubated for 5 min at 37°C before adding 10 µl of 9 mM pyrogallol (dissolved in 10 mM hydrochloric acid), and then it was incubated for exactly 5 min at 37°C. The reaction was stopped with the addition of 300 µl of 2 M formic buffer (pH 3.5) containing 16% Triton-X 100. The absorbance was measured at 540 nm in the spectrophotometer. One unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitro blue tetrazolium by 50%.

Determination of CAT activity

CAT was determined according to the method of Evans and Diplock.[20] Kidney homogenate was diluted with Buffer 2, as already described, to obtain an adequate dilution of the enzyme. Then, 2 ml of the enzyme dilution were added to the cuvette and mixed with 1 ml of 30 mM H_2O_2, and then the absorbance was measured at 240 nm, for 30 sec, in the spectrophotometer. Initial absorbance of the reaction mixture must be around 0.5. The enzyme activity is expressed as the first-order constant that describes the decomposition of H_2O_2 at room temperature.

Determination of GSH-Px activity

Enzymatic activity of GSH-Px was measured using a modified version of the method of Thonson *et al*.[21] All reaction mixtures were dissolved in 20 mM sodium phosphate buffer containing 6 mM ethylenediamine tetraacetic acid (pH 7.0). The reaction mixture consisted of 98.8 µl of phosphate buffer, 700 µl of 2.86 mM GSH, 100 µl of 1 mM sodium azide, 100 µl of 1 mM NADPH and 4.2 µl of GSH reductase (0.5 u). Then, 10 µl of the tissue homogenate supernatant were added to the reaction mixture and incubated at room temperature for 10–15 min. Afterward, 10 µl of 30 mM *t*-butyl hydroperoxide (dissolved in bidistilled water) was added to the reaction mixture and measured at 340 nm for 7 min in the spectrophotometer. A molar extinction coefficient of 6.22×10^3 M/cm was used to determine the activity of GSH-Px. The enzyme activity is expressed as international units of enzymatic activity per milligram of protein. International units are expressed as micromoles of hydroperoxides transformed per minute per milliliter of enzyme.

Lipid peroxidation assay

This assay was used to estimate TBARS levels as described by Ohkawa *et al*.[22] Two hundred milliliters of tissue homogenate supernatant were added to 100 µl of sodium dodecyl sulfate, 750 µl of 20% acetic acid (pH 3.5), 750 µl of 0.6% thiobarbituric acid and 300 µl of distilled water, and were incubated at 95°C for 60 min. The samples were allowed to cool at room temperature. Then 2.5 ml of butanol:pyridine (15:1) and 500 µl of distilled water were added, vortexed, and centrifuged at $2000 \times g$ for 15 min. The absorbance of three ml of the colored layer was measured at 532 nm spectrophotometrically using 1,1,3,3-tetraethoxypropane as standard.

Protein assay

Protein concentrations were determined by the method of Lowry[23] using bovine serum albumin as standard.

Histopathological assessment of renal damage

The left kidneys were quickly removed and fixed in 10% formaldehyde. Tissues were embedded in paraffin, sectioned at 3 µm, stained with hematoxylin and eosin (H/E) and evaluated by light microscopy.

Statistical analysis

Data are expressed as means ± standard error of the mean and analyzed statistically using one-way analysis of variance followed by the Duncan multiple range test for serum Cr determinations, whereas the Kruskall–Wallis test followed by the Mann–Whitney test was applied for the rest of the markers. The 0.05 level of probability was used as statistical significance.

Results

Loss of body weight in all animals was observed 5 days after CDDP injection. The mean reduction in body weight was approximately 16 g, which represents 8% of the initial body weight. This effect on body weight was not reversed in animal groups under O_3 therapy with mixtures of O_3/O_2 administered by rectal insufflation.

The levels of Cr in the blood serum of control and experimental rats are presented in Table 1. After CDDP injection, Cr levels increased about four-fold at day 5 with respect to non-treated controls. However, there were no significant differences in the concentrations of Cr in rat serum between the non-treated controls and those only treated with O_2. No significant differences in Cr levels were found between the group treated with CDDP alone and the other one with CDDP + O_2. In contrast, oxidative preconditioning with O_3/O_2 mixture during 15 days at doses of 0.72 and 1.1 mg/kg but not 0.36 mg/kg induced a remarkable decrease in Cr levels in serum with

respect to the higher values in CDDP group. Greater doses of 1.8 and 2.5 mg/kg did not significantly decrease Cr (Table 1).

The concentration of renal reduced GSH was significantly decreased (58% of non-treated control) in CDDP-treated rats. Ozone therapy (0.72, 1.1, 1.8 and 2.5 mg/kg i.r.) prevented renal GSH depletion induced by CDDP. This effect was greater at doses of 0.72 and 1.1 mg/kg ($p < 0.01$). The lowest dose of O_3 (0.36 mg/kg) was not effective in the prevention of GSH depletion.(Table 1).

The kidney TBARS content used as a measure of lipid peroxidation was significantly increased in rats treated with CDDP alone, as compared with non-treated controls. Oxidative preconditioning with O_3/O_2 mixture during 15 days induced significant decrease in TBARS content at O_3 doses of 0.72 mg/kg ($p < 0.05$), 1.1 mg/kg ($p < 0.001$), 1.8 mg/kg ($p < 0.001$) and 2.5 mg/kg ($p < 0.01$) as compared with CDDP alone. The lower O_3 dose (0.36 mg/kg) did not induce any significant change on renal TBARS content. The data indicate that the induction of lipid peroxidation made by CDDP in the kidney is attenuated by O_3 therapy in a dose-dependent manner (Table 1).

SOD activity in the kidney was significantly decreased (71% of non-treated control) in CDDP-treated rats (Table 1). Pretreatment with O_3/O_2 mixtures (0.72 and 1.1 mg/kg) significantly increased ($p < 0.01$) SOD activity in CDDP-treated animals, which was close to values of non-treated control. However, the lower O_3 dose (0.36 mg/kg) and the greater ones (1.8 and 2.5 mg/kg) did not avoid the decreasing in SOD activity. On the contrary, the greatest O_3 tested dose (2.5 mg/kg) induced a remarkable and significant decrease ($p < 0.01$) of SOD activity (3.05 ± 0.48) versus (6.2 ± 0.9) in the CDDP-treated control, which provides evidence of its deleterious effect on this antioxidant enzyme.

CAT activity in the rat kidney was significantly decreased (63% of non-treated control) in CDDP-treated rats. Pretreatment with O_3 (0.72, 1.1, 1.8 and 2.5 mg/kg, but not 0.36 mg/kg) significantly prevented ($p < 0.05$) the inhibitory effect of CDDP on CAT activity (Table 1).

GSH-Px activity in the kidney was significantly decreased (82% of non-treated control) in CDDP-treated rats. O_3 pretreatment (0.72 and 1.1 mg/kg) significantly increased, almost twice ($p < 0.01$), the GSH-Px activity with respect to both CDDP-treated control rats and non-treated rats. The greater O_3 doses (1.8 and 2.5 mg/kg) induced a minor increase on the enzyme activity (Table 1).

The histopathological changes in the kidney tissue are reported in Fig. 1. Non-treated rats exhibited cellular tumefaction, probably due to the type of euthanasia (asphyxia by ether) (Fig. 1A). Rats treated with CDDP alone revealed intense tubular necrosis, desquamation of renal tubular cells, and cast formation in the lumen, as reported in previous works (Fig. 1B). O_3-treated rats with 1.1 mg/kg showed a moderate cellular tumefaction, revealing no significant differences between them and non-treated rats. At greater doses of 1.8 and 2.5 mg/kg doses, histopathological changes in renal tissue were quite similar to those present in CDDP-treated rats. O_3 oxidative preconditioning provided significant protection against CDDP-induced nephrotoxicity, and the majority of the treated animals showed no significant histopathological alterations (Fig. 1C).

Discussion

The aim of this work was to determine the effect of O_3 oxidative preconditioning on the severity of CDDP-induced nephrotoxicity. In the present study, CDDP-induced acute kidney damage was character-

Table 1. Biochemical determinations in experimental groups

Experimental groups	Serum Cr levels (μM)	GSH (nmol/mg of protein)	TBARS (nmol/mg of protein)	SOD (SOD units/mg of protein)	CAT (k_{15}/g of wet tissue)	GSH-Px (IU/mg of protein)
Non-treated control	67.9＋11.73*	9.3＋0.90*	0.25＋0.014*	8.7＋1.15*	6.8＋0.39 *	6.0＋0.25*
Oxygen-treated control	70.6＋6.61*	8.9＋0.96 *	0.26＋0.012*	8.8＋1.26*	6.7＋0.63*	6.1＋0.14*
Ozone-treated control	69.2＋7.61*	8.5＋0.47*	0.31＋0.034*	8.6＋1.72*	6.7＋1.56*	6.1＋0.14*
CDDP control	280.6＋45.05	5.4＋0.76	0.63＋0.12	6.2＋0.9	4.3＋0.74	5.3＋0.69
O_2−CDDP control	288.4＋44.03	4.9＋0.81	0.55＋0.029	6.6＋0.94	4.3＋0.49	5.5＋0.55
O_3 (0.36 mg/kg)＋CDDP	294.9＋47.70	6.6＋1.34	0.54＋0.077	6.8＋1.23	5.2＋0.79	5.9＋0.98
O_3 (0.72 mg/kg)＋CDDP	106.0＋29.78*	12.3＋1.17*	0.48＋0.049	9.1＋0.81*	5.5＋0.67*	10.5＋1.03*
O_3 (1.1 mg/kg)＋CDDP	130.4＋30.41*	13.4＋2.7*	0.29＋0.062*	9.1＋1.01*	6.0＋0.54*	7.9＋1.32*
O_3 (1.8 mg/kg)＋CDDP	288.4＋47.81	10.9＋1.57*	0.22＋0.03*	6.1＋0.9	5.9＋0.57*	5.8＋0.89 *
O_3 (2.5 mg/kg)＋CDDP	392.9＋47.56	10.6＋1.76 *	0.28＋0.033*	3.0＋0.48*	6.1＋64*	5.7＋0.62 *

Data presented as (mean±standard error of the mean. Serum Cr was measured with the Cr assay kits (Biological Products Enterprise 'Carlos J Finlay', Havana, Cuba). GSH was determined by the method of Beutler *et al.*[18] Enzymatic activity of SOD was determined by a version of the method of Minami and Yoshikawa[19] (one unit of SOD enzymatic activity is equal to the amount of enzyme that diminishes the initial absorbance of nitro blue tetrazolium by 50%). CAT was determined according to the method of Evans and Diplock[20] (the enzyme activity is expressed as the first-order constant that describes the decomposition of hydrogen peroxide at room temperature). Enzymatic activity of GSH-Px was measured using a modified version of the method of Thonson *et al.*[21] (the enzyme activity is expressed as international units of enzymatic activity per milligram of protein). TBARS levels were estimated by Ohkawa *et al.*[22] Protein concentrations were determined by the method of Lowry.[23] * Significant differences with CDDP-treated control groups ($p < 0.05$).
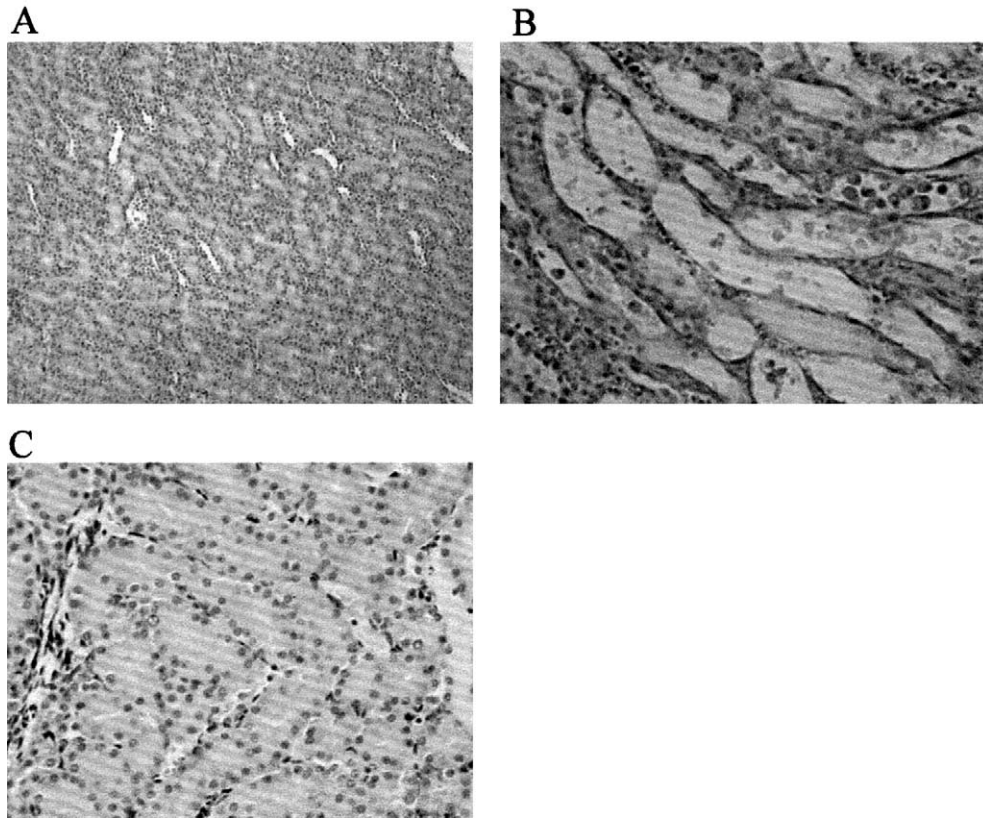
FIG. 1. Histopathological changes in rat kidneys after CDDP administration (6 mg/kg) and under i.r. O_3 therapy. (A) Light micrograph of rat kidneys from the control group. Slight cellular tumefaction ascribed to the type of euthanasia (asphyxia by ether). H/E stain, × 100. (B) Light micrograph from a CDDP-treated rat. Note the widespread tubular necrosis and cast formation in the tubular lumen. H/E stain, × 100. (C) Light micrograph of a rat kidney, which received 15 applications of O_3 (1.1 mg/kg) by rectal insufflation before CDDP injection. Moderate cellular tumefaction was observed. H/E stain, × 100.

ized by a significant increase in serum Cr, decreases in the kidney GSH content and GSH-Px, SOD and CAT activities, and an increase in TBARS production in comparison with the non-treated controls. Histopathological examination of the kidneys of CDDP-treated rats revealed marked tubular necrosis and desquamation of renal tubular cells. These results are in accordance with previous reports.[4–16]

The mechanisms underlying CDDP-induced nephrotoxicity have not been fully elucidated. Several investigators have shown that ROS are closely related to the nephrotoxicity induced by CDDP.[5–7,14] It has also been reported that the inordinate generation of ROS contributes to the initiation and/or maintenance of acute tubular necrosis.[5–7,14] Indeed, several antioxidants such as SOD, ebselen, vitamin E and vitamin C are reported to attenuate cisplatin-induced renal toxicity.[10–16] Several reports demonstrate different mechanisms involved in the CDDP-induced nephrotoxicity including protein kinase C activation,[24] elevation of caspase-3 activity resulting in renal cell apoptosis,[8] enhancement of intrarenal synthesis of thromboxane A_2[25] and dyslipidemia.[26]

Oxidative preconditioning is analogous to other phenomena such as ischemic preconditioning,[27] thermal preconditioning[28] and chemical preconditioning.[29] All of these processes have in common that a repeated and controlled stress is able to protect against a prolonged and severe stress.

Intrarectal O_3 therapy has conferred protection against hepatic ischemia/reperfusion injury by the adenosine accumulation and by blocking the xanthine/xanthine oxidase pathway, decreasing ROS generation after reperfusion.[30–32] Recently, it was demonstrated that intrarectal application of an O_3/O_2 mixture reduced ROS by stimulation and/or preservation of the endogenous antioxidant systems in experimental models of liver and renal ischemia-reperfusion, respectively.[30–32] Also, there is evidence of an increase in the activity of antioxidant enzymes such as SOD and GSH-Px and a decrease of malondialdehyde after O_3 preconditioning in patients with cardiopathy and in rats suffering ischemia/reperfusion damage.[17,33]

Our experimental results clearly demonstrate that oxidative preconditioning with O_3 exerts protective effects in CDDP-induced acute nephrotoxicity in rats. Furthermore, the data provide strong evidence that i.r. O_3 therapy effectively prevented a decrease in the renal antioxidant defense system and certainly avoided the deleterious effect of CDDP on it.

Thus, the pretreatment with O_3/O_2 mixture prevented GSH depletion and the decrease of SOD, CAT and GSH-Px activities induced by CDDP in rat kidney. Also, renal TBARS content was diminished by O_3 therapy. These protective effects of O_3 were dose dependent. The lowest dose of 0.36 mg/kg did not induce any significant change in the former antioxidant markers. In contrast, the doses of 0.72 and 1.1 mg/kg significantly reduced serum Cr and TBARS levels and preserved some constituents of the antioxidant defense, such as GSH content, SOD, CAT and GSH-PX activities, which were levels close to or even greater than those of non treated controls (Table 1).

Furthermore, the preservation of renal SOD, CAT and GSH-PX activities by O_3 pretreatment at lower doses (0.72 and 1.1 mg/kg) shows that this agent can protect these enzymes even 5 days after CDDP administration. Neither significant change of GSH and TBARS renal content nor statistic differences in CAT activity were found at the greater ozone doses of 1.8 and 2.5 mg/kg as compared with that of 1.1 mg/kg, which indicates that the 'plateau effect' in the dose–response curves was reached.

Induction of CAT, SOD, and GSH-Px by O_3 therapy is probably due to hydrogen peroxide produced as result of O_3 decomposition. Hydrogen peroxide has been shown to be one of the major intermediates of O_3 decomposition along with oxygen radicals (OH$^\bullet$ and $O_2{}^\bullet$).

In contrast, the greatest O_3 dose (2.5 mg/kg) induced a marked and significant decrease of SOD activity (3.05 ± 0.48 u/mg of protein) as compared with non-treated controls (8.72 ± 1.15 u/mg of protein), which reveals deleterious effects of ozone treatment on this enzyme, which is probably due to SOD inactivation by H_2O_2 at greater concentrations as it has been demonstrated by Marklund.[34] This author suggested that inactivation of Cu Zn SOD by H_2O_2 appears to be due to a Fenton-type reaction of H_2O_2 with Cu, forming a reactive intermediate that destroys an essential liganding histidine residue of the enzyme. Whiteside and Hassan also demonstrated induction and inactivation of CAT and SOD by ozone in cultures of *Escherichia coli*.[35]

Furthermore, they showed that an increase in the activities of CAT and SOD by O_3 was due to induction of the novo enzyme synthesis rather than activation of pre-existing apoproteins. Thus, the observed induction of SOD, CAT and GSH-Px in response to O_3 pretreatment provides further evidence that there is a correlation between antioxidant enzyme biosynthesis and O_3 exposure.

Additionally, these authors also showed that the antioxidant enzymes are subjected *in vivo* to the damaging effects of O_3 at relatively high doses. Therefore, in concordance with our results this observation provides evidence that O_3, besides

inducing the biosynthesis of these enzymes, could also cause inactivation of them by the mechanism previously explained.

In addition, O_3 oxidative preconditioning might prevent CDDP-induced acute renal failure through attenuation of renal tubular damage and enhancement of the regenerative response of the damaged tubular cells. As shown in Fig. 1, animals treated with i.r. O_3 therapy before CDDP recovered the normal structure of its kidneys 5 days after CDDP administration, showing a moderate cellular tumefaction, in contrast with CDDP-treated rats, which showed severe acute tubular necrosis.

In conclusion, a single dose of CDDP leads to the inhibition of renal antioxidant enzyme activity, depletion of renal GSH levels, increased serum Cr and enhanced renal lipid peroxidation, which cause nephrotoxicity. Our results support the evidence that part of the mechanism of nephrotoxicity in CDDP-treated rats is related to depletion and inhibition of the antioxidant system. Therefore, the prevention of this inhibition in rats protected by i.r. O_3 therapy support the potential use of it at low doses to ameliorate CDDP-induced renal injury.

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