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Scavenging Action of Zinc and Green Tea Polyphenol on Cisplatin and Nickel Induced Nitric Oxide Generation and Lipid Peroxidation in Rats

SEEMA JOSHI[‡], S. K. HASAN[+], RAMESH CHANDRA[+], M. M. HUSAIN[+], AND R. C. SRIVASTAVA [*]

[+]*Industrial Toxicology Research Centre, P. O. Box. 80, M. G. Marg, Lucknow 226 001, India;*
[‡]*Isabella Thoburn P. G. College, Faizabad Road, Lucknow 226 007, India*

Objective Toxic metal ions have been implicated in the generation of reactive oxygen species (ROS) and nitric oxide (NO). Metallothionines (MT) and plant flavonoids have been reported in the intervention against oxidative damage. We investigated the effect of zinc induced MT and green tea polyphenol (GTP) in reducing the oxidative responses induced by nickel and platinum. **Methods** Zinc (10 mg/kg b. wt, sc) was administered to rats twice at a gap of 24hrs and GTP (10 mg/100 mL in drinking water) was fed ad libitum for 8 days. Nickel chloride (150 umol/kgb.wt, ip) and cisplatin (50 μmol/kg b.wt, sc) was administered to rats 24 h after Zn or GTP pre-treatment. Animals of all the groups were sacrificed 16 hrs after treatment and biochemical markers for toxicity were monitored. **Results** Zinc or GTP pre-treatment caused significant protection against nickel or cisplatin enhanced mortality in rats, and reduction in lipid peroxidation and NO. **Conclusion** It is proposed that inhibition of ROS and NO by GTP and zinc may prove useful as a selective pharmacological agent in the amelioration of metal toxicity.

Key words: Green tea polyphenol; Zinc; Nitric oxide; Lipid Peroxidation; Nickel; Cisplatin

INTRODUCTION

Nitric Oxide pathways indicate a general regulatory function, which if altered, could contribute to the genesis of a wide variety of diseases[1-5]. Lipid peroxidation is a sensitive marker of oxidative damage to cellular membranes involving ROS and/or NO.

Cis-diamminedichloroplatinum (cisplatin), a drug of choice for the treatment of tumors, selectively and persistently inhibits the synthesis of DNA and RNA[6-8]. However the adverse effects and toxicity to organs, such as kidney, gastrointestinal tract, bone marrow, etc. limits its clinical usefulness[9-13]. We have provided evidence that NO may play a crucial role towards the development and/or exacerbation of cisplatin induced pathological conditions[11,12].

Studies have demonstrated that nickel salts, an industrial health hazard, enhance

[*] Correspondence should be addressed to Dr. R. C. Srivastava, Scientist 'F' and Area Coordinator, Analytical Toxicology, Industrial Toxicology Research Centre, P. O. Box 80, M. G. Marg, Lucknow, 226 001, UP., INDIA. Phone (Nos):+91-522-2213786, 2227586-246 and 2213175 (D). Fax: +91-522-228227, 22847. E-mail: rc_srivastava1 @rediffmail.com

Biographical note of the first author: Dr (Mrs) Seema JOSHI, Ph. D., is associate professor in the department of chemistry, I. T. College, Lucknow University, Lucknow, India. Research interest is in the field of Environmental and Chemical Toxicology.

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peroxidation of lipid rich membrane[14-17]. There are reports indicating that ROS are the proximal lipid oxidants which may have relevance to nickel mediated carcinogenecity[14,18-21]. Nickel salts have also been shown to enhance NO production *in vitro* and i*n vivo* situations[16,22]. This had led to investigations of possible therapeutic intervention aimed at reducing the extent of oxidative injury through antioxidants.

Naturally occurring plant phenols, such as tannic acid, quercitin and green tea polyphenols (GTP), possessing antioxidant, antimutagenic and anticarcinogenic activities have been shown to modulate NO production and other biochemical responses under various predisposing conditions[23-25]. In view of the low toxicity and potential therapeutic significance of GTP, we studied its antioxidant potential in modulating cisplatin and nickel induced NO production and lipid peroxidation in rats and compared its efficacy with physiological antioxidant, Zn-MT.

MATERIALS AND METHODS

Chemicals

Nickel (II) chloride hexahydrate was obtained from Fluka (Switzerland) and Dowex-5OW (200-400, 8% cross linked Na^+ form) from Biorad, USA. Cisplatin, L-NMMA, dithiothreitol (DTT), ethylene glycol- bis-(β-aminoethyl ether)-N,N,N,N, tetraacetic acid (EGTA), and ethylene diaminetetraacetic acid sodium salt (EDTA), were obtained from Sigma-Aldrich (St. Louis, USA). GTP was obtained from Natural Resources and Products International (Rockville, Maryland, USA). L-[^{14}C] arginine (sp. activity 300 mCi/m mol) was obtained from Amersham, UK. All the other chemicals of highest purity grade including zinc (II) sulphate were obtained from BDH (India) or SRL (India).

Animals and Treatments

Eight week-old male albino wistar rats were obtained from the Industrial Toxicology Research Centre (ITRC) Breeding colony and had free access to pellet diet (Nav Maharashtra Chakan Oil Mill Ltd., Pune, India) and water. Animal experimentation was approved in accordance with the policy laid down by the Animal Care Committee of ITRC, Lucknow. The summary of the experimental design and its objective are given in Table 1.

To evaluate the protective effect of GTP and Zn on nickel or cisplatin-induced biochemical parameters in rats, animals were randomly divided into nine groups consisting of 8 rats in each. Animals in first three groups respectively received saline, nickel (150 μmole/kg, bwt, ip) or cisplatin (50 μmole/kg, bwt, sc) only to serve as controls. The animals of group 4, 5, and 6 were administered two consecutive doses of Zn (II) sulphate (10 mg/kg, bwt. subcutaneously) at a gap of 24 h. Previous studies have shown that zinc-metallothionein (Zn-MT) level peaks 24 h after second dose of zinc (II) sulphate administration and remains elevated upto 72 h. Twenty four h after the second dose of zinc, animals of group 5 and 6 received respectively single injection of nickel (150 μmole/kg, bwt, ip) and cisplatin (50 μmole/kg bwt, sc) while the animals of group 4 received normal saline and served as a positive control. Animals in the remaining three groups (7, 8, and 9) received oral feeding of GTP (10 mg/100 mL, w/v, *ad libitum*) in drinking water consecutively for eight days. Animals of group 8 and 9 received respectively identical dose of nickel or cisplatin on day 9, post GTP treatment while animals of group 7 received normal saline and served as a positive control. Animals of all the groups were sacrificed 16 h after the treatment, and their organs

were removed immediately, cleaned free of extraneous material, homogenized and processed for sub-cellular fractionation.

TABLE 1

Summary of Experimental Design and Their Objectives

Group	Treatment(s) Schedule and Dose/kg B. wt.					
	Saline	$NiCl_2$	Cisplatin	Zinc	GTP	Objectives
1	Saline	-	-	-	-	To Serve as Control
2	Saline	150 μmol	-	-	-	To Evaluate the Effect of Nickel Alone on Mortality in Rats, Lipid Peroxidation and NO-synthase Activity.
3	Saline	-	50 μmol	-	-	To Evaluate the Effect of Cisplatin Alone on Mortality in Rats, Lipid Peroxidation and NO-synthase Activity.
4	Saline	-	-	2×10 mg	-	To Serve as Positive Control
5	Saline	150 μmol	-	2×10 mg	-	To Evaluate the Protective Effect of Zinc Pre-treatment on the Nickel Induced Mortality in Rats, Lipid Peroxidation and NO-synthase Activity.
6	Saline	-	50 μmol	2×10 mg	-	To Evaluate the Protective Effect of Zinc Pre-treatment on the Cisplatin Induced Mortality in Rats, Lipid Peroxidation, NO-synthase. Activity.
7	Saline	-	-	-	10 mg/100 mL in Drinking Water *ad libtum* for 8 Day	To Serve as Positive Control
8	Saline	150 μmol	-	-	10 mg/100 mL in Drinking Water *ad libtum* for 8 Day	To Evaluate the Protective Effect of GTP on Nickel Induced Mortality in Rats, Lipid Per-oxidation and NO-synthase Activity.
9	Saline	-	50 μmol	-	10 mg/100 mL in Drinking Water *ad libtum* for 8 Day	To Evaluate the Protective Effect of GTP Pre-treatment on Cisplatin Induced Mortality in Rats, Lipid Per-oxidation and NO-synthase Activity.

Note. *$NiCl_2$ or cisplatin was administered as a single dose. Zinc was administered twice at a gap of 24 h. Single dose of $NiCl_2$ and cisplatin was administered to rats 24 h after Zn or GTP treatment.

To evaluate the protective effect of zinc or GTP pre-treatment on nickel or cisplatin induced mortality in rats, similar protocol was followed as described for biochemical parameters (Table 1) except that all animals were observed for survival upto day 14 post-metal salt treatment. Mean survival time (MST) of the treated animals was calculated as given in Table 2.

Lipid peroxidation was measured in whole homogenate of liver and kidney in terms of thiobarbituric acid reacting species (TBARS) as described earlier[17]. The results are expressed as n mol malondialdehyde formed /g wet tissue. Metallothionein concentration was estimated by Cd – haem method in liver tissue as described earlier[17]. The activity of Ca^{2+}-dependent nitric oxide synthase (c-NOS) and Ca^{2+}-independent nitric oxide synthase (i-NOS) activity in the cytosol fraction of kidney and liver was quantified by measuring the conversion of L [^{14}C]arginine to L[^{14}C]citrulline in the presence of saturating concentrations of the enzyme and co-factors essentially as described earlier[11,12]. The activity of the c-NOS was determined from the difference between L-[^{14}C]citrulline produced from control samples and samples containing 1 mmol/L EGTA. The activity of i-NOS was determined

from the difference between samples containing 1 mmol/L EGTA and samples containing 1 mmol/L EGTA and 1 mmol/L L-NMMA. The analysis of significance of difference between the groups was performed by means of Student's *t*-test[26].

TABLE 2

Effect of Zn and GTP Pre-treatment on the Protection Against Cisplatin or Nickel-mediated Mortality in Rats

Group	Treatment(s)	No. of Survivors/ Total No. of Rats	% Survival	MST In Days
1	Saline	12/12	100	14.0
2	Ni	7/12	58	8.9
3	Cisplatin	5/12	41	6.2
4	Zn	12/12	100	14.0
5	Zn+Ni	10/12	83	12.1
6	Zn+Cisplatin	8/12	67	10.4
7	GTP	12/12	100	14.0
8	GTP+Ni	10/12	83	12.7
9	GTP+Cisplatin	9/12	75	11.8

Note. Animals were observed for their survival upto day 14 after treatment(s). The dose and duration of exposure are given under material and methods. Mean survival time (MST) was calculated by counting the total no. of deaths occurring each day and the figure was multiplied by the number of days that the animals remained alive. The process of computation was continued until day 14, when all surviving animals were scored as having succumbed on that day. The MST of a group of animals is expressed as a quotient obtained by dividing the total number of days rats were observed by the number of animals in the group.

RESULTS

Administration of Zn(II) SO_4 (2×10 mg zinc/kg bwt. sc) to rats caused 12 fold induction in the level of hepatic MT (150±12.8 mg/g wet tissue) at 48 h compared to the saline treated control (Table 2, Gp. -4). The dose of GTP was well tolerated by all the animals with no apparent sign of toxicity (Table 2, Gp. -7).

The results as shown in Table 2 reveal that administration of nickel or cisplatin caused 42% and 59% mortality in rats respectively by day 14 compared to the saline treated control. Zinc pre-treatment resulted in the significant protection against nickel or cisplatin enhanced mortality in rats as evidenced by 83% and 67% survival of rats. Oral feeding of GTP (10 mg /100 mL, w/v, in drinking water for eight days *ad libitum*) was equally effective in enhancing the survival of rats (83% and 75%) as evidenced by the enhanced MST of nickel and cisplatin treated rats (Table 2).

The results shown in Fig. 1 reveal that nickel administration significantly enhanced hepatic lipid peroxidation by 4.5 fold and cisplatin by two fold compared to the normal saline treated groups. Zinc pre-treatment caused significant reduction in nickel or cisplatin induced hepatic lipid peroxidation (49% and 21%) and renal peroxidation (16% and 23%). GTP pre-treatment also caused significant reduction in nickel and cisplatin enhanced hepatic (66% and 33%) and renal (32% and 41%) lipid peroxidation.

Results shown in Fig. 2 indicate that the administration of nickel significantly induced only i-NOS activity in both liver and kidney. The increase in the nickel induced i-NOS activity in liver was more than three fold compared to kidney. Cisplatin treatment also led to significant enhancement of i-NOS activity in both liver and kidney. Zinc and GTP pre-treatment was also effective in reducing i-NOS activity in both liver and kidney of

nickel or platinum treated rats.

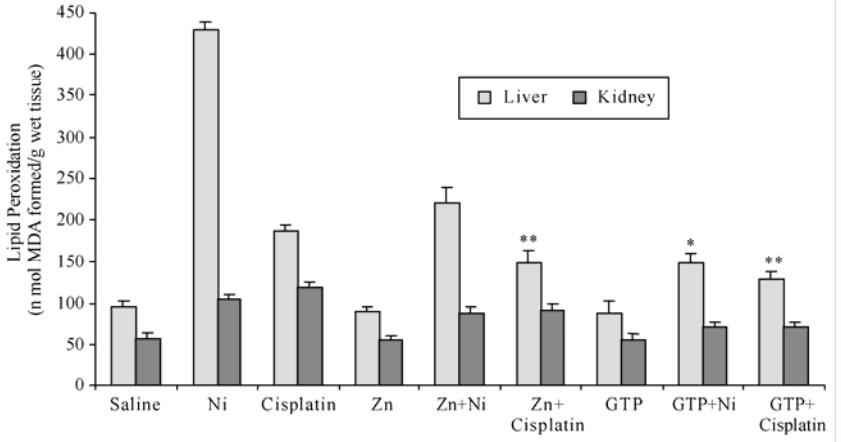

FIG. 1. Effect of Pre-treatment of Zn and GTP on the protection against cisplatin or Nickel enhanced hepatic and renal lipid peroxidation in rats. (Each value represents $\bar{x} \pm s$ of six animals). [a]$P<0.05$ when compared to nickel treated rats; [b]$P<0.05$ when compared to cisplatin treated rats; * = a, ** = b.

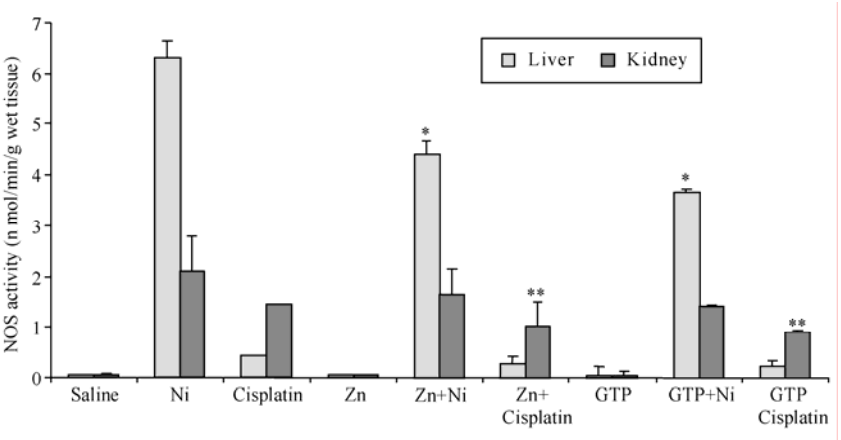

FIG. 2. Effect of Pre-treatment of Zn and GTP on the protection against cisplatin or nickel enhanced hepatic and renal nitric oxide synthase activity in rats (Each value represents $\bar{x}+s$ SE of six animals). [a]$P<0.05$ when compared to nickel treated rats; [b]$P<0.05$ when compared to cisplatin treated rats; *=a, **=b.

DISCUSSION

Substantial evidence exists for the involvement of metal ions in the generation of ROS in biological systems[14,15,20,27,28]. A few reports have demonstrated the role of metals in the modulation of NO[11,12,15,16,22]. Despite known physiological functions of NO, its role in cytotoxic events is acquiring increased significance. The most striking feature of NO is its reactivity with O^-_2 to form peroxynitrite anion ($ONOO^-$), a powerful oxidant. Under physiological pH, $ONOO^-$ dissociates to produce OH^- radical and NO_2 radical, the molecules with high potency to damage cells/tissues[29].

Lipid peroxidation is a sensitive marker of injury to cellular membranes involving oxygen-derived free radicals. The present observations on the significant enhancement of lipid peroxidation along with the enhanced production of NO clearly demonstrate the role of NO, and possibly of superoxide radical (O^{-}_{2}) as well, in the development of nickel or cisplatin-induced toxic responses. We have earlier hypothesized that NO production may be responsible for the development of complications associated with exposure of cisplatin to rats[11,12]. These results are in close agreement with earlier reported observations that blocking the production of NO by NOS inhibitors either prevented or reduced the toxic response of a chemical[2,5,12,16].

Results of the present study confirm earlier observation that Zn-MT acts as an efficient interceptor of OH^{-} and O^{-}_{2} hence serving as physiological antioxidant in nickel and cisplatin- induced peroxidation of lipid rich membrane[17]. The attenuation of nickel or cisplatin- induced i-NOS activity by GTP or Zn-MT further supports their role as an effective scavenger of NO[30]. Thus, GTP and Zn-MT may prove to be a better scavenger of oxidative response in view of their ability to scavenge OH, O^{-}_{2}, and NO radicals.

The results showing protective effect of Zn-MT suggest that an enhanced concentration of Zn in the target organs prior to cisplatin or nickel treatment may be responsible for reducing toxic responses of the latter. Zn-MT has been shown to be an efficient physiological antioxidant due to high content of cysteine residues[17]. The significant inhibition of renal and hepatic lipid peroxidation induced by cisplatin or nickel on Zn-MT treatment adds support for proposed role for Zn in the stabilization of membrane integrity possibly through the displacement of the redox active metal ions such as Fe and Cu from the ROS sensitive sites in the liver and kidney[11,17].

The protective effect of oral intake of GTP, a phenolic antioxidant with known anti-carcinogenic properties, has been accounted for at least in part, by their ability to prevent oxygen and nitrogen radical-induced cytotoxicity[23-25,31]. A significant protection afforded by GTP pre-treatment to nickel or cisplatin-treated rats, as evidenced by the inhibition in lipid peroxidation, may be attributed to the lowering in the concentration of ROS. Marked inhibition of nickel or cisplatin-induced i-NOS activity has strengthened our assumption that these phenolic antioxidants may not only act as ROS scavengers but also play a role in destabilizing the free radical reaction involving NO and O^{-}_{2}. However, in the absence of the measurement of anti-oxidant enzymes in the present study, it is premature to indicate which combination of catalytic alterations is responsible for its anti-oxidant behaviour during nickel or cisplatin toxicity.

The physiological antioxidant appears more effective than the known inhibitors of NO in view of their ability of scavenging O^{-}_{2} and $OH^{\cdot}$ etc. In view of the wide spread roles of NO in pathogenesis, it is proposed that inhibition of i-NOS by GTP or zinc treatment may prove useful as a selective pharmacological agent because of their ability to inhibit the *in vivo* production of NO.

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