Nutrition and Cancer
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t775653687

Diallyl Disulfide Prevention of Cis-Diammine Dichloroplatinum-Induced Nephrotoxicity and Leukopenia in Rats: Potential Adjuvant Effects

Jéssica P. Chiarandini Fiore [a]; Silvia L. Fanelli [a]; Elida C. de Ferreyra [a]; José A. Castro [a]
[a] Centro de Investigaciones Toxicológicas (CEITOX): CITEFA/CONICET, Buenos Aires, Argentina

Online Publication Date: 01 November 2008

To cite this Article Chiarandini Fiore, Jéssica P., Fanelli, Silvia L., de Ferreyra, Elida C. and Castro, José A.(2008)'Diallyl Disulfide Prevention of Cis-Diammine Dichloroplatinum-Induced Nephrotoxicity and Leukopenia in Rats: Potential Adjuvant Effects',Nutrition and Cancer,60:6,784 — 791

To link to this Article: DOI: 10.1080/01635580802100869

URL: http://dx.doi.org/10.1080/01635580802100869

Nutrition and Cancer, 60(6), 784–791

ISSN: 0163-5581 print / 1532-7914 online
DOI: 10.1080/01635580802100869

Routledge
Taylor & Francis Group

Diallyl Disulfide Prevention of Cis-Diammine Dichloroplatinum–Induced Nephrotoxicity and Leukopenia in Rats: Potential Adjuvant Effects

Jéssica P. Chiarandini Fiore, Silvia L. Fanelli, Elida C. de Ferreyra, and José A. Castro

Centro de Investigaciones Toxicológicas (CEITOX): CITEFA/CONICET, Buenos Aires, Argentina

Cisplatin (CisPt) is an effective chemotherapeutic agent against several human cancers, but it produces important nephrotoxicity, leukopenia, and mortality. In this work, we report initial results on the potential ability of diallyl disulfide (DADS) to block these toxicities without compromising chemotherapy. Male Sprague Dawley rats were used (control, DADS, CisPt, and CisPt/DADS). CisPt was administered sc as a single dose (10.5 mg/kg) in saline. DADS was given daily intragastrically in olive oil (292.5 mg/kg) 1 h before CisPt administration the first day and 146.25 mg/kg during the next 3 days. The animals were sacrificed at the fifth day after CisPt administration. DADS significantly decreased CisPt-induced nephrotoxicity as evaluated by histology and by seric urea (CisPt: 11.05 $\pm$ 3.59; CisPt/DADS: 6.53 $\pm$ 1.74) and creatinine (CisPt: 24.74 $\pm$ 3.03; CisPt/DADS: 14.83 $\pm$ 2.07). DADS also decreased leukopenia (CisPt: 13.5% and CisPt/DADS: 43.4% respect the control), and mortality (CisPt: 50%; CisPt/DADS: 29%). DADS showed ability to interact with reactive oxygen species (H_2O_2, hydroperoxides, $OH^{\bullet}$) and with iron. DADS treatment does not change Platinum levels in kidney (CisPt: 15.2 $\pm$ 5.1; CisPt/DADS: 13.9 $\pm$ 4.5). Because DADS is known to inhibit cellular replication and to promote apoptosis of tumor cells, results suggest that DADS merit to be tested as a potential coadjuvant of CisPt chemotherapy in tumor-bearing animals.

INTRODUCTION

Cisplatin [cis-Diamminedichloroplatinum (CisPt)] is one of the most effective chemotherapeutic agents used in the treatment of a wide range of solid tumors (testes, ovary, head and neck, bladder, prostate, and other organs) (1–3).

However, CisPt has many side effects that limit its use that include nephrotoxicity, myelotoxicity, ototoxicity, peripheral neuropathy, hypomagnesemia, hematological toxicity reactions, and gastrointestinal side effects (4,5).

Submitted 23 August 2007; accepted in final form 30 December 2008.

Address correspondence to José A. Castro, Centro de Investigaciones Toxicológicas, CEITOX (CITEFA-CONICET), Juan B. de La Salle 4397, B1603ALO Villa Martelli, Buenos Aires, Argentina. Phone: 54-11-4709-5911. E-mail: jcastro@citefa.gov.ar

Nephrotoxicity is recognized as the main collateral effect, and it is the most important limiting factor of its clinical use (6,7).

There is significant evidence suggesting that in the cytotoxic activity of CisPt, the formation of reactive oxygen species (ROS) play an important role and that CisPt nephrotoxicity is closely associated with an increase of lipid peroxidation in the kidney and with inhibitory activity against antioxidant defenses (8–14).

It is currently believed that the antitumor mechanism of action of CisPt involves as the essential event the formation of a variety of adducts with nuclear DNA, which include interstrand and intrastrand DNA cross-links and DNA-protein cross-links (15,16).

As part of an extensive screening effort directed to find preventive agents against CisPt major undesirable toxic effects, potential preventive compounds having antioxidant or iron chelating properties and known to have proapoptotic effects on cancer cells were tested.

One of those chemicals, diallyl disulfide (DADS) exhibited in preliminary studies promising preventive properties against CisPt-induced nephrotoxicity and leukopenia.

DADS is a very well-known component of garlic essential oil (17). Methyl and allyl sulfides and trisulfides are among many biologically active sulfur-containing compounds in garlic essential oil. Dausch and Nixon (18) reported that the principal volatile sulfides in garlic oil are diallyl sulfide (DAS) and DADS, the former accounting as much as 14% and the latter 60% of it. The results of those initial studies are described in this work.

MATERIALS AND METHODS

Chemicals

CisPt, DADS, deferoxamine mesylate, and tert-butylhydroperoxide were from Sigma-Aldrich (Steinheim, Germany). All other chemicals were of the best quality available.

Animals and Treatments

Noninbred male Sprague Dawley rats were used. The procedures used for breeding, housing, and handling our stock animals were those described by the Food, Drug and Medical Technology National Administration (ANMAT, Argentina). They were kept in a controlled room with a 12 h light–dark cycle (light phase 06:00–8:00). The temperature was at 23 ± 2°C and the relative humidity at 45–65%. Food and water were available ad libitum.

Four groups of male, noninbred, Sprague-Dawley male rats (280–290 g) were used in these experiments: control, DADS, CisPt, and CisPt/DADS. Groups of 8 animals were used in each experiment design. CisPt was administered subcutaneously as a single dose (10.5 mg/kg) in saline. DADS was given daily intragastrically in olive oil (292.5 mg/kg) 1 h before CisPt administration the 1st day and 146.25 mg/kg during the next 3 days (19). Control received saline and olive oil. The DADS selected dosing regimen was made because it was known to be suitable for repetitive administration such as the one needed for future long-term studies in CisPt-treated, tumor-bearing animals. Rats were sacrificed at the 5th day after CisPt. Animals were anaesthetized with ether, blood samples were taken, and serum was isolated. Kidneys were rapidly excised and processed. Reported results derived from several similar studies in which the beneficial effects of DADS treatment were tested (see details in **Results** for each given parameter).

Determination of Serum Urea and Creatinine

Serum samples from animals of each group (control, DADS, CisPt, and CisPt/DADS) were collected for measuring serum urea and creatinine. They were determined using commercially available colorimetric kits from Wiener Lab (Rosario, Argentina). The procedures involved in the manufacturer's instructions were those described by Stegemann and Loeschcke and Butler, respectively (20,21). Urea levels in g/l and creatinine levels in mg/l were expressed as mean ± SD.

Histopathological Examinations

Kidneys were fixed in Bouin, dehydrated with ethanol, clarified with xylol, and embedded in paraffin. Then they were sectioned at 5 μm. Histological sections of kidney from all the treated groups were stained with hematoxylin and eosin. The microscopic scoring of the kidney sections was carried out in a blinded fashion by 2 observers who were unaware of the treatment groups. They assigned a score as described, which represents the approximate extent of necrotic area in the critical proximal tubules in a scale ranging from 0–4 (0, *no necrosis;* 1, *a few focal necrotic spots;* 2, *necrotic area was about one half;* 3, *necrotic spots was about two thirds;* 4, *nearly the entire area was necrotic*).

The photomicrographs of kidney section shown in Fig. 1 are representative average of observations.

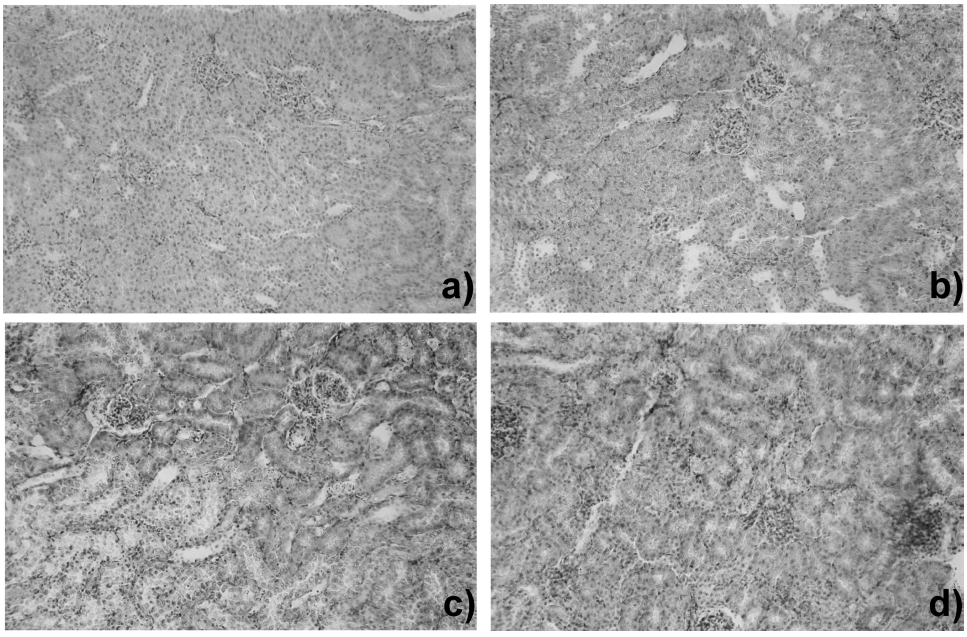

FIG. 1. Depicted photomicrographs are representative of the observations made by 2 independent workers in 7 similar experiments. Photomicrographs are of kidney samples of the control group (a); DADS-treated group (b); CisPt-treated group (c), and DADS-treated CisPt group (d). See **Methods** for details. Kidney sections from Control and DADS rats show normal glomeruli and tubular structure. Sections from CisPt-treated animals show severe tubular necrosis and dilatation of the Bowman's space. Sections taken from the DADS-treated animals show a significant prevention of both the tubular necrosis and the deleterious effects on the glomeruli.

Determination of Platinum Content in Renal Tissue

Kidney tissue was dried in an oven at 160°C for 3 h, then it was burnt under oxidant conditions and calcined in a muffle furnace at 750°C until the carbonaceous material disappeared. Fuming HNO_3 was added to the sample, and it was heated to dryness at 140°C. The residue was dissolved in HNO_3 (22). Pt content was determined by inductively plasma emission spectroscopy (ICD-OES) in a Perkin Elmer (Germany) Model Optima 2000 DV at 203.646 nm. The selected wavelength was one of the three major Pt spectral bands and the one not exhibiting spectral interferences. Percentage of recovery from tissue sample was 93.3%. The correlation coefficient (r^2) of the calibration curve was 0.999994. The precision was 1.69%. The measurements were made by the Analytical Chemistry Section from the Department of Chemistry of CITEFA (Instituto de Investigaciones Científicas y Tecnicas para la Defensa, Villa Martelli, Buenos Aires, Argentina).

Determination of Tert-Butylhydroperoxide-Induced Chemiluminescence in Renal Tissue

Chemiluminescence was measured in a Wallac-Rack Beta 1214 (Pharmacia Wallac O. Y., Turku, Finland) liquid scintillation counter at room temperature in an out of coincidence mode (23). Rat kidneys were homogenized (15.4–16.9 mg protein/ml) in 0.25 μM sucrose, 50 uM deferoxamine in TKM buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM KCl), pH 7.5. The 600 *g* supernatant was kept at 37°C for 10 min in a Dubnoff-shaker. Chemiluminescence measurement was started by addition of 3 mM tert-butylhydroperoxide (TBHP). Deferoxamine was included to attempt to remove metals such as iron. Three samples per group were run, each consisting of a homogenate from a separate lot of pooled renal tissue (8 animals each). Results are expressed as arbitrary units (Area $\times 10^6$/mg protein) obtained by quantification of the area under the curve of emission as a function of time.

Interaction Between DADS and ROS

1. To visualize the potential interaction between DADS with the different components of a ROS-generating system, the behavior of DADS against each and the total components of a Fenton reaction was tested.

   The "Fenton system" used (24) was prepared essentially as described, briefly: 5 mM ferrous sulfate (prepared freshly in N_2-purged water), 5 mM hydrogen peroxide in 50 mM NaH_2PO4; pH 7.4). Controls including only DADS (0.34 mM) and DADS with every single component or with the full Fenton system were also made.
2. In addition, we analyzed the ability of interaction between DADS and tert-butylhydroperoxide (TBHP). The studied system contained 5.7 mM TBHP and 0.34 mM DADS in 50 mM NaH_2PO4 (pH 7.4). Control without TBHP was also made.

The mixtures derived from incubations described in 1. or 2. were centrifuged at 15,000 rpm for 2 min. After 25 min of reaction at 20°C, the ability of interaction between DADS and ROS was tested determining the unreacted DADS still present in the incubation mixture by HPLC. To that end, an aliquot of the sample was analyzed at 40°C in a Hewlett Packard model 1090 series II HPLC with an HP ODS-Hypersil column (200 mm × 2.1 mm I.D., 5 μm particle size) and diode array detector. The mobile phase, consisting of acetonitrile/water/tetrahydrofurane (70/27/13), was delivered at a constant flow rate (0.2 ml/min), and the column effluent was monitored at 240 nm. DADS was quantified by peak-area radio with respect to a calibration curve ($r^2 = 0.9984$).

White Blood Cell Count (WBC)

The total WBC (leukocytes) was performed in a Neubauer chamber using the Turk liquid as a diluent. Results were expressed as N° leukocytes/mm^3 of blood.

Protein Concentration Determination

Protein concentrations were determined by the method of Lowry et al. (25) using bovine serum albumin as standard.

Statistics

The significance of the differences between mean values was assessed by analysis of variance test and Tukey–Kramer posttest (26). Calculations were performed using Graph Pad software (GraphPad Software Inc., San Diego, CA, USA). Differences were considered significant when $P < 0.05$.

RESULTS

Effect of DADS on the Mortality and Animals Weight Induced by CisPt

DADS administration significantly decreased this CisPt induced mortality (CisPt: 50%; CisPt/DADS: 29%). The weight changes of the animals during the experimental period were as follows: Control and DADS groups gained weight (24.55 ± 6.42 g and 18.22 ± 4.81 g, respectively; $P > 0.05$), whereas CisPt- and CisPt/DADS-treated groups exhibited a significant weight lost (48.05 ± 6.85 g and 45.34 ± 5.68 g, respectively; $P > 0.05$). When we compared CisPt and CisPt/DADS values with control and DADS values, there were significant differences ($P < 0.001$). Results are the mean from values obtained in 7 similar experiments.

Determination of Serum Urea and Creatinine

CisPt decreased glomerular filtration rate, which was correlative with serum urea and creatinine values. Serum urea and creatinine concentrations (Table 1) were significantly higher at the 5th day after administration of a single dose of CisPt when compared to those of the control group. DADS treatment of CisPt-poisoned rats significantly reduced serum urea and creatinine increased levels (Table 1). Results are the mean from values obtained in 4 similar experiments.

TABLE 1
Seric urea and creatinine values in DADS-treated cisplatin (CisPt) poisoned rats[a]

	Urea (g/l)	Creatinine (mg/l)
Control	0.53 ± 0.03	8.39 ± 2.01
DADS	0.41 ± 0.12	2.48 ± 1.97^{c}
CisPt	11.05 ± 3.59^{b}	24.74 ± 3.03^{b}
CisPt/DADS	$6.53 \pm 1.74^{b,c}$	14.83 ± 2.07^{b}

[a] Abbreviation is as follows: DADS, diallyl disulfide. CisPt was administered subcutaneously as a single dose (10.5 mg/kg) in saline. DADS was given daily intragastrically in olive oil (292.5 mg/kg) 1 h before CisPt administration the 1st day and 146.25 mg/kg during the next 3 days. Animals were sacrificed at the 5th day after CisPt. Seric urea and creatinine were determined as described in **Materials and Methods**. Groups of 8 animals were used. Values are the means ± SD from results obtained in 4 similar experiments.

[b] $P < 0.001$. Urea: Control vs. CisPt, DADs vs. CisPt/DADS; Creatinine: Control vs. CisPt, CisPt vs. CisPt/DADS, DADS vs. CisPt/DADS.

[c] $P < 0.05$. Urea: CisPt vs. CisPt/DADS; Creatinine: Control vs. DADS.

Histopathological Examinations

The impaired renal function induced by CisPt was further confirmed by histological examination of kidney (Table 2).

As shown by Figs. 1a and 1b, the kidney from control and DADS rats showed no abnormality, whereas kidney in CisPt-treated animals revealed a marked proximal tubules necrosis (Fig. 1c). DADS administration decreased the CisPt induced tubular necrosis (Fig. 1d). Reported results are the mean of the observations made by 2 independent workers in 7 similar experiments.

Platinum Content in Renal Tissue

The Platinum content in renal tissue at the 5th day of poisoning was not significantly affected by DADS administration (CisPt: 15.2 ± 5.1; CisPt/DADS: 13.9 ± 4.5; $P > 0.05$).

TABLE 2
Semiquantitative analysis of histology of rats' kidney[a]

Treatment	Score
Control	0+
DADS	0+
CisPt	3+
CisPt/DADS	1–2+

[a] Abbreviations are as follows: DADS, diallyl disulfide; CisPt, cisplatin. Rats were treated with CisPt and DADS, and histological grading was performed as discussed in **Materials and Methods**. Reported results are the mean of the observations made by 2 independent workers in 7 similar experiments.

TBHP-Induced Chemiluminescence in Homogenates of Renal Tissue From Rats Treated With CisPt

The hydroperoxide-induced chemiluminescence in rat renal homogenates from CisPt-treated rats was significantly more intense than in controls. DADS administration to the CisPt-poisoned animals significantly decreased the CisPt enhancement of hydroperoxide induced chemiluminescence (Fig. 2). These DADS decreasing effects involved the area under the curve of emission against time but without changing its shape. The chemiluminescence emission in samples treated with DADS alone was not significantly different from that derived from control animals.

Interaction Between DADS and ROS

Results obtained in experiments in which DADS interacted with each component of the Fenton system or with the full mixture showed that DADS was able to react with either Fe^{2+} or H_2O_2 or with the $OH^{\bullet}$generating full Fenton mixture as evidenced the Fenton assay. In effect, DADS content decreased significantly not only when incubated with the complete Fenton system but also when exposed to any single component of the Fenton reaction system individually (Fig. 3).

DADS was also able to react with hydroperoxides as shown by the decreases in DADS content in incubation mixtures containing TBHP (Fig. 4).

Effect of CisPt Intoxication on White Blood Cell Count (N° leukocytes/mm^3) and Its Prevention by DADS Administration

Blood leukocyte counts were significantly lower at the 5th day after administration of a single dose of CisPt ($1{,}785.71 \pm 753.72$) when compared to those of the control group ($13{,}243.75 \pm 2{,}216.24$; $P < 0.05$). Counts from DADS group ($12{,}420.00 \pm 726.80$) were not significantly different from the control group. DADS treatment of CisPt-poisoned rats ($5{,}750.00 \pm 1{,}960.73$; $P < 0.05$) significantly increased the white cell count of the CisPt-intoxicated animals.

DISCUSSION

In agreement with previous studies from other laboratories, CisPt induced severe kidney damage in experimental animals (6–14). These observations are of high significance to clinical reports about toxic side effects of CisPt to humans. Those toxic effects include nephrotoxicity, ototoxicity, neurotoxicity, and bone marrow depression, but its most dose-limiting side effect is nephrotoxicity (6,7). Furthermore, cumulative hematological, renal, and neurological toxicities can result in increased mortality (8). A significant mortality and leukopenia were also observed in our experiments. Despite these undesirable side effects of CisPt and the availability of other newer less toxic platinum drugs, CisPt remains as an important antineoplastic drug for the treatment of solid tumors. Reasons for that rest in its proven effectiveness as a chemotherapeutic agent and in

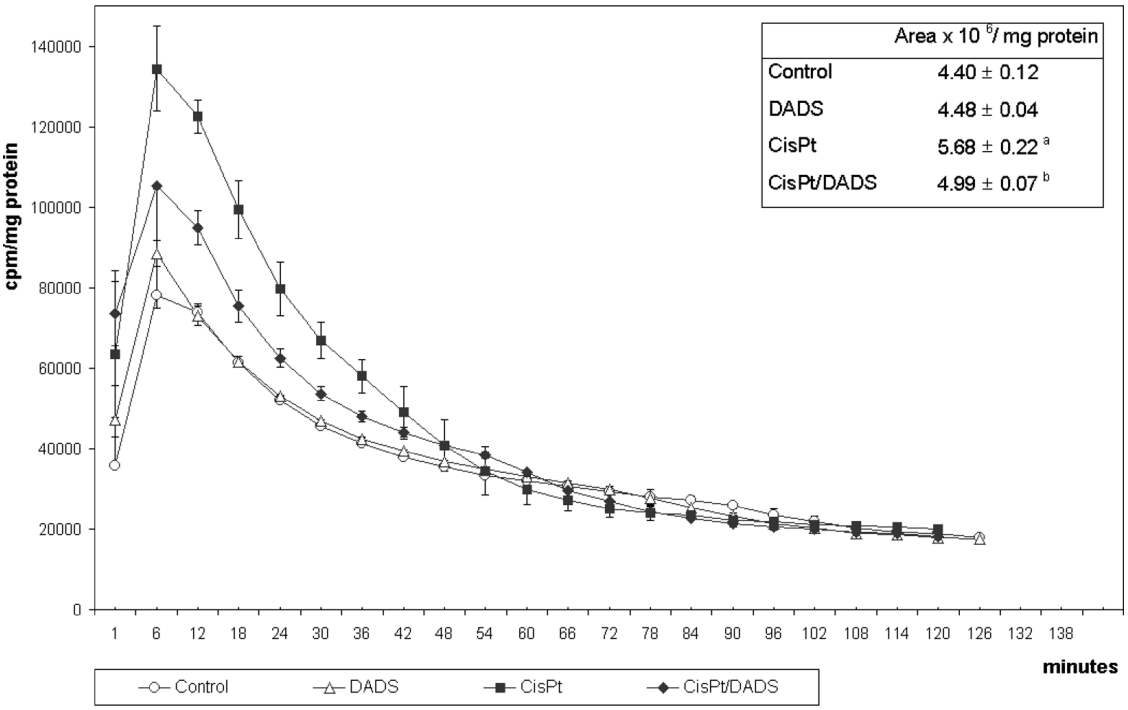

FIG. 2. Tert-butylhydroperoxide-induced chemiluminescence of rat renal tissue homogenates. Kidneys were homogenized (15.4–16.9 mg protein/ml) in 0.25 M sucrose, 50 uM deferoxamine in TKM buffer (50 mM Tris-HCl, 5 mM $MgCl_2$, 2.5 mM KCl), pH 7.5. The 600 *g* supernatant was kept at 37°C for 10 min in a Dubnoff-shaker. Chemiluminescence measurement was started by addition of 3 mM TBHP. Three samples per group were run, each consisting of a homogenate from a separate lot of pooled renal tissue (8 animals each). $^a P < 0.001$ (control vs. CisPt). $^b P < 0.05$ (CisPt vs. CisPt /DADS)

the existing experience with it (27). Many attempts to anchorite the CisPt toxicity have been described in literature because of their potential clinical interest (27,28). However, those preventive treatments should not compromise the antitumoral effects of CisPt. CisPt toxicity is believed to be related to its ability to promote an oxidative stress condition in kidney tissue (8–14) after a process of bioactivation involving renal CyP2E1 and generation of ROS in an iron-mediated process. The chemotherapeutic effect of CisPt instead is believed to involve the formation of platinum containing DNA adducts (15,16). These initial considerations led us to test several chemicals being either antioxidants or inhibitory against CyP2E1 action or iron chelators but having proved proapoptotic properties against tumor cells as potentially effective in preventing CisPt toxicity. One chemical having promising properties in that respect was diallyldisulfide (DADS). DADS is a relevant component of garlic essential oil (17) proved to be an antioxidant (29,30) and to inhibit CyP2E1 in liver microsomes (31,32). More important, it is known to exert inhibitory effects in cultured human tumoral proliferation and to prevent cancer in laboratory animals induced by a variety of chemical carcinogens and the growth of transplanted tumor xerographs in vivo by inducing apoptosis and/or perturbing cell cycle progression (33–36). However, a garlic-containing diet that may be achieved through human consumption and able to lead to some or all of the DADS favorable effects previously reported by others and mentioned above (29–36) would be unreal. This suggested a feasible use as a dietary supplement having a potential coadjuvant action.

In the present study, we found that DADS given during the CisPt intoxication process partially prevented the nephrotoxic effects and the leukopenia provoked by the chemotherapeutic drug. The preventive effects appear to be partially related to its ability to enhance the antioxidant defenses in kidney tissue as suggested by our studies on the TBHP-induced chemiluminescence in which we observed a significant decrease in the intensity of the hydroperoxide-promoted emission of chemiluminescence in the samples derived from the DADS treated animals. Low level chemiluminescence reflects the occurrence of excited states generated by oxidative reactions and to determine the presence of ROS. The TBHP-stimulated chemiluminescence of tissue samples provides an assay that integratively estimates the amount of prooxidants and antioxidants in a preparation under those circumstances (23). Then, the obtained results should be interpreted as showing that the DADS treatment improved those defenses. Several different possibilities could be envisaged as involved in that preventive effect. For example, previous studies from other laboratories have shown that DADS treatments was able to increase GSH levels and the activity of GSH related enzymes (31). Another allyl sulfur compound present in garlic essential oil preparations such as S-allylcysteine (SAC) was also effective in favorably modifying the response to CisPt but altered cellular GSH in a biphasic manner (37). However,

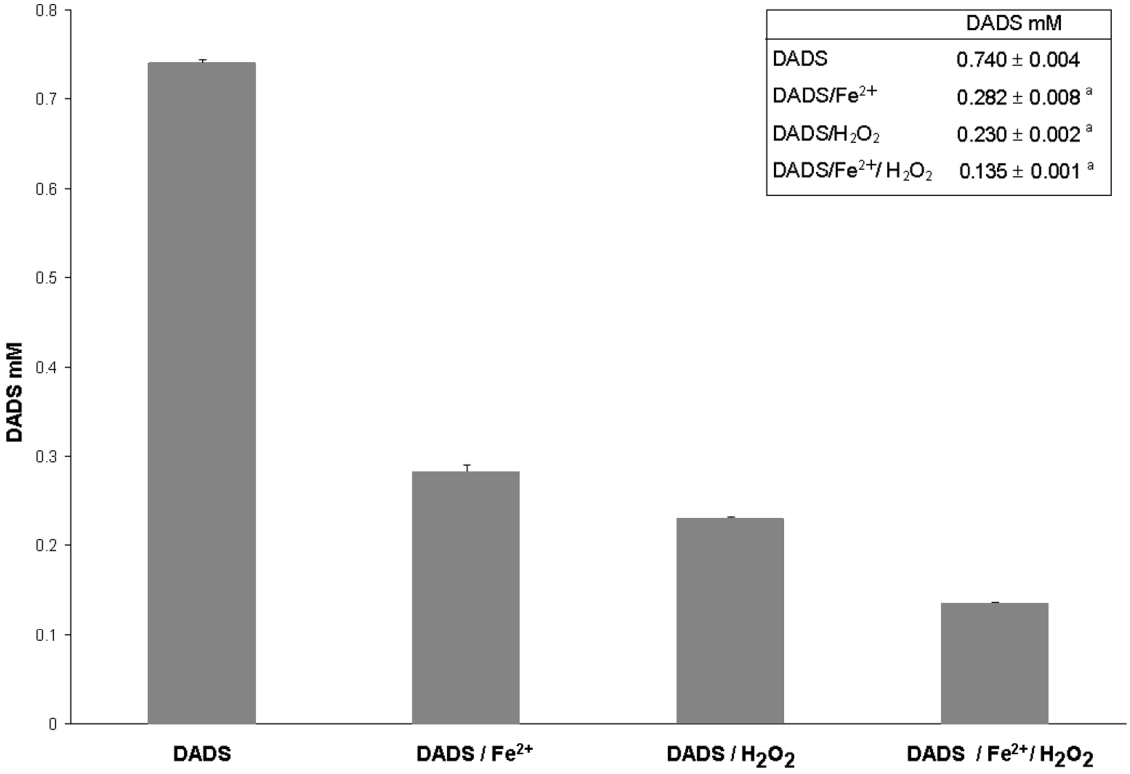

FIG. 3. Interaction between DADS and reactive oxygen species (ROS) from the Fenton system. The full "Fenton system" studies for interactions with DADS used was as follows : 5 mM ferrous sulfate (prepared freshly in N_2-purged water), 5 mM hydrogen peroxide and DADS in 50 mM NaH_2PO4 (pH 7.4). Controls including only DADS (0.34 mM) and DADS with every single component were also made. The mixture was centrifuged at 15,000 rpm for 2 min. After 25 min of reaction at 20°C, the interaction between DADS and reactive oxygen species (ROS) or iron was tested determining the DADS content remaining by HPLC diode array detection at 240 nm. See **Methods** for details. Values are the means ± SD of three individual samples per group. [a] $P < 0.001$ (DADS vs. DADS/Fe, DADS vs. DADS/H_2O_2, DADS vs. DADS/Fe/H_2O_2; DADS/H_2O_2 vs. DADS/Fe/H_2O_2; DADS/Fe vs. DADS/Fe/H_2O_2)

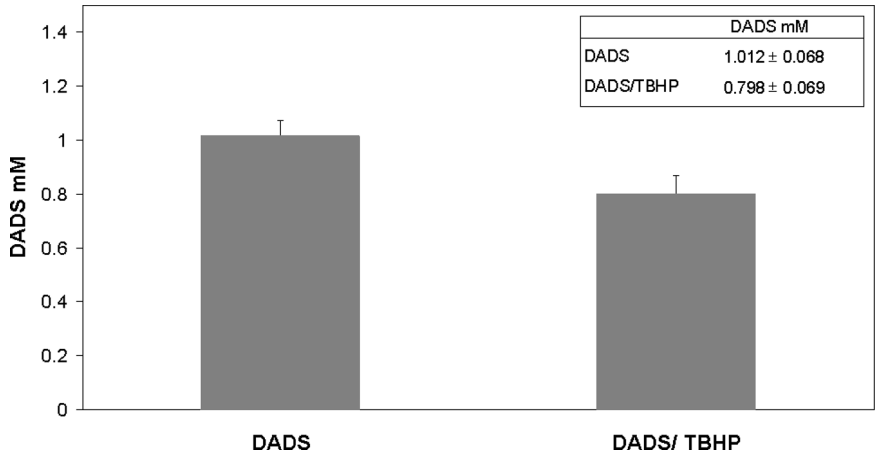

FIG. 4. Interaction between DADS and tert-butylhydroperoxide. Incubation mixtures contained 5.7 mM TBHP and 0.34 mM DADS in 50 mM NaH_2PO4 (pH 7.4). Control without TBHP was also made. The mixture was centrifuged at 15,000 rpm for 2 min. After 25 min of reaction at 20°C, the interaction between DADS and the hydroperoxide was tested determining the DADS content remaining by HPLC and diode array detection at 240 nm. See **Methods** for details. Values are the means ± SD of three individual samples per group; $P < 0.005$.

in contrast to the well established inhibitory effects of DADS on cancer cells growth (33–36), SAC was found by Yellin et al. (37) to have no effect on cell growth.

Also, previous studies from others and from our laboratory have shown that DADS is able to inhibit lipid peroxidation (29,30). In the present studies, the ability of DADS to interact with hydroperoxides and with Fe^{2+}, H_2O_2, and the complete hydroxyl radical Fenton-generating system further suggests that DADS treatment could be effective, at least in part, because of its ability to react with ROS and/or the ROS generating reactions.

On behalf of this hypothesis is the fact that a highly specific iron chelator such as deferoxamine was found in previous studies from other laboratories to be an effective treatment against cisplatin nephrotoxicity (38). In fact, previous work by Baliga et al. (11) provided in vitro and in vivo evidence suggesting a role of iron in cisplatin-induced nephrotoxicity.

Further, recent studies of Jiang et al. (14) evidenced that specific hydroxyl free radical scavengers such as dimethylthiourea and N-acetylcysteine were preventive against cisplatin nephrotoxicity.

The DADS treatment does not appear to potentially compromise the CisPt antitumoral effects. That hypothetical expectation rests on the fact that the Platinum content in kidney samples from DADS-treated animals was not significantly different from that of CisPt-only treated animals. CisPt chemotherapeutic effects depend on the formation of several stable Platinum-DNA adducts (15,16), which apparently could have remained uncharged. On the other hand, these observations also exclude the possibility that DADS-prevented toxicity were related to increases of excretion of soluble forms promoted by the organosulfur compound.

The potential possibility that DADS was a promising, active coadyuvant of CisPt, however, still remains far from being established and must require far more experiments, particularly in tumor-bearing animals.

ACKNOWLEDGMENTS

J. P. Chiarandini Fiore has a doctoral fellowship from the National Research Council from Argentina (CONICET).

REFERENCES

1. LebWohl D and Canetta R: Clinical development of platinum complexes in cancer therapy: an historical perspective and an update. *Eur J Cancer* **34,** 1522–1534, 1998.
2. Wong E and Giandomenico CM: Current status of platinum-based antitumor drugs. *Chem Rev* **99,** 2451–2466, 1999.
3. Jakupec MA, Galanski M, and Keppler, BK: Tumor-inhibiting platinum complexes: state of the art and future perspectives. *Rev Physiol Biochem Pharmacol* **146,** 1–54, 2003.
4. Ferguson LR and Pearson AE: The clinical use of mutagenic anticancer drugs. *Mutat Res* **355,** 1–12, 1996.
5. O'Dwyer PJ, Stevenson JP, and Johnson SW: Clinical Status of cisplatin, carboplatin and other platinum-based antitumor drugs. In: *Cisplatin: Chemistry and Biochemistry of a Leading Anticancer Drug,* Lippert B (ed.). Verlag Helvetica Chimica Acta, Zürich, Switzerland, Wiley-VCH. 1999, pp. 31–69.
6. Arany I and Safirstein RL: Cisplatin nephrotoxicity. *Semin Nephrol* **23,** 460–464, 2003.
7. Taguchi T, Nazneen A, Abid MR, and Razzaque MS: Cisplatin associated nephrotoxicity and pathological events. *Contrib Nephrol* **148,** 107–121, 2005.
8. Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, et al.: Reduction of Cis-Platinum induced nephrotoxicity by zinc histidine complex: the possible implication of nitric oxide. *Biochem Mol Biol Int* **36,** 855–862, 1995.
9. Matsushima H, Yonemura K, Ohishi K, and Hishida A: The role of oxygen free radicals in cisplatin-induced acute renal failure in rats. *J Lab Clin Med* **131,** 518–526, 1998.
10. Sadzuka Y, Shoji T, and Takino Y: Effect of cisplatin on the activities of enzymes which protect against lipid peroxidation. *Biochem Pharmacol* **43,** 1873–1875, 1992.
11. Baliga R, Zhang Z, Baliga M, Ueda N, and Shah SV: In vitro and in vivo evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int* **53,** 394–401, 1998.
12. Khynriam D and Prasad SB: Changes in glutathione related enzymes in tumor-bearing mice after cisplatin treatment. *Cell Biol Toxicol* **18,** 349–358, 2002.
13. Baek SM, Kwon CH, Kim JH, Woo JS, Jung JS, et al.: Differential roles of hydrogen peroxide and hydroxyl radical in cisplatin-induced cell death in renal proximal tubular epithelial cells. *J Lab Clin Med* **142,** 178–186, 2003.
14. Jiang M, Wei Q, Pabla N, Dong G, Wang CY, et al.: Effects of hydroxil radical scavenging on cisplatin-induced p53 activation, tubular cell apoptosis and nephrotoxicity. *Biochem Pharmacol* **73,** 1499–1510, 2007.
15. Reed E: Alkylating agents and platinum: is clinical resistance simply a tumor cell phenomenon? *Curr Opin Oncol* **3,** 1055–1059, 1991.
16. Lawley PD and Philips DH: DNA adducts from chemotherapeutic agents. *Mutat Res* **355,** 13–40, 1996.
17. Block E: The organosulfur chemistry of the Genus Allium—implications for the organic chemistry of sulfur. *Angew Chem Int Ed Engl* **31,** 1135–1178, 1992.
18. Dausch J and Nixon W. Garlic: a review of its relationship to malignant disease. *Prev Med* **19,** 346–361, 1990.
19. Guyonet D, Belloir C, Suschetet M, Siess MH, and Le Bon AM: Antimutagenic activity of organosulfur compounds from Allium is associated with phase II enzyme induction. *Mutat Res* **495,** 135–145, 2001.
20. Stegemann H and Loeschcke V: Microdetermination of nitrogen as indophenol blue by cloramine-T oxidation. *Hoppe-Seyler's Z Physiol Chem* **329,** 241–248, 1962.
21. Butler AR: The Jaffé reaction: identification of the coloured species. *Clin Chem Acta* **59,** 227–232, 1975.
22. Basinger MA, Jones MM, and Holscher MA: L-methionine suppress pathological sequelae of cis-platinum in the rat. *Fundam Appl Toxicol* **14,** 568–577, 1990.
23. Boveris A, Fraga CG, Varsavsky AI, and Koch OR: Increased chemiluminescence and superoxide production in liver of chronically ethanol treated rats. *Arch Biochem Biophys* **227,** 534–541, 1983.
24. Castro GD, Costantini MH, Delgado de Layño AM, and Castro JA: Rat liver microsomal and nuclear activation of methanol to hydroxymethyl free radicals. *Toxicol Lett* **129,** 227–236, 2002.
25. Lowry OH, Rosebrough NJ, Farr AL, and Randall, RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* **193,** 265–275, 1951.
26. Gad SC: Statistics for toxicologists. In: *Principles and Methods of Toxicology,* Hayes AW (ed.). 4th ed. Philadelphia: Taylor & Francis, 2001, pp. 285–364.

27. Ali BH and Al Moundhri MS: Agents ameliorating or augmenting the nephrotoxicity of cisplatin and other platinum compounds: a review of some recent research. *Food Chem Toxicol* **44,** 1173–1183, 2006.
28. Kosmider B and Osiecka R: Flavonoid compounds: a review of anticancer properties and interactions with cis-diamminedichloroplatinum (II). *Drug Dev Res* **63,** 200–211, 2004.
29. Fanelli SL, Castro GD, Toranzo EGD, and Castro JA: Mechanisms of the preventive properties of garlic components in the carbon tetrachloride-promoted oxidative stress: diallyl sulfide, diallyl disulfide, allyl mercaptan and allyl methyl sulfide. *Res Commun Mol Pathol Pharmacol* **102,** 163–174, 1998.
30. Dwivedi C, John LM, Schimidt DS, and Engineer FN: Effect of oil-soluble organosulfur compounds from garlic on doxorubicin-induced lipid peroxidation. *Anticancer Drugs* **9,** 291–294, 1998.
31. Siess MH, Le Bon AM, Canivenc-Lavier MC, and Suschetet M: Modification of hepatic drug metabolizing enzymes in rats treated with alkyl sulfides. *Cancer Lett* **120,** 195–201, 1997.
32. Shimada M, Liu L, Nussler N, Jonas S, Langrehr JM, et al.: Human hepatocytes are protected from ethanol-induced cytotoxicity by DADS via cyP2E1 inhibition. *Toxicol Lett* **163,** 242–249, 2006.
33. Knowles LM and Milner JA: Possible mechanism by which allyl sulfides suppress neoplastic cell proliferation. *J Nutr* **131,** 1061S–1066S, 2001.
34. Herman-Antosiewicz A and Shivendra SV: Signal transduction pathway leading to cell cycle arrest and apoptosis induction in cancer cells by Allium vegetable-derived organ sulfur compounds: a review. *Mutat Res* **555,** 121–131, 2004.
35. Wu XJ, Kassie F, and Mersch-Sundermann V: Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds. *Mutat Res* **589,** 81–102, 2005.
36. Arunkumar A, Vijayababu MR, Venkataraman P, Senthilkumar K, and Arunakaran J: Chemoprevention of rat prostate carcinogenesis by diallyl disulfide, an organsulfur compound of garlic. *Biol Pharm Bull* **29,** 375–379, 2006.
37. Yellin SA, Davidson BJ, Pinto JT, Sacks PG, Qiao C, et al.: Relationship of glutathione and glutathione-S-transferase to cisplatin sensitivity in human head and neck squamous carcinoma cell lines. *Cancer Lett* **85,** 223–232, 1994.
38. Kameyama Y and Gemba M: The iron chelator deferoxamine prevents cisplatin-induced lipid peroxidation in rat kidney cortical slices. *Jpn J Pharmacol* **57,** 259–262, 1991.