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Chemoprotective Effect of a Nuclear Factor-κB Inhibitor, Pyrrolidine Dithiocarbamate, Against Cisplatin-Induced Testicular Damage in Rats

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ABSTRACT: The objective of this study was to evaluate inducible nitric oxide synthase (iNOS) and nuclear factor-κB inhibitor (NF-κB) expression and the potential chemoprotective effects of an NF-κB inhibitor, pyrrolidine dithiocarbamate (PDTC), against cisplatin-induced testicular damage in rats. Rats were divided into 4 equal groups: group 1, control; group 2, injected with cisplatin (CIS) for 5 days (7 mg/kg/day intraperitoneally [IP]); group 3, injected with PDTC alone; group 4, injected with CIS plus PDTC (100 mg/kg IP). Body and testicular weights, plasma testosterone levels, and histopathologic structure of the testicular tissue were determined. The iNOS and NF-κB activity were evaluated immunohistochemically by staining p65 to define NF-κB activity. Malondialdehyde (MDA), reduced glutathione (GSH), and nitric oxide (NO) levels and glutathione peroxidase (GSH-Px) activity were assessed in testicular tissue. Body and testicular weights, plasma testosterone levels, activity of GSH-Px, and GSH levels were all significantly decreased, whereas the levels of MDA and NO were significantly increased in rats of the CIS group. PDTC treatment increased plasma testosterone levels. A significant increase in GSH levels and GSH-Px activity and a decrease in MDA and NO levels in testicular tissue were observed in the CIS + PDTC group. Immunohistochemically, there was a marked staining for iNOS and NF-κB/p65 expression in rats injected with CIS compared with the control ($P < .001$). CIS caused irregular seminiferous tubules, reduction of seminiferous epithelial layers, significant arrest of maturation, and perivascular fibrosis. Moreover, PDTC administration to CIS-treated rats significantly prevented these histopathologic chances, as well. CIS induces iNOS expression through activation of NF-κB/p65, and CIS-induced testicular toxicity may be prevented by PDTC, which is a selective NF-κB inhibitor.

Key words: Oxidative stress, antioxidant, testicular damage.
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Cytotoxic chemotherapy has improved the survival rates in many conditions, particularly testicular malignancies. Treatment is, however, associated with significant morbidity, and testicular dysfunction is among the most common long-term side effects of this therapy (Howell and Shalet, 2001). Effective anticancer therapy with cytotoxic drugs such as cisplatin (CIS) is limited by the reproductive toxicity that has been documented in various experimental studies (Cherry et al, 2004). Particularly, spermatogenic cells are targeted by this agent because of their high mitotic activity.

The cellular mechanism of testicular damage induced by CIS is poorly understood. Traditionally, the biochemical basis of CIS toxicity is believed to be associated with the oxidative stress through generation of free radicals and reactive oxygen species (ROS), and these oxidants have been considered to exert their effects through a direct toxic effect on target cells (Ateşşahin et al, 2006a,b). However, recent studies have shown that these species activate the nuclear factor-κB (NF-κB) family of transcription factors, which are key regulators for inflammatory gene expression (eg, inducible nitric oxide synthase [*iNOS*]), and NF-κB can also be activated by oxidative stress (Ghosh et al, 1998). To the best of our knowledge, our study is the first one that has evaluated the role of NF-κB activation in chemotherapeutic-induced testicular damage.

Pyrrolidine dithiocarbamate (PDTC) is an antioxidant. Recent studies have suggested that the pro-oxidant and metal-chelating properties of PDTC could also be involved in its ability to inhibit NF-κB (Gukovsky et al, 1998; Liu et al, 1999; Bowie and O'Neil, 2000). Some studies showed that PDTC might inhibit NF-κB activation via stabilization of IκB-α (Virlos et al, 2003) or inhibition of the ubiquitin-proteasome pathway (Si et al, 2005). There are a lot of studies concerning the selective NF-κB inhibitor properties of PDTC (Schreck et al, 1992).

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The aim of this study was to evaluate the role of NF-κB and iNOS in oxidative testicular injury throughout the pathogenesis of testicular damage induced by CIS and to investigate the effects of PDTC, known as a selective NF-κB inhibitor.

Materials and Methods

Chemicals

CIS was obtained from Mayne Pharma Plc (Warwickshire, United Kingdom), and PDTC was obtained from Sigma-Aldrich Chemical Corp (St Louis, Missouri).

Animals

In this study, 24 healthy adult male Wistar albino rats (5–6 weeks; 340–350 g) were used. The animals were kept under standard laboratory conditions (12 hours light/dark cycle; 26°C–28°C) for at least 1 week before the experiment, and those conditions were preserved until the end of the experiment. Animal cages were kept clean, and food and water were given regularly every day. All experiments in this study were performed in accordance with the guidelines for animal research issued by the National Institutes of Health and were approved by the local committee on animal research.

Study Design and Treatment

The rats were randomly divided into 4 groups, each consisting of 6 animals. CIS was injected intraperitoneally (IP) at 7 mg/kg/day for 5 consecutive days. The dose of CIS was selected according to previous studies that demonstrated significant testicular toxicity in rats (Ateşşahin et al, 2006a). PDTC dissolved in distilled water and injected IP at 100 mg/kg/day for 5 consecutive days. The doses of PDTC were selected based on the results of recent studies in which the antioxidant and anti-inflammatory action of this agent was apparent (Tian et al, 2006; Tugcu et al, 2006). Group 1 served as control and received a single dose (IP) injection of 1 mL of distilled water. Group 2 rats were treated with CIS alone. In group 3, rats were treated with PDTC alone. Rats in group 4 received CIS + PDTC. The total dose of PDTC administrated to rats was separated into 2 doses and injected 2 hours before and after the administration of CIS.

Sample Collection

The testicular toxicity of CIS can be detected within 7 weeks after treatment with a sufficient dose in rats (Karashima et al, 1988; Pogach et al, 1989). Therefore, 40 days after receiving the last dose of CIS, rats were weighed and sacrificed under anesthesia (ketamine 200 mg/kg body wt IP). The abdomen was opened with a middle-line incision, and orchiectomy was performed. After the testes were weighed, one of them was stored at −80°C for enzymatic evaluation. The other testis was fixed with Bouin solution for histopathologic examination. Blood samples were collected from the aorta and separated for biochemical examination.

Biochemical Studies

Plasma Testosterone Level—The plasma testosterone level was used to evaluate chemotherapy-associated hypoandrogenism and was measured with an immunoenzymatic method according to the protocol described by Srivastava (2001).

Lipid Peroxidation Level—Testicular tissue was removed and homogenized in a Teflon-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (wt/vol) whole homogenate. Malondialdehyde (MDA), which forms as an end product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA, also referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrofluorometer, as described previously (Wasowicz et al, 1993). The MDA level was expressed as nmol/g of wet tissue.

Glutathione Peroxidase Activity—Glutathione peroxidase (GSH-Px) activity was measured according to Paglia and Valentine (1967) by monitoring the oxidation of reduced NADPH at 340 nm. Enzyme units were defined as the number of μmol of NADPH oxidized per minute and calculated using the extinction coefficient of NADPH at 340 nm (6.22×10^6/mol/cm). Results were reported as nmol/g of wet tissue.

GSH Level—Reduced GSH was estimated by the method of Moron et al (1979), in which the color developed is read at 412 nm. Protein concentrations in all samples were measured using the method of Lowry et al (1951). Results were reported as μmol/g of wet tissue.

NO Level—Total nitrite was quantified by the Griess reaction after incubating the supernatant with *Escherichia coli* nitrate reductase to convert NO_3 to NO_2. Griess reagent (1 mL of 1% sulfanilamide, 0.1% naphthyl-ethylenediamine hydrochloride, and 2.5% phosphoric acid; Sigma-Aldrich) was then added to 1 mL of supernatant. The absorbance was read at 545 nm after a 30-minute incubation. The absorbance was compared with the standard graph of $NaNO_2$, obtained from the reduction of $NaNO_3$ (1–100 μmol/L). The accuracy of the assay was checked in 2 ways; the interassay and intra-assay coefficients of variation were 7.52% and 4.61%, respectively. To check conversion of nitrate to nitrite (recovery rate), predetermined amounts of nitrate were added to control plasma samples; these samples were deproteinized and reduced as above. Results were expressed as nmol/g of wet tissue.

Histopathologic Examination

Evaluation of Spermatogenesis—Testicular tissue (approximately 5–10 mg) was prepared for histologic examination. Semithin paraffin wax testicular tissue sections (4 μm thick) fixed in Bouin solution were stained with hematoxylin and eosin and were examined under a light microscope (Olympus, Tokyo, Japan) under ×100, ×200, and ×400 magnification using standard techniques. To evaluate spermatogenesis, at least 40 seminiferous tubules were examined per slide, and each slide were scored using the Johnson score (Johnson et al, 1980). Seminiferous tubules were scored on a scale of 1 to 10, with complete inactivity of tubules scored as 1 and those with maximum activity (≥5 spermatozoa in the lumen) scored as 10.

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Evaluation of Fibrosis—To evaluate testicular fibrosis, specimens obtained from testis were embedded in paraffin, cut into 4-μm sections, and stained with Masson trichrome. Specimens were scored briefly after staining as follows: (−) no fibrosis; (+) fibrosis in fewer than 25% of total testicular tissue (mild); (++) fibrosis in 25% to 50% of total testicular tissue (moderate); (+++) fibrosis in more than 50% of total testicular tissue (serious) (Ayyıldız et al, 2004).

Immunohistochemical Studies

For immunohistochemical evaluation, specimens were processed for light microscopy and sections were incubated at 60°C overnight and then dewaxed in xylene for 30 minutes. After rehydrating in a decreasing series of ethanol baths, sections were washed with distilled water and phosphate-buffered saline (PBS) for 10 minutes. Sections were then treated with 2% trypsin in 50 mM Tris buffer (pH 7.5) at 37°C for 15 minutes and washed with PBS.

Sections were delineated with a Dako pen (Dako, Glostrup, Denmark) and incubated in a solution of 3% H_2O_2 for 15 minutes to inhibit endogenous peroxidase activity. Then sections were incubated with NF-κB/p65 (Rel A) Ab-1 (R-B-1638-R7 Neomarkers; Labvision, Fremont, California) and iNOS Ab-1 (R-B-1605-R7 Neomarkers; Labvision) antibodies. The Ultravision horseradish peroxidase/3-amino-9-ethylcarbazole staining protocol (Labvision) was used at this stage.

Sections prepared for each case were examined by light microscopy. Positive and negative controls were conducted in parallel with NF-κB– and iNOS-stained sections. Staining of sections with commercially available antibodies served as the positive control. Negative controls included staining tissue sections with omission of the primary antibody.

According to the diffuseness of the staining, sections were graded as 0 (no staining), 1 (staining <25%), 2 (staining between 25% and 50%), 3 (staining between 50% and 75%), or 4 (staining >75%). According to staining intensity, sections were graded as follows: 0 (no staining), 1 (weak but detectable staining), 2 (distinct staining), or 3 (intense staining). Immunohistochemical values were obtained by adding the diffuseness and intensity scores.

Statistical Analyses

Statistical analyses of the histopathologic and immunohistochemical evaluations of the groups were carried out with the χ^2 test, and analyses of the biochemical data were completed with the Mann-Whitney *U* test. Results of all groups are shown as means ± SD. All results were compared one by one with other groups' results and with control group results. $P < .05$ was recognized as statistically significant.

Results

The biochemical, histopathologic, and immunohistochemical results were similar for control and PDTC alone–treated rats, and we decided to consider them without distinction and to report only the control group.

Body and Testicular Weights

Significant body and testicular weight losses were observed in the group that received CIS alone compared with the control group ($P < .05$). Coadministration of PDTC in CIS-treated rats preserved body and testicular weights.

Biochemical Variables in Plasma and Tissue

Plasma testosterone levels were significantly lower in rats treated with CIS alone compared with those in the control group ($P < .001$); coadministration of PDTC in CIS-treated rats significantly increased plasma testosterone levels ($P < .001$) (Figure 1A).

The GSH-Px activity and GSH levels in testicular tissue of rats treated with CIS alone were significantly lower than those in the control group ($P < .01$), and treatment with PDTC significantly elevated the GSH-Px activity and GSH level in group 4 rats ($P < .01$) (Table).

The MDA and NO levels in the testicular tissue were found to be significantly higher in rats treated with CIS alone than those in the control group ($P < .01$), and treatment with PDTC prevented elevation of MDA and NO levels significantly in CIS-treated rats ($P < .01$) (Table).

Histologic Examination

Spermatogenesis—Rats in the control group showed normal testicular morphology with a regular course of spermatogenesis and Sertoli cells (Figure 2A). All rats treated with CIS alone were characterized by a depletion of germ cells, irregular seminiferous tubules exhibiting Sertoli cell only type and a few spermatogonia. Reduced seminiferous epithelial layers were found in numerous tubules, and irregular and diminished tubules containing a few germ cells were also seen (Figure 2B). Significant maturation arrest and low Johnson scores were also observed in the group that received CIS alone compared with the control group. Spermatogenesis was significantly preserved in the rats treated with CIS + PDTC. The morphologic characteristics of the testes were comparable to those in the control group (Figure 2C).

Testicular Fibrosis—The perivascular fibrosis and hyalinization of intertubular connective tissue were not determined in the control group and the group that received PDTC + CIS (Figure 3A). In the rats that received only CIS, mild perivascular fibrosis and hyalinization of intertubular tissue were observed (Figure 3B).

Immunohistochemical Studies

On immunohistochemical evaluation, there was more intense expression of iNOS (Figure 4A through F) and p65 in rats subjected to CIS alone compared with controls ($P < .01$) (Table). There was poor or slight

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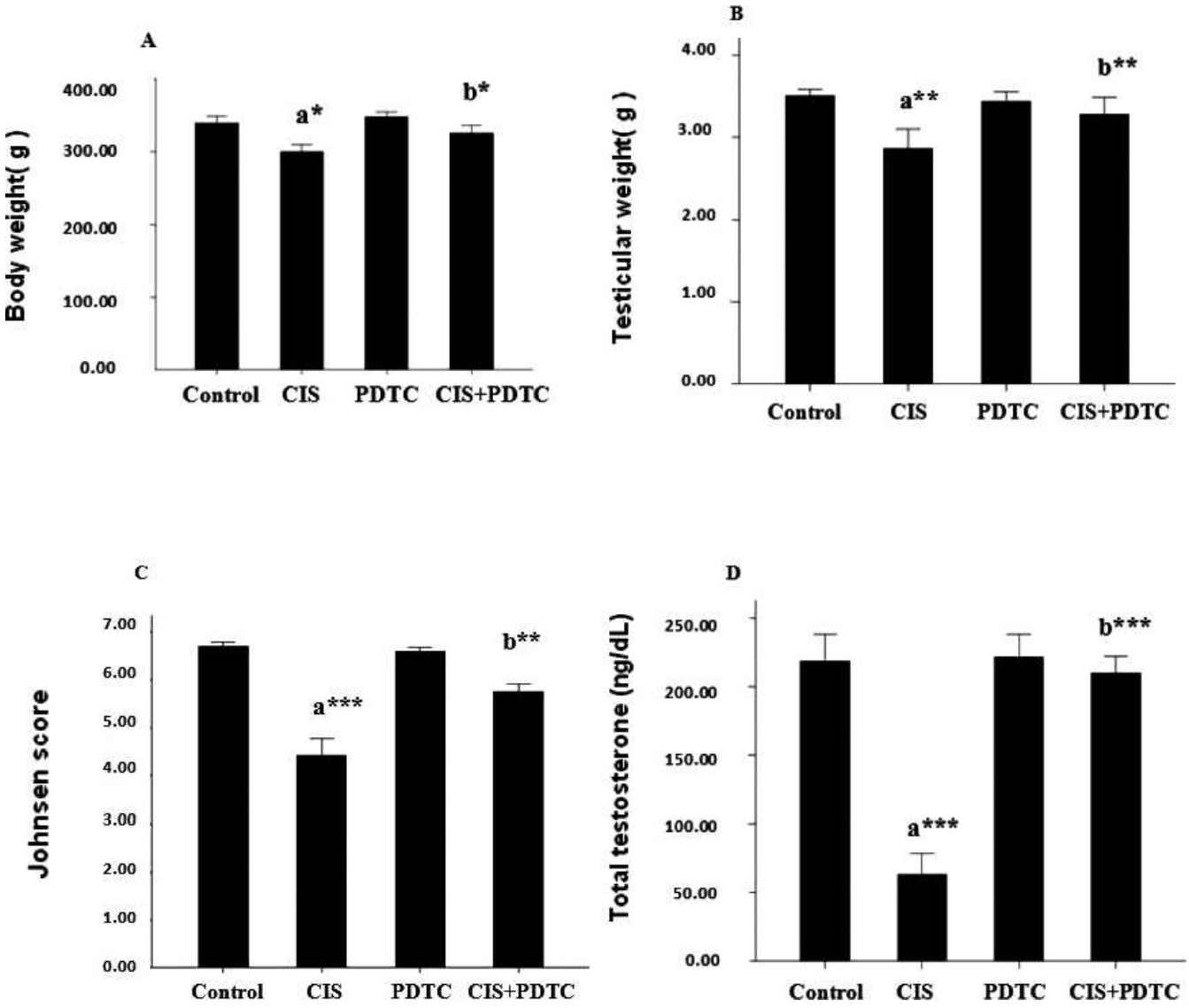

Figure 1. (**A**) Body weight, (**B**) testicular weight, (**C**) Johnson score, and (**D**) total testosterone levels in control, cisplatin (CIS), and CIS+ pyrrolidine dithiocarbamate (PDTC) groups. a indicates compared with control group; b, compared with CIS group. Control vs CIS + PDTC and $P > .05$. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.

expression of iNOS and p65 in the CIS + PDTC and control groups, but there was no significant difference between these 2 groups (Table).

Discussion

CIS is a highly effective antineoplastic DNA-alkylating agent used in the treatment of various solid tumors, including cancer of the testis, bladder, ovary, cervix, endometrium, head, neck, and lung. Its wide spectrum of clinical use is limited because CIS is known to cause adverse effects on testes, kidneys, peripheral nerves, and the inner ear; the impairment of kidney function by CIS is recognized as the main side effect and the most important dose-limiting factor (Stadnicki et al, 1975; Ateşşahin et al, 2005).

It has been reported that male rats receiving CIS exhibit decreases in reproductive organ weights and impaired fertility along with alterations in the growth and development of next generations (Sharma and Agarwal, 1996; Cherry et al, 2004). In the present study, administration of CIS reduced weights of testes when compared with controls, thereby showing consistency with previous reports that claimed that CIS decreases reproductive organ weights (Türk et al, 2008). Reductions in testis weight was due to marked parenchymal atrophy, and histopathologic examination showed

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Table. *GSH-Px activity; GSH, MDA, and NO levels; and immunohistochemical staining scores in control, CIS, PDTC, and CIS + PDTC groups*[a]

Variable	Control	CIS	PDTC	CIS + PDTC
GSH-Px, nmol/g of wet tissue	8.5 ± 0.8	4.5 ± 0.5[b**]	8.6 ± 0.3	8.7 ± 0.2[c**]
GSH, μmol/g of wet tissue	1.45 ± 0.08	0.95 ± 0.1[b**]	1.42 ± 0.2	1.8 ± 0.3[c**]
MDA, nmol/g wet tissue	35.7 ± 3.6	57 ± 8[b**]	37.3 ± 2.1	38.3 ± 5.7[c**]
NO, nmol/g wet tissue	44.3 ± 1.16	91.6 ± 5.4[b***]	43.4 ± 1.2	46.6 ± 2.1[c***]
Histopathology				
iNOS				
0	...	...	...	...
1	...	...	...	...
2	5	...	4	...
3	1	...	2	3
4	...	...	...	3
5	...	...	...	...
6	...	4	...	...
7	...	2	...	...
p65				
0	...	...	...	...
1	...	...	...	...
2	3	...	5	...
3	3	...	1	2
4	...	...	...	4
5	...	1	...	...
6	...	3	...	...
7	...	2	...	...

Abbreviations: CIS, cisplatin; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; NO, nitric oxide; PDTC, pyrrolidine dithiocarbamate.

[a] Values are expressed as means ± SD for 6 rats in each group.

[b] Compared with control group.

[c] Compared with CIS group.

Control group compared with CIS + PDTC group and $P > .05$.

$^{**}P < .01$, $^{***}P < .001$.

severe degeneration, necrosis, reductions in seminiferous tubules, and germinal cell thickness in the testes of rats treated with CIS alone. The effects of this agent on the testis might be due to its specific toxic effects on the target organ and not as a result of its general toxicity. We also observed body weight loss in rats treated with CIS alone. The reduction in body weight may be due to malnutrition occurring as a consequence of systemic toxic effects of CIS. In the present study, PDTC treatment prevented a reduction in both body and testes weight in the CIS + PDTC group when compared with the group subjected to CIS alone.

Plasma testosterone levels were significantly decreased in CIS-treated rats in the present study. The lower plasma testosterone level in CIS-treated rats could be attributed to impaired Leydig cells. Coadministration of PDTC in CIS-treated rats significantly increased the testosterone levels. The spermatogenic inhibition observed among CIS-treated rats in the present study may be the result of decreased plasma testosterone levels. Besides hormonal alteration, the spermatogenic inhibition may also be due to the formation of free radical products in the testicular tissue because they exert a detrimental effect on spermatogenesis.

In traditional studies on the mechanisms of testicular injury induced by alkylating agents such as CIS and cyclophosphamide, investigators focused on the effects of oxidative stress and free radicals on target organs (Fadıllıoğlu et al, 2003). Oxidative stress is induced by oxidant substances commonly known as ROS (Sheweita et al, 2005). The main ROS that have potential implication in reproductive biology are the superoxide anion, the hydroxyl radical, and H_2O_2 (Sikka, 2001). Normally the balance between ROS produced by prooxidants and the ROS scavenged by antioxidants is maintained; however, cellular damage arises when this equilibrium is disturbed (Sharma and Agarwal, 1996). On the other hand, it has been reported that various antioxidants or free radical scavengers, including lycopene, melatonin, vitamin C, and vitamin E, decrease CIS-induced testicular damage significantly (Giri et al, 1998; Choudhury and Jagdale, 2002; Ateşşahin et al, 2006a,b).

Lipid peroxidation (LPO) has been suggested to be closely related to CIS-induced testicular damage, and MDA is a good indicator of the degree of LPO (Ateşşahin et al, 2006b). In the present study, we observed significant increases in MDA content of testicular tissues in rats treated with CIS alone and

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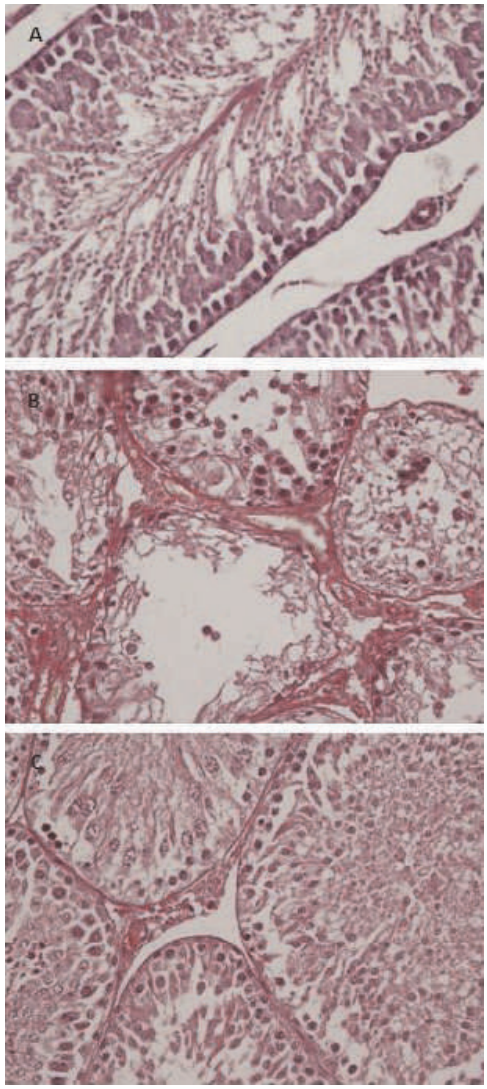

Figure 2. Light microscopy of testicular tissue in rats treated with cisplatin (CIS) alone or with pyrrolidine dithiocarbamate (PDTC). (**A**) Regular seminiferous tubules, normal intertubular gaps, and advanced-stage cells of spermatogenesis in the control group (hematoxylin and eosin stain, ×400). (**B**) Seminiferous tubules containing only Sertoli cells, spermatogoniums, and irregular seminiferous tubules in CIS alone group (hematoxylin and eosin stain, ×400). (**C**) Highly regular seminiferous tubules showing spermatogenesis at the level of spermatocytes in the CIS + PDTC group (hematoxylin and eosin stain, ×400). Color figure available online at www.andrologyjournal.org.

found out that PDTC administration reduced MDA levels.

GSH is one of the most important molecules in the cellular defense mechanism against chemically reactive toxic compounds or oxidative stress (Yu and Anderson, 1997). Decreased cellular GSH levels and capacity for GSH synthesis sensitize cells to certain drugs. GSH synthesis is induced in cells exposed to oxidative stress as an adaptive process. Therefore, interest has been focused on compounds that act as antioxidants and are capable of stimulating GSH synthesis. In the present study, treatment with PDTC partially reserved the increase in GSH levels. The present study also showed a decrease in the activity of antioxidant enzymes such as GSH-Px, along with a drop in GSH levels in rats treated with CIS alone. The decrease in activity of antioxidant enzymes may predispose sperm to increased free radical damage because GSH-Px has been considered the primary scavenger of H_2O_2 (Vernet et al, 2004).

The production of ROS is also affected by NO. Excessive production of NO causes vasodilatation and hypotension leading to organ hypoperfusion, edema, and organ dysfunction. NO can interact with ROS to form peroxynitrite, which is a powerful oxidant and cytotoxic agent and may play an important role in the cellular damage associated with the overproduction of NO. It is generally thought that NO derived from endothelial NOS regulates physiologic vasodilatation at low levels, whereas excessive NO production owing to elevated expression of iNOS can cause cytotoxic effects in surrounding cells. The contribution of NO to tissue damage can be a direct effect mediated by NO itself (Davis et al, 2001) and an indirect effect mediated by reactive nitrogen species produced by the interaction of NO with superoxide anions or oxygen (Wink and Mitchell, 1998; Davis et al, 2001). In the present study, we found the activity of GSH-Px as an antioxidant enzyme decreased and the level of MDA (as a lipid peroxide) increased, indicating the role of NO on the free radical reaction and suggestive of the fact that the oxidation response could intensify CIS-induced injury.

Moreover, recent studies have shown a role of NF-κB in injuries of organs such as the kidney, liver, and pancreas (Frossard et al, 2001; Sun and Andersson, 2002; Orfila et al, 2005; Tugcu et al, 2006). Hence, the role of NF-κB in oxidative damage in testes has attracted more and more attention. NF-κB is a rapid response transcription factor in the cytoplasm and consists of 2 subunits of 50 kd and 65 kd that are bound to an inhibitor protein called IκB. This phosphorylated inhibitor unit is tagged by ubiquitin for subsequent proteolysis, and eventually the freed NF-κB complex is able to translocate into the nucleus where it transactivates target genes (Lee and Burckart, 1998). This is a common end point of various signal transduction pathways (Kolesnick and Golde, 1994). It has been suggested that NF-κB mediates lipopolysaccharide and γ-interferon induction of NOS in rat macrophages (Sakurai et al, 1996; Goligorsky et al, 2002). Furthermore, the presence of potential NF-κB binding sites in the *iNOS* gene has been reported (Xie et al, 1994). The expression of iNOS is mainly controlled by activation of its transcriptional factors, including

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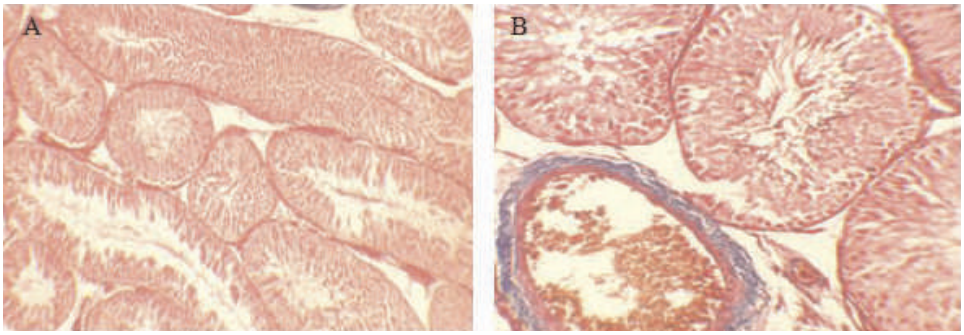

Figure 3. Light microscopy of rat testes from the (**A**) control and **(B)** cisplatin (CIS) groups. (**A**) Regular and fibrosis-free seminiferous tubules (Masson trichrome stain, ×100). (**B**) Fibrosis in perivascular and interstitial area in the CIS group (Masson trichrome stain, ×200). Color figure available online at www.andrologyjournal.org.

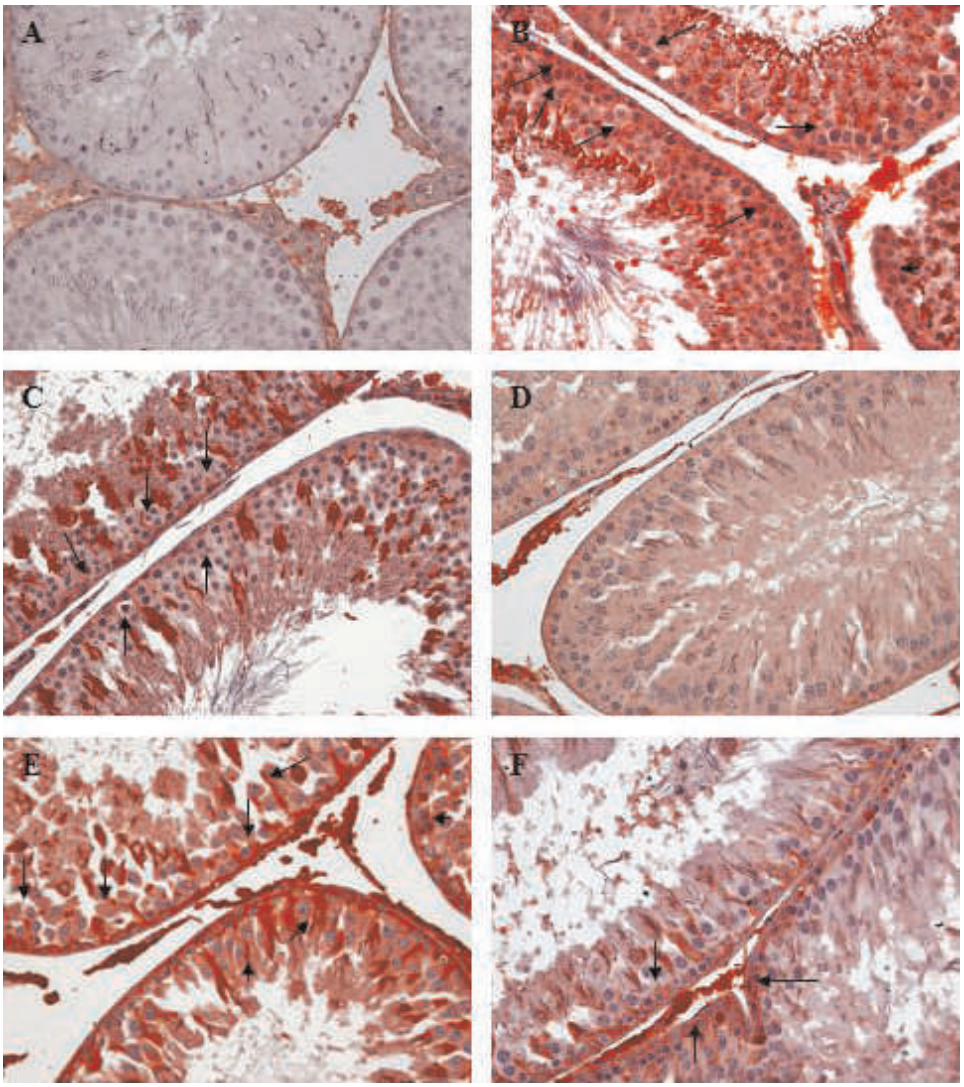

Figure 4. Immunohistochemical staining showing inducible nitric oxide synthase (iNOS) and nuclear factor-κB inhibitor (NF-κB) expression. (**A**) Focal mild staining (score 2) with iNOS in controls (×400). (**B**) Diffuse iNOS staining (score 7) in cisplatin (CIS) group (×400). (**C**) Mild iNOS staining (score 3) in CIS + pyrrolidine dithiocarbamate (PDTC) (×400). (**D**) Low NF-κB/p65 positivity (score 2) in controls (×400). (**E**) Diffuse, intensive NF-κB/p65 positivity (score 6) in CIS group (×400). (**F**) Low NF-κB/p65 positivity (score 3) in CIS + PDTC (×400). Color figure available online at www.andrologyjournal.org.

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NF-κB. In the present study, we immunohistochemically showed that iNOS expression was increased in rats subjected to CIS alone treatment, but iNOS expression was decreased in rats treated with CIS + PDTC. The present study suggests that iNOS expression can be inhibited by a NF-κB inhibitor. Reverse transcriptase polymerase chain reaction or Western blotting analyses are functional assays that can measure the actual activity of iNOS. Western blotting provides a more quantitative way of measuring iNOS and NF-κB p65 subunit activity. Therefore, the absence of Western blotting analyses may be a limitation of this study. However, there are a number of studies in the literature that have made use of immunohistochemical grading of iNOS to evaluate NO activity (Jang et al, 2006).

Nakashima et al (1999) reported inhibition of active NF-κB by dexamethasone, acetylsalicylic acid, or PDTC caused by suppression of only the p65 subunit of NF-κB and reported no inhibition of the p50 subunit by the NF-κB inhibitors. We studied only the p65 subunit immunohistochemically. In the current study, the lower expression of the NF-κB p65 subunit in the CIS + PDTC group indicates that the administration of PDTC may inhibit NF-κB activation.

Histopathologic examination showed significant maturation arrest and low Johnson scores, and mild perivascular fibrosis and hyalinization of intertubular tissue were observed in the group that received CIS alone compared with the control group. The result was consistent with those of previous investigations describing testicular histologic alterations in CIS-induced testicular injury (Ateşşahin et al, 2006b; Türk et al, 2008). PDTC treatment improved the CIS-induced histologic testicular changes.

There is a contentious issue on whether antioxidant supplementation alters the efficacy of cancer chemotherapy. Some authors argue that antioxidants scavenge the ROS integral to the activity of certain chemotherapy drugs (Heaney et al, 2008), thereby diminishing treatment efficacy. Others suggest that antioxidants might be mitigating toxicity and thus allowing for uninterrupted treatment schedules and a reduced need for lowering chemotherapy doses (Lee et al, 2008). There is no study reported in the literature that compares the tumoricidal effect of CIS + PDTC with the antitumoral effect of PDTC alone in an experimental animal tumor model.

The transcription factor NF-κB is reportedly activated by anticancer chemotherapeutic agents in many cancer cell lines, and NF-κB activation is one mechanism by which tumors become resistant to apoptosis (Camp et al, 2004). Enhanced activation of NF-κB can induce chemoresistance in cancer cells either through the up-regulation of multidrug resistance genes or through the inhibition of the tumor suppressor gene p53 (Tergaonkar et al, 2002). In addition to metal chelating and antioxidant properties, PDTC is the most potent NF-κB inhibitor (Schreck et al, 1992). The NF-κB inhibitory property of PDTC is independent of its antioxidant behavior (Woods et al, 1999). PDTC has been shown to enhance the cytotoxicity of the chemotherapeutic agent 5-fluorouracil in animal models of colorectal cancer (Chinery et al, 1997; Bach et al, 2000). PDTC has also been shown to induce proapoptotic and antiproliferative effects in prostate cancer (Chen et al, 2005), renal cancer (Morais et al, 2006), leukemia (Arima et al, 2004), lymphoma (Meli et al, 2003), and gastric cancer cells (Li et al, 2005). Moreover, PDTC has been shown to induce apoptosis in tumor cells by inhibiting the proteasomal activity (Milacic et al, 2008). It has been reported that PDTC harbors potent anticancer activity against CIS-resistant neuroblastoma cells (Zhang et al, 2008).

In light of the information provided above, because PDTC has an inhibitor property alongside inducing apoptosis in tumor cells by inhibition of proteasomal activity, we believe that its use in combination with CIS can increase the chemotherapeutic efficiency of CIS and that it can prevent CIS-induced toxicity owing to its antioxidant properties.

In conclusion, the findings of the present study reinforce the significant role of ROS in the pathogenesis of CIS-induced testicular toxicity and demonstrate that blockade of NF-κB activation by an antioxidant could be an effective strategy for prophylaxis of CIS-induced testicular damage. PDTC has a potent protective effect against the testicular toxicity of this agent and might be clinically useful. However, there is a need for further studies on this issue before clinical application can be recommended.

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