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Nickel-Induced Oxidative Stress in Testis of Mice: Evidence of DNA Damage and Genotoxic Effects

KODIPURA DORESWAMY, BALAKRISHNA SHRILATHA, THIMAPPA RAJESHKUMAR, AND MURALIDHARA

From the Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, India.

ABSTRACT: Oxidative stress (OS) mechanisms are speculated to play a significant role in nickel-induced toxic effects and their carcinogenic potency. Although nickel-induced oxidative damage in somatic tissues is well demonstrated, evidence of the involvement of a similar mechanism(s) in nickel-induced testicular dysfunction and associated genotoxic effects is scarce. Hence, the present study aimed to investigate the nickel-induced OS response in testis and the associated genotoxic implications in vivo. Initially, the toxicity profile of nickel chloride was determined in adult albino mice (CFT-Swiss) following administration (intraperitoneal) of single doses. Subsequently, multiple sublethal doses (1.25, 2.5, and 5.0 μmol/100 g of body weight per day for 3 days) were used to characterize effects on testicular histoarchitecture, lipid peroxidation (LPO) in testis (homogenates, microsomal or mitochondrial fractions) and epididymal sperm, DNA damage, induction of apoptosis in testis, and incidence of sperm head abnormalities. Although short-term doses of nickel induced only a minimal LPO response, multiple doses elicited a moderate (15% to 30%) increase in LPO in whole homogenates and higher dose-related increases in both mitochondrial (20% to 50%) and microsomal fractions (25% to 60%). This was associated with a significant increase in DNA damage in the testis as evidenced by increased single-strand breaks (fluorimetric analysis of DNA unwinding assay). Further, at higher doses, nickel-induced apoptosis was demonstrable in the testis biochemically. Although caudal sperm counts determined at all sampling weeks showed no alterations, analysis for head abnormalities revealed a nearly 3- to 4-fold increase in the percentage of abnormal sperms among the nickel-treated males during the first 3 weeks. Furthermore, mating of nickel-treated (2.5 μmol/100 g of body weight per day for 5 days) males sequentially for a period of 5 weeks with untreated females resulted in a significant increase in male-mediated dominant lethal–type mutations (the frequency of dead implantations) during the first 3 weeks, suggesting a stage-specific effect on postmeiotic germ cells. These findings suggest that testicular toxicity of nickel compounds may be related to enhanced production of reactive oxygen species, probably mediated through oxidative damage to macromolecules, including damage to DNA.

Key words: Nickel chloride, oxidative damage, apoptosis, abnormal sperms, dominant lethal mutations.

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Nickel salts are considered an industrial health hazard, since many nickel compounds reach the human environment (Venugopal and Luckey, 1978). Nickel has been classified as a human carcinogen based on epidemiological evidence, which shows high incidence of nasal and lung cancers in refinery workers (Coogan et al, 1989; Goyer, 1991), and its potency to induce tumors in a variety of mammalian species (Sunderman, 1987, 1989). Although the toxicity and carcinogenicity of nickel compounds in humans and experimental animals are well demonstrated, the underlying mechanisms of their action remain unclear (Sunderman et al, 1985; Stohs and Bagchi, 1995). The most plausible mechanism that may be operative in vivo is the generation of reactive oxygen species (ROS), which may initiate lipid peroxidation (LPO), oxidative damage to critical macromolecules such as proteins or DNA, and cell damage or death. LPO constitutes a free radical oxidation process in which polyunsaturated fatty acids of the cell membrane decompose to yield, among others, highly reactive lipid hydroperoxides, H_2O_2, hydroxyl radicals, and malondialdehyde (MDA) (Pryor, 1985; Halliwell and Gutteridge, 1989). MDA has been demonstrated to cause cross-linking and polymerization of membrane components and may contribute to mutagenic, genotoxic, and carcinogenic effects (Ueda et al, 1985; Vaca et al, 1988; Wang and Liehr, 1995).

In mammalian cells, induction of DNA single-strand breaks, DNA protein cross-links, sister chromatid exchanges, and chromosomal aberrations has been demonstrated with various nickel salts (Kawanishi et al, 1989; Torreilles and Gurein, 1990; Kasprzak, 1991). Since nickel ions bind weakly to purified DNA in vitro, indirect mechanisms such as generation of ROS have been previously proposed to explain nickel-induced DNA damage (Patierno and Costa, 1985; Zhong et al, 1990; Huang et al, 1993). Further, nickel salts are shown to enhance LPO in various somatic tissues, such as blood, muscle, liver, and kidney of rats (Athar et al, 1987; Kasprzak and Her-

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Correspondence to: Dr Muralidhara, Department of Biochemistry and Nutrition, CFTRI, Mysore 570 020, India (e-mail: mura16@yahoo.com).

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nandez, 1989; Misra et al, 1990; Stinson et al, 1992; Chen et al, 1998).

Effects of nickel compounds on reproduction in rodent models are well documented (Pandey et al, 1999; Das and Dasgupta, 2000). Although bioaccumulation of nickel in testis is well demonstrated, the exact mechanisms of nickel-induced male reproductive toxic effects are not clear (Kakela et al, 1999; Obone et al, 1999). Further, the involvement of oxidative stress (OS) mechanisms in nickel-induced testicular toxicity has not been unequivocally demonstrated. In addition, studies on the relationship between induction of LPO and DNA damage in testis in vivo and susceptibility of epididymal sperm (ES) to nickel-induced OS are totally lacking. More importantly, to our knowledge, the ability of nickel salts to induce apoptosis (programmed cell death) and DNA damage in testis, implications on sperm morphology and development, and their possible genotoxic effects have not been previously addressed.

Accordingly, in the present study, we investigated the propensity of nickel to induce OS in testis and ES of mice following administration of single or multiple sublethal doses. Using similar doses, induction of DNA damage in testis or ES and apoptosis (in terms of DNA fragmentation) in testis was also investigated. Furthermore, nickel-induced genotoxic effects were ascertained by examining their effects on caudal sperms (counts and head abnormalities) and their ability to induce male-mediated dominant lethal (DL) mutations. Our results suggest that OS induced by multiple low doses of nickel chloride in subcellular fractions (mitochondrial or microsomal) of testis is associated with significant DNA damage and apoptosis. Further, nickel chloride has the propensity to cause stage-specific genotoxic effects measured in terms of a higher percentage of sperm head abnormalities and DL-type mutations discernible only during specific posttreatment weeks.

Materials and Methods

Chemicals

Nickel chloride, thiobarbituric acid, 1,1,3,3-tetramethoxypropane, ethidium bromide, and agarose were obtained from Sigma Chemical Co (St Louis, Mo). All other chemicals were of analytical grade.

Animals and Care

Adult mice (10 weeks old) of CFT-Swiss strain of both sexes were randomly drawn from the stock colony of our animal house facility and were held in groups of 5 in polypropylene cages under standard conditions. During a week of acclimatization and throughout the studies, they were maintained on commercial mouse pellets (Gold Mohur, supplied by Lipton India Pvt Ltd, India) ad libitum and had free access to water. The cages were kept on racks built of slotted angles and were housed in a controlled atmosphere with a temperature of 25°C ± 5°C and a mean relative humidity of 50% ± 5%.

Animal Treatment

To assess the induction of OS in testis, groups of male mice were initially administered (intraperitoneally) nickel chloride (dissolved in distilled water) at single dosages (highest dose, 5 μmol/100 g of body weight). For all other investigations, a multiple exposure regimen (0.625, 1.25, 2.5, and 5.0 μmol/100 g of body weight for either 3 or 5 consecutive days) was used. These dosages were based on our earlier study in which the toxicity profile of nickel chloride was computed (Doreswamy et al, 2001). Mice were killed 24 hours after the last dose (biochemical studies, DNA damage, and fragmentation experiments) and at 1, 2, 3, and 5 weeks following the beginning of the treatment (sperm head abnormality study). Body weights were recorded on the days of treatment and autopsy. Immediately after euthanatizing, testes and epididymis were excised and weights were recorded. One testis and its correspondent epididymis were fixed in Bouin fixative and processed for histopathological examination.

Induction of LPO in Testis and ES

Induction of OS was ascertained by measuring the extent of LPO in testicular homogenates, microsomes, mitochondrial fractions, and ES of adult mice at 24 hours after the last dose in both single- and multiple-exposure regimens. For homogenate preparation, testes were decapsulated, weighed, and homogenized with a glass Teflon grinder at 4°C in phosphate buffer, pH 7.4. Microsomes were prepared following the method of Shimoji and Aniya (1994) with minor modifications, and mitochondrial fractions were prepared as per the method described by Trounce et al (1996). ES suspensions were prepared as per the procedure described earlier (Muralidhara and Narasimhamurthy, 1991). LPO was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS) by following the method of Ohkawa et al (1979) using 1,1,3,3-tetramethoxy propane as the standard.

Measurement of Antioxidant Enzyme Activity

Testis samples were homogenized in 50 mM phosphate buffer (pH 7.4) and sonicated over ice. The activities of enzymes (catalase, glutathione peroxidase, and glutathione S-transferase) were determined in 1000 × *g* supernatants of testis homogenates. Catalase (EC 1.11.1.6) activity was assayed by the method of Aebi (1984), and the activity was expressed as micromoles of H_2O_2 consumed per minute per milligram of protein. The activity of glutathione peroxidase (EC 1.11.1.9) was determined using the tbHP as the substrate according to the method described by Flohe and Gunzler (1984), and the activity was expressed as nanomoles of NADPH oxidized per minute per milligram of protein (e340 = 6.22 mM/cm). Glutathione S-transferase (EC 2.5.1.18) was assayed at 340 nm by measuring the rate of enzyme catalyzed conjugation of reduced glutathione with 1-chloro-2-4-dinitro benzene according to the method of Guthenberg et al (1985). Enzyme activity was expressed as nanomoles of s-2,4-dinitrophenyl glutathione formed per minute per milligram

Table 1. *Body and testicular weights of mice administered multiple doses of nickel chloride sampled at various posttreatment weeks**

	Posttreatment Week							
	1		2		3		5	
Group	BW	TW	BW	TW	BW	TW	BW	TW
Control	40.0 ± 2.6	659 ± 52	44.4 ± 3.5	545 ± 58	45.4 ± 2.7	571 ± 10	47.5 ± 2.6	500 ± 51
Ni^1	41.7 ± 2.6	644 ± 40	45.3 ± 2.9	500 ± 94	42.5 ± 2.4	586 ± 23	42.2 ± 4.9	555 ± 40
Ni^2	40.7 ± 2.1	604 ± 25	46.4 ± 2.4	565 ± 28	45.3 ± 4.3	544 ± 15	45.6 ± 2.7	639 ± 11
Ni^3	42.5 ± 2.5	624 ± 15	45.4 ± 1.4	545 ± 25	45.8 ± 3.3	574 ± 18	44.8 ± 2.7	619 ± 11

* Values are mean ± SD of 4 animals each. Data analyzed by Student's *t* test. No significant differences were noted between the treatment and control groups. BW indicates body weight (in grams); TW, testis weight (in milligrams per 100 g of body weight); Ni^1, 1.25 μmol/100 g of body weight; Ni^2, 2.5 μmol/100 g of body weight; and Ni^3, 5.0 μmol/100 g of body weight.

of protein. Protein level was determined using bovine serum albumin as the standard as per the method of Lowry et al (1951).

Quantification of DNA Damage

The fluorimetric analysis of DNA unwinding (FADU) assay was performed according to the procedure described by Birnboim (1990). Testicular cell suspensions were prepared as described previously from our laboratory (Rajeshkumar et al, 2002). Final cell pellet was suspended in a known volume of KRBS. An aliquot of testicular cell suspension (approximately 1×10^6 cells) or ES (approximately 2×10^6) was transferred into test tubes and cells lysed for 10 minutes. The assay was performed in triplicate. The pH was increased by adding, successively and carefully, the alkaline solutions to allow DNA unwinding. Following neutralization, the percentage of double-stranded DNA (ds-DNA) formed was detected by measuring the fluorescence of samples after addition of ethidium bromide. Measurements were performed in a Shimadzu F-2000 fluorescence spectrophotometer with 520 and 575 as excitation and emission wavelengths, respectively. The percentage of ds-DNA that remained after the unwinding process was calculated by determining the following ratio: (unwound DNA fluorescence − denatured DNA fluorescence)/(native DNA fluorescence − denatured DNA fluorescence).

Agarose Gel Electrophoresis of Testicular DNA

DNA from testis of both control and nickel-treated mice was isolated following the method of Hughes and Gorospe (1991). Briefly, testes were decapsulated and teased in lysis buffer, and following the addition of diethyl pyrocarbonate, the samples were digested with proteinase K. After repeated extraction with phenol-chloroform, the DNA in the supernatant was precipitated by adding 3 M sodium acetate and an equal volume of ice cold absolute alcohol. The samples were kept at −20°C for DNA precipitation. The DNA was pelleted and washed with 70% alcohol. The pellet was dried and dissolved in Tris EDTA buffer (pH 8.0). The DNA was quantified spectrophotometrically, and 5 μg of DNA was loaded onto a 1.8% agarose gel and electrophoresed at 50 V in Tris borate EDTA buffer. The gel was stained with ethidium bromide and visualized on a UV transilluminator.

ES Counts and Sperm Head Abnormalities

Following administration of multiple doses of nickel chloride, male mice were killed by cervical dislocation at 1, 2, 3, and 5 weeks after treatment. Fresh cauda epididymis held in 0.9% NaCl was processed according to the method described previously (Muralidhara and Narasimhamurthy, 1991) to determine the sperm counts. Aliquots of sperm suspensions were stained in 1% eosin Y, and the smears were examined for abnormal sperms (a minimum of 1000 sperms per animal) and expressed as the percentage of abnormal sperms based on the original method (Wyrobeck and Bruce, 1978).

DL Mutation Response

For this study, 10 adult males were administered (intraperitoneally) nickel chloride (2.5 μmol/100 g of body weight) for 5 consecutive days and mated with untreated (1:1) virgin females each week sequentially for 5 weeks. Successful mating was ascertained by the presence of vaginal plugs, and all the pregnant females were humanely killed 16 to 17 days after detection of plugs and assessed for the degree of postimplantation embryolethality as described previously (Muralidhara and Narasimhamurthy, 1996). Males that were administered (intraperitoneally) a single dose of cyclophosphamide (100 mg/100 g of body weight) and mated with virgin females for 5 consecutive weeks served as the positive control.

Statistical Analyses

Data on both LPO and DNA damage were analyzed using Student's *t* test, and $P < .05$ was set as the minimum level of statistical significance. However, data on ES count, sperm head abnormalities, and 3 variables of DL assay (pregnancy, total implantations, and postimplantation deaths) were analyzed using a nonparametric test, the Mann-Whitney *U* test (Snedecor and Cochran, 1980).

Results

Body Weights, Testicular Weights, and Pathology

Administration of nickel chloride at multiple sublethal doses did not induce any clinical signs of toxicity or mortality at any of the doses. Data on the body and testicular weights presented in Table 1 showed no treatment-related effects. Further there were no alterations in the relative weights of epididymis, among the treated mice (data not shown). Microscopic examination of both testis and epi-

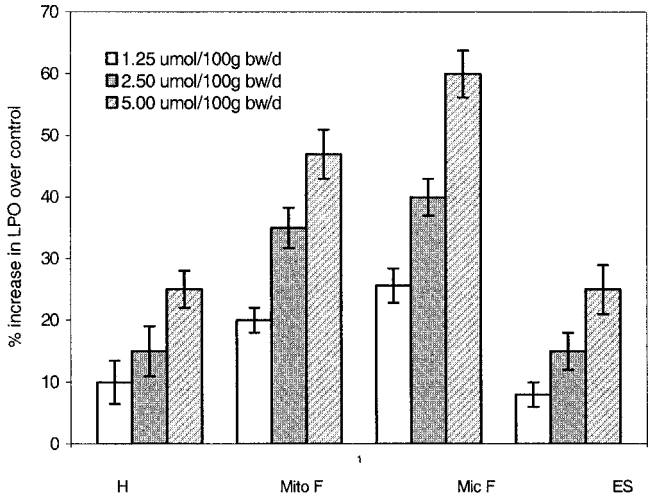

Figure 1. Percent increase in lipid peroxidation (LPO) in whole homogenates (H), mitochondrial fractions (Mito F) and microsomal fractions (Mic F) of testis, and epididymal sperm (ES) of mice treated with multiple doses (1.25, 2.5, and 5.0 μmol/100 g of body weight per day for 3 days) of nickel chloride.

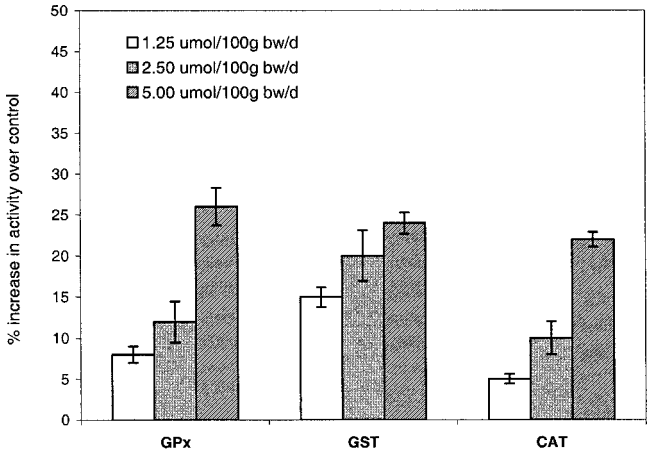

Figure 2. Percent increase in enzyme activities of glutathione peroxidase (GPx), glutathione S-transferase (GST), and catalase (CAT) in cytosolic fractions of testis of mice treated with multiple doses (1.25, 2.5, and 5.0 μmol/100 g of body weight per day for 3 days) of nickel chloride.

didymis showed no obvious treatment-related pathological lesions.

Induction of LPO in Testicular Homogenates, Microsomes, Mitochondrial Membranes, and ES

Following single doses, only a marginal increase (15%) in the endogenous TBARS (an index of in vivo LPO) levels was evident in the testis at 24 hours after exposure at the highest dosage of 5 μmol/100 g of body weight only (data not shown). However, there were significant increases in the TBARS levels in whole homogenates and mitochondrial and microsomal fractions following multiple exposure regimens (Figure 1) measured at 24 hours after the last dose. Although the degree of LPO induction was marginal at the lowest dose (1.25 μmol), a nearly 15% to 30% increase was evident at the higher doses (2.5 and 5 μmol/100 g of body weight). However, the mitochondrial fractions showed a dose-related increase in LPO induction, which ranged from a 20% to 50% increase over the control levels. Further, nickel treatment resulted in a relatively higher degree of LPO induction (25% to 60%) in the microsomal fractions at similar doses (Figure 1). Interestingly, there was a 10% to 20% increase in the LPO in the ES during the first week with nickel treatment (Figure 1).

Antioxidant Enzymes in Testis

Alterations in the activities of antioxidant enzymes measured in testis following nickel treatment are presented in Figure 2. Dose-related elevations were evident in the activities of all antioxidant enzymes. A significantly enhanced activity of glutathione peroxidase was evident at the highest dosage (5.0 μmol). The activities of glutathione S-transferase were elevated significantly (15% to 26%) at all the doses. Although the increase was marginal at the lowest dose, the catalase activities were enhanced significantly (10% to 25%) at the higher doses.

DNA Damage in Testis and ES

The effect of multiple doses of nickel chloride on DNA damage in testis and ES quantified at 24 hours after the last dose is presented in Table 2. The formation of strand breaks (as measured by the FADU assay) that led to a decreased percentage of ds-DNA was evident only at higher doses (2.5 and 5 μmol/100 g of body weight). In the testis, the background DNA damage was 17%. However, treatment with nickel chloride resulted in increased dose-related DNA damage. A similar trend was evident in ES, and only a marginal increase in DNA damage was observed at the highest dose.

Agarose gel electrophoresis of genomic DNA extracted from both control and nickel-administered mice as visualized by ethidium bromide fluorescence is shown in Fig-

Table 2. *Percentage of double-stranded DNA in testis and epididymal sperms of mice administered (intraperitoneally) with multiple doses of nickel chloride**

	Double-Stranded DNA, %	
Group	Testis	Epididymal Sperms
Control	83 ± 5	90 ± 4
Ni^1	80 ± 6	85 ± 5
Ni^2	65 ± 8†	82 ± 4‡
Ni^3	62 ± 6†	80 ± 5‡

* Values are mean ± SD of 4 animals each. Data analyzed by Student's *t* test. Ni^1 indicates 1.25 μmol/100 g of body weight; Ni^2, 2.5 μmol/100 g of body weight; and Ni^3, 5.0 μmol/100 g of body weight.
† $P < .05$.
‡ $P < .02$.

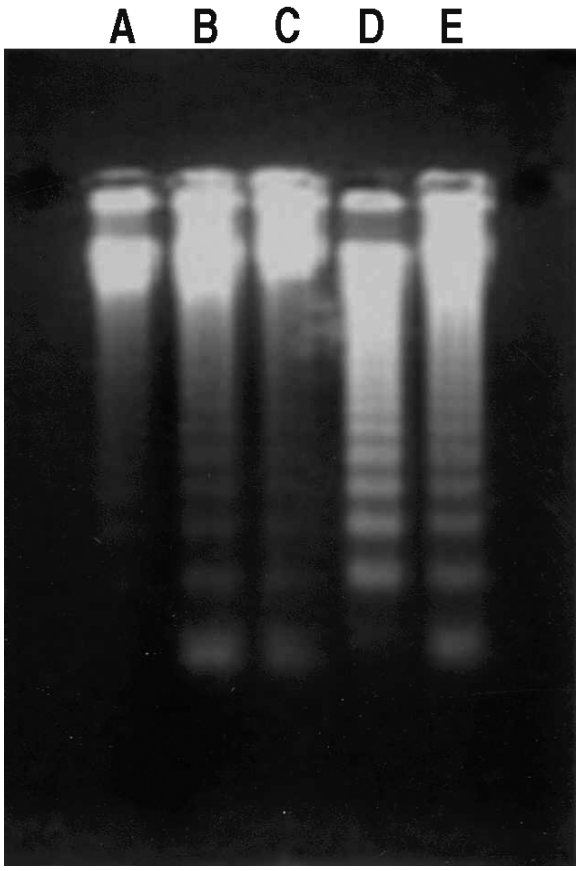

Figure 3. 1.8% agarose gel electrophoresis of DNA extracted from mice testis treated with multiple doses of nickel chloride. Lanes A through E are 0, 0.625, 1.25, 2.5, and 5.0 μmol of nickel chloride per 100 g of body weight per day for 3 days, respectively.

ure 3. As evident from the figure, the control samples showed only a minimal degree of DNA damage (lane A). However, nickel treatment induced significant apoptosis, since typical ladder patterns of DNA fragmentation were discernible at all doses. Although the laddering of DNA appeared to be weak at the lower doses (0.625 and 1.25 μmol/100 g of body weight; lanes B and C), it was marked at the higher doses (2.5 and 5 μmol/100 g of body weight; lanes D and E).

ES Counts and Incidence of Sperm Head Abnormalities

The sperm caudal counts among nickel-treated mice were on par with those of controls at all sampling periods (data not shown). Data on the frequency of sperms with head abnormalities determined during various posttreatment weeks are presented in Table 3. The spontaneous incidence of the percentage of abnormal sperms among controls ranged from 1.4 to 2.0 and was within the background incidence reported previously for the CFT-Swiss mice from our laboratory. However, nickel treatment induced a significant increase in the percentage of abnormal sperms only during the first 3 weeks and the frequency

Table 3. *Incidence of abnormal sperms in mice administered (intraperitoneally) multiple doses of nickel chloride**

	Week			
Group	1	2	3	5
Control	2.0 ± 0.12	2.1 ± 0.15	1.8 ± 0.22	2.3 ± 0.16
Ni^1	2.1 ± 0.18	2.2 ± 0.21	1.9 ± 0.18	2.2 ± 0.15
Ni^2	5.9 ± 0.24†	7.3 ± 0.25†	5.0 ± 0.28†	3.6 ± 0.19‡
Ni^3	5.9 ± 0.26†	5.7 ± 0.27†	6.9 ± 0.18†	4.2 ± 0.25‡

* Values are mean ± SD of 4 animals each. Data analyzed by Mann-Whitney *U* test. Ni^1 indicates 1.25 μmol/100 g of body weight; Ni^2, 2.5 μmol/100 g of body weight; and Ni^3, 5.0 μmol/100 g of body weight.
† $P < .001$.
‡ $P < .002$.

normalized thereafter. Nickel treatment induced a 3- to 4-fold increase during weeks 1, 2, and 3. The major head abnormalities consisted of amorphous heads, balloon heads, and big heads and hammerheads as described originally by Wyrobeck and Bruce (1978).

Assessment of DL Mutation Response

Administration of nickel chloride at 2.5 μmol/100 g of body weight per day on 5 consecutive days seemed to marginally affect the mating efficiency of males only during the first 3 weeks. The percentage of induced pregnancies during weeks 1, 2, and 3 were 75%, 55%, and 75%, respectively. During weeks 4 and 5, it was comparable to those of negative controls (80% to 90%). However, no significant difference was found with regard to the total number of implantations among pregnancies that resulted from nickel-treated males during all the 5 weeks (Table 4). The total implantations per litter in the nickel group ranged from 10.9 to 11.4. However, there was a marked decrease in the number of live implantations among the nickel group during weeks 1, 2, and 3. Further, there was no decrease in the number of live implantations in the nickel-treated group during weeks 4 and 5. The number of live implantations per litter during weeks 1, 2, and 3 was 9.0, 8.2, and 9.00, respectively. As a consequence, the mean incidence of dead implantations per litter during these weeks increased to 1.9, 3.2, and 2.2, respectively, which was statistically significant. The dead implantations per litter expressed as a percentage of total implantations (Table 5) showed a significant increase (approximately 17%, 28%, and 20%) compared with the controls (<10%). For comparison, data on the size of increase in the incidence of dead implantations following treatment of males with a single dose of cyclophosphamide (a positive DL mutagen) was also obtained. The cyclophosphamide-treated males induced nearly a sevenfold increase in the dead implantations per litter during the first 2 weeks and a fourfold increase during the third week.

Table 4. *Dominant lethal response in mice administered (intraperitoneally) multiple doses of nickel chloride (2.5 μmol/100 g of body weight per day for 5 days)*

Index*	Treatment	Weeks†				
		1	2	3	4	5
TI litter	Control	11.5	11.2	11.3	11.4	11.6
	Nickel	10.9	11.4	11.2	11.2	10.9
	Cyclophosphamide‡	8.7	11.4	10.1	11.3	10.8
LI litter	Control	10.5	10.3	9.9	10.3	10.4
	Nickel	9.0	8.2	9.0	10.1	9.7
	Cyclophosphamide	3.4	5.0	6.5	10.3	9.8
Total DI litter	Control	1.0	0.9	1.4	1.1	1.2
	Nickel	1.9§	3.2‖	2.2‖	1.1	1.2
	Cyclophosphamide	5.3¶	6.33¶	3.6¶	1.4	1.0

* TI indicates total implantations; LI, live implantations; and DI, dead implantations.
† Data analyzed by Mann-Whitney *U* test.
‡ Cyclophosphamide was given at 10 mg/100 g of body weight in a single dose.
§ $P < .05$.
¶ $P < .002$.
‖ $P < .001$.

Discussion

Epidemiological findings (ie, increase in sperm oxidation and poor antioxidant levels in semen under certain situations of OS in humans such as ascorbic acid deficiency and smoking) have emphasized a potential relationship between oxidative damage in testis and sperms and testicular dysfunction (Dawson et al, 1992; Fraga et al, 1996). Currently, it is well appreciated that damage to testicular male germ cells induced by various xenobiotics, products of abnormal metabolism, or ROS can result in testicular dysfunction, leading to infertility (Aitken, 1994, 1995; Stohs and Bagchi, 1995; Sikka, 2001). In this regard, we have previously reported the development of a hydroperoxide model of OS in testis and examined the correlation among LPO in testis, DNA damage, and genotoxic implications (Rajeshkumar and Muralidhara, 1999; Rajeshkumar et al, 2002).

Although much evidence indicates the involvement of OS mechanisms in nickel-mediated toxic effects in somatic organs (Sunderman et al, 1985; Athar et al, 1987; Sunderman, 1989; Misra et al, 1990; Stinson et al, 1992; Chen et al 1998), studies on the propensity of nickel to induce OS in testis are scarce. More importantly, data on the consequences of DNA damage on sperm morphology and development and their possible genotoxic implications are nonexistent. In the present study, low, single doses of nickel failed to induce any notable increase in LPO in both testicular homogenates and ES. However, significant induction was evident with multiple sublethal doses, clearly indicating the importance of repeated exposures. Although the degree of induction was relatively low in homogenates, significant increases were evident in both mitochondrial and microsomal fractions, suggesting the higher susceptibility of these membranes to nickel intoxication.

In the present study, we also observed moderate elevations in the activities of few antioxidant enzymes (glutathione peroxidase, glutathione S-transferase, and catalase) in testis, suggesting the induction of OS. Previously, we reported significant increases in protein carbonyls and iron levels in rat testis following administration of sublethal multiple doses of nickel chloride (Doreswamy et al, 2001). Further, we found no evidence of pathological lesions (necrosis) in testis due to nickel treatment (data not shown). However, nickel induced a significant degree of apoptosis in testis, which was assessed biochemically (in terms of DNA laddering). Although this is the first report, to our knowledge, on nickel-induced apoptosis in testis, other authors have documented similar cell death in testis of experimental animals following exposure to cadmium (Xu et al, 1996), cyclophosphamide (Cai et al, 1997), and various other apoptogenic agents (Blanco-Rodriguez and Martinez-Garcia, 1998). Further studies are essential to understand the mechanisms related to

Table 5. *Induction of dominant lethal mutation response in mice administered (intraperitoneally) multiple doses of nickel chloride (2.5 μmol/100 g of body weight per day for 5 days)**

Treatment	Week				
	1	2	3	4	5
Control	8.69	8.03	10.9	9.64	10.3
Nickel	16.5‡	28.00¶	19.64¶	9.82	11.0
Cyclophosphamide†	60.27§	55.86§	35.00§	11.5	11.1

* Data are percentage of dead implantations per litter expressed as percentage of total implants. Data analyzed by Mann-Whitney *U* test.
† Cyclophosphamide was given at 10 mg/100 g of body weight in a single dose.
‡ $P < .05$.
§ $P < .002$.
¶ $P < .001$.

nickel-induced apoptotic cell death in testis and its implications.

In the present study, nickel induced significant DNA damage only at higher doses in testis. Our data are consistent with earlier reports on nickel chloride–induced DNA strand breaks in rat liver (Stinson et al, 1992) and in vitro cell models (Patierno and Costa, 1985; Kawanishi et al, 1989). Although the mechanism of nickel-induced DNA damage is not clear, it may be caused by the induction of Fenton-generated hydroxyl radicals as suggested earlier in somatic cells (Misra et al, 1990; Stinson et al, 1992). Other authors (Athar et al, 1987) have hypothesized that nickel-induced accumulation of iron in hepatic tissue may be directly responsible for the oxidative damage to macromolecules. Although speculative, similar mechanisms may also be operating in germ cells, since we also noted significant increases in iron in the rat testis following nickel intoxication (Doreswamy et al, 2001).

The susceptibility of epididymal spermatozoa to OS induced by nickel is evident, since a significant increase in LPO was observed following multiple exposures. Although sperms produced in the testis are reasonably well protected by the microenvironment of Sertoli cells, they are less protected against the oxidant environment in epididymis, owing to the slow transit time and prolonged storage (Cummins et al, 1994). In the current study, the sperm counts were in the normal range, suggesting the absence of any detrimental effect on total production of sperms. However, other authors have reported reduced sperm counts in experimental animals (Obone et al, 1999; Pandey et al, 1999) administered nickel sulfate at higher doses and for a longer time. Since higher doses of nickel tend to induce severe pathological lesions and have a direct effect on spermatogenesis, we chose to experiment with very low doses for 3 or 5 days.

Interestingly, nickel-induced OS in testis was associated with significant genotoxic effects measured in terms of production of abnormal sperms and DL mutations. The higher frequency of abnormal sperms during weeks 1, 2, and 3 clearly suggests a stage-specific effect on the development of sperms and also indicates the higher susceptibility of postmeiotic germ cells to nickel intoxication. These data are consistent with earlier observations that many germ cell mutagens induce genotoxic effect by producing a higher incidence of abnormal sperms in mice (Ehling, 1977). Furthermore, in our DL assay, administration of nickel (2.5 μmol/100 g of body weight per day for 5 days) failed to induce any detrimental effect on the incidence of implantations. However, a marked increase of dead implantations among pregnancies arising from nickel-treated males clearly suggested a specific induction of DL-type mutations. The peak DL response observed in weeks 2 and 3 can probably be interpreted as a direct effect on the spermatids that appear to be the most susceptible stage to nickel exposure.

In conclusion, multiple doses of nickel exposure produced moderate OS in testis of mice, which was apparently associated with apoptotic cell death and DNA damage in testis and ES. The genotoxic effects (ie, increased frequency of ES with abnormal heads and higher percentage of DL-type mutations) can be interpreted as a specific effect on spermatozoa and spermatids (early or late), which can play a significant role in the development of male infertility. Further, these data taken together suggest that nickel-induced testicular dysfunction at lower sublethal doses is wholly or partly mediated through oxidative damage to macromolecules, including damage to DNA.

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