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Comparison of Effects of 0.5 and 3.0 Gy X-Irradiation on Lipid Peroxidation and Antioxidant Enzyme Function in Rat Testis and Liver

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ABSTRACT: The prooxidant effect of X-irradiation on rat testis and liver tissue was studied with doses of 0.5 and 3.0 Gy; the latter dose kills the proliferating spermatogonia and causes a maturation-depletion process in the germ cells. The level of lipid peroxidation, measured by the formation of diene conjugates and thiobarbituric acid-reactive substances (TBARS) and the activities of the antioxidant enzymes were determined 0.5 hours, 1 day, 7 days, and 31 days after the exposure. In the liver, increased levels of diene conjugation (+36%, $P < 0.05$) in the group of 3.0 Gy at 0.5 hours indicated increased lipid peroxidation. At the same time, TBARS were increased (+25%, $P < 0.05$) in the group of 0.5 Gy, but not in the 3.0-Gy group. In the testis, diene conjugation was not determined at 0.5 hours postirradiation, and at day 1 it was at the control level. The level of TBARS in the testis was below control (−11%, $P < 0.01$) in the 3.0-Gy group at day 1. At day 31 after 3.0 Gy in the testis, an increase in the amount of conjugated dienes (+24%, $P < 0.01$) was observed in parallel with a decreased level of TBARS (−15%, $P < 0.01$). The activity of superoxide dismutase (SOD) was decreased in the testis at 0.5 hours postirradiation (−28%, $P < 0.05$, and −29%, $P < 0.05$, in the groups of 0.5 and 3.0 Gy), whereafter it returned to normal by day 7. In the liver, such inactivation of SOD was not observed. At day 1 postirradiation, a decreased level of catalase activity was detected in the liver (−18%, not significant, and −26%, $P < 0.05$) but not in the testis. Inductions of glutathione transferase (GSH-Tr) in the testis (+31%, $P < 0.05$, and +38%, $P < 0.01$) and glutathione peroxidase (GSH-Px) in the liver (+26%, $P < 0.05$, and +33%, $P < 0.01$) were seen at day 7. By day 31, increased levels of GSH-Px (+32%, $P < 0.05$) and GSH-Tr (+41%, $P < 0.01$) were observed in the testis after 3.0 Gy but not after 0.5 Gy. In this study, the changes in diene conjugates are suggested to reflect changes in lipid peroxidation, whereas those in TBARS are not. SOD activity of rat testis was found to be sensitive to inactivation by 0.5 and 3.0 Gy X-irradiation, which makes testis vulnerable to oxidative stress. In addition, germ cell depletion 31 days after 3.0 Gy increased the level of lipid peroxidation and the activities of lipid peroxide detoxifying enzymes GSH-Px and GSH-Tr in the rat testis. In the rat liver, 3.0-Gy X-irradiation leads to a transient increase in lipid peroxidation, but SOD is not inactivated.

Key words: reactive oxygen, oxidative damage, superoxide dismutase, antioxidant enzymes, ionizing radiation.

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Low amounts of potentially toxic reactive oxygen species are produced in normal cellular metabolism, and the synergistic action of the antioxidant enzyme system is essential for the defense against oxidative injury. Prooxidant states have been proposed to play a role in various pathological processes affecting the testis: infertility due to defective sperm function (Jones et al, 1979; Alvarez et al, 1987; Aitken et al, 1989), and testicular atrophy in cryptorchidism (Ahotupa and Huhtaniemi, 1992), as well as after administration of testicular toxicants such as ethanol (Rosenblum et al, 1985), cadmium (Kojima et al, 1990), or polycyclic aromatic hydrocarbons (Georgellis et al, 1987). Physiological changes in oxygen radical production have been suggested as occurring in the process of cellular differentiation (see Allen and Balin, 1989).

Ionizing radiation causes a prooxidant state as a result of the intracellular generation of reactive oxygen species (Gerschman et al, 1954). Antioxidant enzymes have been shown to be implicated in protection from radiation damage (Gray and Stull, 1983; Gee et al, 1985; Peng et al, 1986; Scott et al, 1989). Exposure of the rat testis to 3.0 Gy of X-rays kills most of the cycling spermatogonia, and thus causes a maturation-depletion process in which the number of subsequent germ cell types is progressively decreased (Dym and Clermont, 1970; Kanagasniemi et al, 1990). This model can be used to study the interactions between germ cells and the other testicular cells and the cellular localization of various phenomena occurring in the testis. However, ionizing radiation exposure also has effects in other testicular cells than cycling spermatogonia. Thus, it is difficult to know if the findings observed are

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due to changes in the numbers of the specific cell types or to the toxic effect of radiation on the function of the cells. In the present study, we have investigated the effect of ionizing radiation on various parameters of oxidative stress and the function of the enzymatic antioxidant defense in rat testes. As an indicator of oxidative stress, peroxidation of cellular lipids was measured by two different methods. To gain information about germ cell depletion vs. the other effects of irradiation, we used whole-body irradiation at exposure doses of 0.5 and 3.0 Gy, and for comparison, the same measurements were also carried out in the liver, where no alterations in the numbers of the cell types are caused by irradiation.

Materials and Methods

Chemicals

1,1,3,3-Tetraethoxypropane, 1-chloro-2,4-dinitrobenzene, butylated hydroxytoluene, cumene hydroperoxide, epinephrine, glucose-6-phosphate, hydrogen peroxide (H_2O_2), linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid, sodium salt), lucigenin (*bis*-*N*-methylacridinium nitrate), reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide diphosphate (NADPH), xanthine, as well as the enzymes catalase (bovine liver), GSH reductase (Baker's yeast), superoxide dismutase (SOD; Cu/Zn-form, bovine erythrocytes), and xanthine oxidase (buttermilk) were all purchased from Sigma Chemical Co. (St. Louis, Missouri).

Animals and X-Irradiation

Male Sprague-Dawley rats were fed standard laboratory rat chow and water *ad libitum* and kept at 20°C to 22°C with a light : dark cycle of 14:10 hours. Four to six animals at once were whole-body irradiated without anesthesia in a cardboard box (30 × 40 × 12 cm) situated 100 cm from the focus, using a 40 × 40-cm field size. The dose rate was 2.0 Gy per minute of 4 MeV X-rays produced by a Clinac 4/100 linear accelerator (Varian, Palo Alto, California). Exposed and control rats (four per group) were killed by decapitation under carbon dioxide anesthesia at 0.5 hours (25–40 minutes), 1 day, 7 days, and 31 days postirradiation.

Tissue Preparation

The testes and livers were dissected out, rinsed in 0.25 mol/L sucrose, and weighed. A 33% (w/v) testis homogenate was prepared with an Ultra-Turrax homogenizer (Janke & Kunkel KG, IKA-Werk, Staufen, Germany) and a 20% (w/v) liver homogenate with a Potter-Elvehjem glass-Teflon homogenizer, both in a 0.25 mol/L sucrose solution (at 0°C). A postmitochondrial supernatant was prepared by centrifugation (10,000 × *g* for 10 minutes at 4°C).

Lipid Peroxidation

The level of lipid peroxidation was estimated: (1) by the amount of conjugated diene double bonds in centrifuged supernatant fluid and (2) by the amount of thiobarbituric acid-reactive substances (TBARS) in tissue homogenate. In the assay of diene conjugation, lipids extracted by chloroform–methanol were dried under a nitrogen atmosphere and then redissolved in cyclohexane immediately before measuring. Samples were scanned from 300 to 220 nm with a spectrophotometer. The absorbance at 300 nm was subtracted from the absorbance at 233 nm, which represents the conjugated-diene signal (Corongiu et al, 1983). Unpromoted thiobarbituric acid test was performed using 100 μl of homogenate, which was added to 400 μl of phosphate buffer, pH 7.4. Standard samples contained 1,1,3,3-tetraethoxypropane instead of homogenate. Thereafter, 500 μl of 30% trichloroacetic acid and 50 μl of 2% (w/v) butylated hydroxytoluene in ethanol were added to the reaction mixture. To start the reaction, 500 μl of thiobarbituric acid (375 mg/50 ml H_2O) was added, and the tubes were heated in a boiling water bath for 15 minutes. Then the tubes were cooled, and after centrifugation (2,000 × *g* for 10 minutes) the absorbance was measured at 535 nm (Bird and Draper, 1984). Coefficients of variation for the TBA test were 17.3% (interassay) and 5.0% (intraassay).

Enzyme Assays

At 0.5 hours postirradiation, SOD (Cu/Zn-form; EC 1.15.1.1) was assayed spectrophotometrically by inhibition of epinephrine autoxidation (Misra and Fridovich, 1972). Samples were added to the reaction mixture made in sodium carbonate buffer, pH 10.2, and the absorbance change was recorded at 480 nm. In the other groups, SOD activity was measured with Bio-Orbit 1251 Luminometer (Bio-Orbit Ltd., Turku, Finland) by inhibition of xanthine–xanthine oxidase-dependent chemiluminescence enhanced by lucigenin and linoleate (Laihia et al, 1993). Testis homogenate samples were diluted 1:2,500 or 1:5,000 and liver homogenate samples 1:5,000 in assay medium, potassium phosphate buffer, pH 10.0. Reaction mixture was made by adding 55 μl of the sample dilution, 20 μl of xanthine oxidase (420 mU/ml), 20 μl of 0.1 mM lucigenin, and 20 μl of 200 mM linoleic acid to 385 μl of assay medium. The superoxide-producing reaction was initiated by automated dispensing of 60 μl of 1.45 mM xanthine. Chemiluminescence was measured for 10 minutes at 35°C. An integral value was calculated to get the inhibition of chemiluminescence as a percentage of the control value of buffer as a sample. In both assays, standard curves for the activity of SOD were drawn by using purified SOD preparation. One microgram of the enzyme preparation corresponds to 3.6 U. These two SOD assays are fully comparable. The activity of catalase (EC 1.11.1.6) was determined by measuring the rate of disappearance of 15 mmol/l H_2O_2 at 240 nm (Beers and Sizer, 1952). Glutathione peroxidase (GSH-Px; EC 1.11.1.9) was assayed by measuring the oxidation of $NADPH^+$ at 340 nm, with cumene hydroperoxide as the substrate (Paglia and Valentine, 1967). In the assay of glutathione transferase (GSH-Tr; EC 2.5.1.18), 1-chloro-2,4-dinitrobenzene was used as the substrate, and the absorbance change was recorded at 340 nm (Habig et al, 1974). The activity of the hexose monophosphate shunt (HMS) was assayed using glucose-6-phosphate as the substrate and 340 nm as the recording wavelength (Glock and McLean, 1953). Because the total NADPH production was measured, the HMS activity represents the sum of glucose-6-phosphate dehydroge-

Table 1. *Weights and protein concentrations of rat testes and liver at 0.5 hours, 1 day, 7 days, and 31 days postirradiation*

	Time postirradiation*			
	0.5 hours	1 day	7 days	31 days
Testes weight (g)				
Control	3.38 ± 0.02	3.70 ± 0.08	3.59 ± 0.04	3.78 ± 0.29
0.5 Gy	3.58 ± 0.07	3.49 ± 0.14	3.66 ± 0.12	3.60 ± 0.18
3.0 Gy	3.46 ± 0.13	3.21 ± 0.03[a]	3.58 ± 0.06	2.38 ± 0.03[b]
Liver weight (g)				
Control	16.32 ± 0.51	19.23 ± 2.12	17.20 ± 1.65	16.41 ± 0.92
0.5 Gy	18.92 ± 1.46	13.86 ± 0.64	14.98 ± 21.7	17.11 ± 0.96
3.0 Gy	17.74 ± 1.09	16.36 ± 0.55	18.25 ± 1.67	19.40 ± 1.19
Testes protein concentration (mg/g wet weight)				
Control	191 ± 11.45	139 ± 8.00	132 ± 3.15	163 ± 16.59
0.5 Gy	182 ± 23.33	127 ± 6.13	124 ± 6.44	172 ± 6.70
3.0 Gy	173 ± 8.20	127 ± 2.77	124 ± 3.21	191 ± 7.78
Liver protein concentration (mg/g wet weight)				
Control	270 ± 13.47	342 ± 32.06	319 ± 9.43	281 ± 19.37
0.5 Gy	300 ± 6.48	262 ± 24.30	289 ± 15.00	308 ± 25.35
3.0 Gy	278 ± 14.58	283 ± 9.15	289 ± 22.18	373 ± 35.15

* Values are mean ± SE of four rats per group. Significantly different from control group: [a]$P < 0.05$; [b]$P < 0.01$.

nase and 6-phosphogluconate dehydrogenase activities. SOD and catalase activities were determined in tissue homogenates, whereas centrifuged supernatant fluid was used in GSH-Px, GSH-Tr, and the HMS assays.

Protein

Protein content was measured by the biuret method (Layne, 1957) with bovine serum albumin as the reference protein.

Statistical Analysis

One-way analysis of variance followed by Scheffe's F-test was used to compare the 0.5- and 3.0-Gy groups to the respective control group. A P-value less than 0.05 was selected as the limit of statistical significance.

Results

Organ Weights and Protein Concentrations

Ionizing radiation caused no statistically significant alterations in the liver weights, though a slight decline was observed at day 1 postirradiation (Table 1). The protein concentrations in the liver tissues remained unchanged during the follow-up period (Table 1).

Testis weights were not affected by 0.5 Gy irradiation, whereas those exposed to 3.0 Gy irradiation were reduced by 13% ($P < 0.05$) from control levels by day 1 postirradiation (Table 1). By day 7, the testicular weights in this group were normalized, but 31 days after exposure to 3.0 Gy, a secondary reduction by 37% ($P < 0.01$) was observed, and testicular atrophy was evident. Irradiation exposure did not affect the protein concentrations in the testes (Table 1).

Lipid Peroxidation

In liver tissue, the level of diene conjugates was significantly increased at 0.5 hours in the 3.0-Gy group (+36%, $P < 0.05$), but not in the 0.5-Gy group (Fig. 1). On the contrary, at 0.5 hours TBARS were increased in the 0.5-Gy group (+25%, $P < 0.05$), but not in the 3.0-Gy group. At day 1 these determinations did not differ significantly from control levels, and by day 7 TBARS were decreased below the control in the group of 3.0 Gy (−16%, $P < 0.05$). At day 31 postirradiation, no effect was observed in the determinations of lipid peroxidation in the liver.

In testis tissue, the amount of TBARS was below control levels at 0.5 hours postirradiation in both exposure groups, but the difference was statistically not significant (Fig. 1). In the 3.0-Gy group it remained reduced until day 1 (−11%, $P < 0.01$) and recovered to control levels by day 7, whereas in the 0.5-Gy group, the level of TBARS was normal at day 1 and elevated at day 7 (+15%, $P < 0.05$). At day 31 postirradiation, the amount of TBARS was normal in the 0.5-Gy group and below control level in the 3.0-Gy group (−15%, $P < 0.01$). There were no changes in conjugated dienes in the testis until day 31, when an increase of 24% ($P < 0.01$) above control levels was observed in the 3.0-Gy group (Fig. 1).

Activities of the Antioxidant Enzymes

In the liver, the activity of SOD was increased by 32% (statistically not significant) from control levels in the 3.0-Gy group by 0.5 hours postirradiation (Fig. 2). Thereafter its level was near the control. Testicular SOD activity was clearly reduced by 0.5 hours postirradiation: −28% ($P < 0.05$) in the 0.5-Gy group and −29% ($P < 0.05$) in the

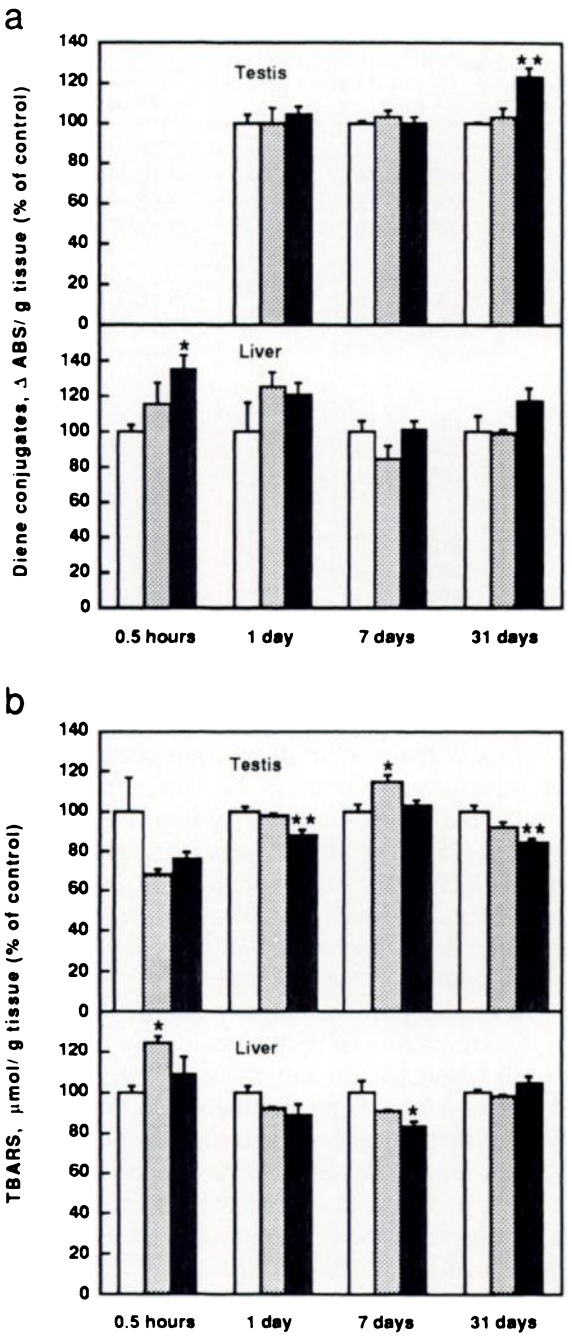

FIG. 1. The levels of diene conjugation **(a)** and thiobarbituric acid-reactive species **(b)** as the mean percent (± SE) of control values in rat testis and liver at 0.5 hours, 1 day, 7 days, and 31 days postirradiation. Open bars represent control rats ($n = 4$), and stippled (0.5 Gy; $n = 4$) and filled bars (3.0 Gy; $n = 4$) irradiated rats. The asterisks indicate a significant difference from the control: $^{*}P < 0.05$; $^{**}P < 0.01$.

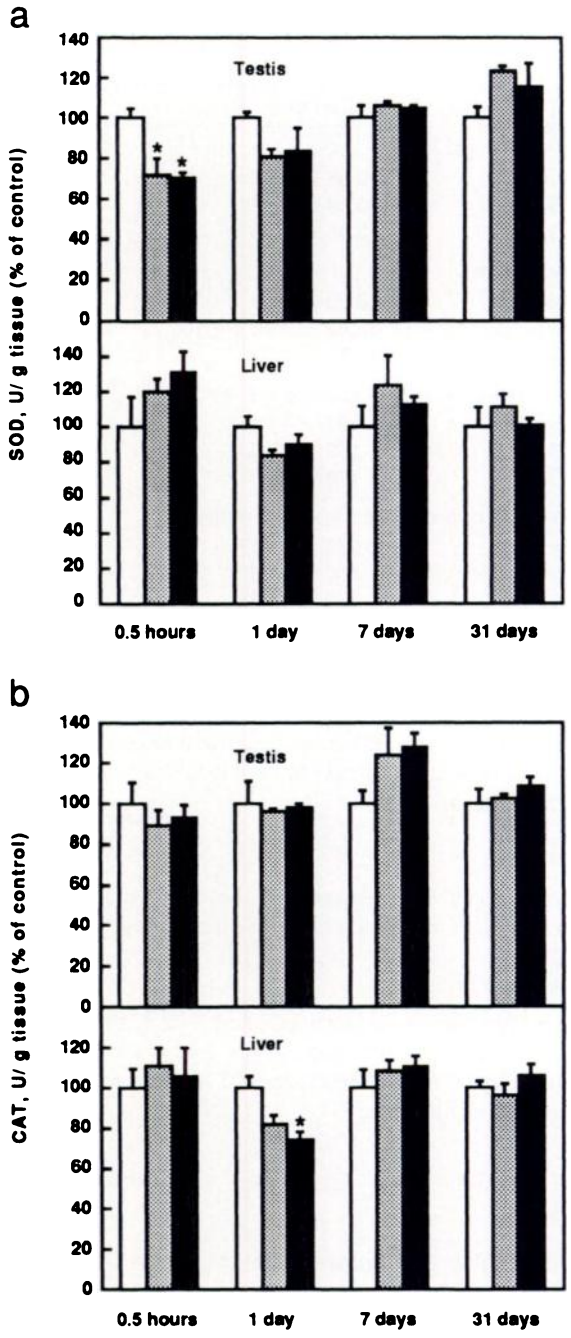

FIG. 2. Activities of superoxide dismutase **(a)** and catalase **(b)** as the mean percent (± SE) of control values in rat testis and liver at 0.5 hours, 1 day, 7 days, and 31 days postirradiation. Open bars represent control rats ($n = 4$, except $n = 3$ for SOD in the liver at day 31), and stippled (0.5 Gy; $n = 4$) and filled bars (3.0 Gy; $n = 4$) irradiated rats. The asterisks indicate a significant difference from the control: $^{*}P < 0.05$; $^{**}P < 0.01$).

3.0-Gy group (Fig. 2). It recovered to normal by day 7 and was slightly elevated at 31 days after irradiation in both exposure groups.

The only changes observed in the hepatic catalase activities were the decreased values detected at day 1: −26% ($P < 0.05$) and −18% (not significant) below the control in the 3.0-Gy and 0.5-Gy groups, respectively (Fig. 2). The catalase levels slightly increased in the testis by day 7 in both irradiation groups and returned to control levels by day 31 postirradiation (Fig. 2).

Hepatic GSH-Px activities increased by day 7 postirradiation, being then 33% ($P < 0.01$, in the 3.0-Gy group)

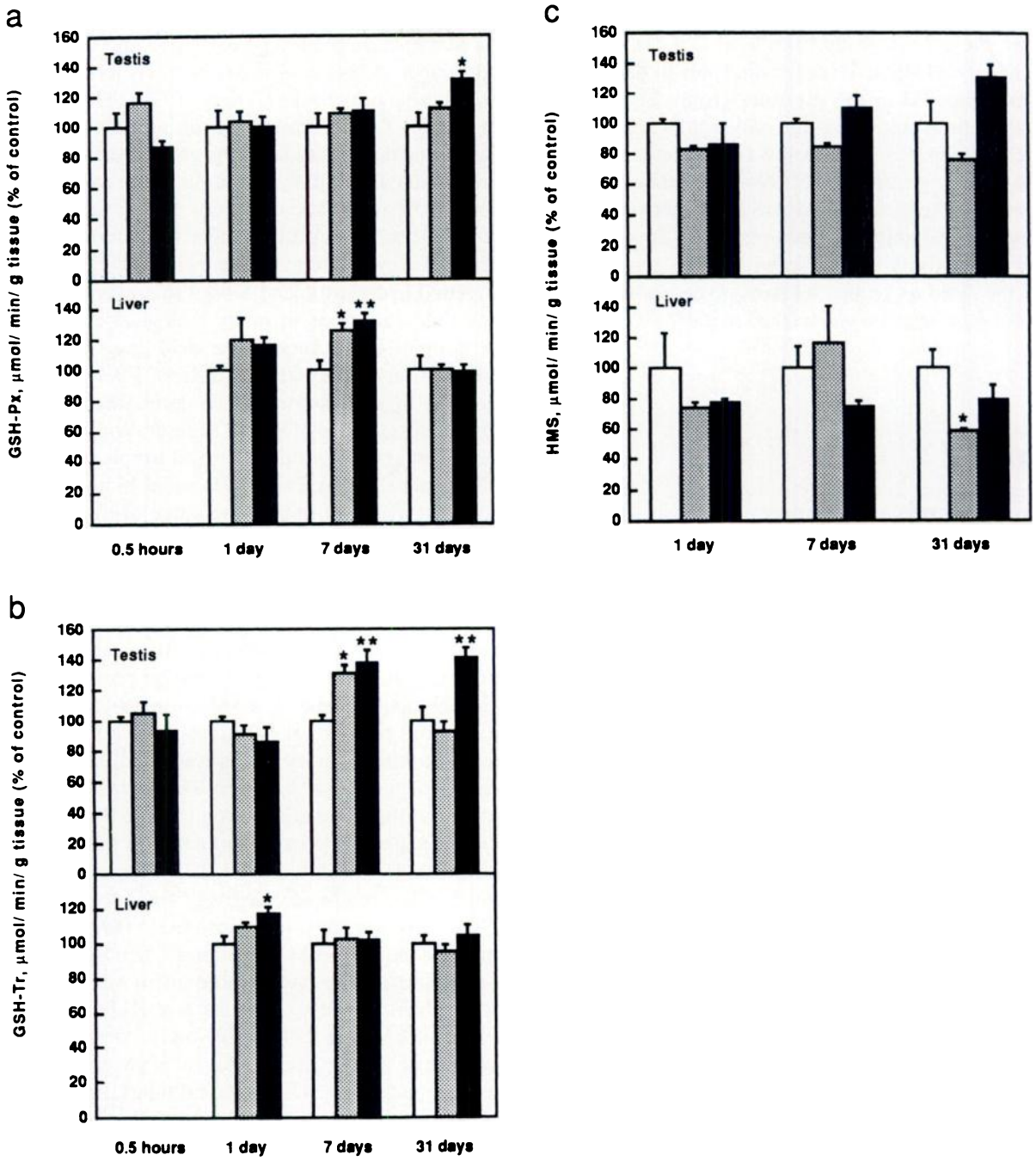

FIG. 3. Activities of glutathione peroxidase **(a)**, glutathione transferase **(b)**, and hexose monophosphate shunt **(c)** as the mean percent (± SE) of control values in rat testis and liver at 0.5 hours, 1 day, 7 days, and 31 days postirradiation. Open bars represent control rats ($n = 4$), and stippled (0.5 Gy; $n = 4$) and filled bars (3.0 Gy; $n = 4$) irradiated rats. The asterisks indicate a significant difference from the control: $^{*}P < 0.05$; $^{**}P < 0.01$.

and 26% ($P < 0.05$, in the 0.5-Gy group) above the control (Fig. 3). Thereafter, GSH-Px level returned in the liver to normal by the end of the follow-up period. In the testis this kind of increase was not observed, whereas by day 31 the activity of GSH-Px was increased 32% ($P < 0.05$) above control levels in the 3.0-Gy group. The level in the 0.5-Gy group remained unchanged (Fig. 3).

The hepatic GSH-Tr activity was slightly increased at day 1 postirradiation (+18%, $P < 0.05$, in the 3.0-Gy group); thereafter it returned to normal levels and was not affected during the follow-up (Fig. 3). In the testis, the activity of this enzyme was normal at 0.5 hours and 1 day after irradiation and increased then clearly by day 7 in both exposure groups (+31%, $P < 0.05$, in the 0.5-Gy group, and +38%, $P < 0.01$, in the 3.0-Gy group) (Fig. 3). By day 31 postirradiation, GSH-Tr activity was

returned to normal in the 0.5-Gy group but remained elevated in the 3.0-Gy group (+41%, $P < 0.01$).

In the hepatic HMS activities, a tendency to decreased values was observed in the exposure groups at most of the follow-up time points, but the only significant change was a decline 41% ($P < 0.05$) below control levels by day 31 in the 0.5-Gy group (Fig. 3). No significant changes were detected in the testicular HMS levels between irradiated and control animals; however, at 31 days postirradiation this activity was elevated (+32%, not significant) in the 3.0-Gy group, whereas a decreased level (−24%, not significant) was observed in the 0.5-Gy group (Fig. 3).

Discussion

Although the testis is an extremely radiosensitive organ, there are certain cell types that are radioinsensitive: the interstitial cells, the cells comprising the tubular wall, and the Sertoli cells. The intermediate and type B spermatogonia have the highest sensitivity to radiation. If their sensitivity is indicated by 1, the relative sensitivities of other spermatogenic cell types are as follows: Type A spermatogonia 70, spermatocytes 400, spermatids 300, and spermatozoa 10,000 (Ellis, 1970). The human testes respond to localized radiation as low as 0.35 Gy (Sandeman, 1966), and we have found a significant mutagenic effect with 0.5 Gy (Lähdetie and Parvinen, 1981). According to quantitative analyses, most of the spermatogonia are reduced in number at 7 days after 3.0 Gy, while most of the spermatocytes and early spermatids are reduced in number 31 days after 3.0 Gy (Dym and Clermont, 1970; Kangasniemi et al, 1990). The irradiation dose of 3.0 Gy also has been observed to induce meiotic micronuclei in early spermatids (Lähdetie and Parvinen, 1981) and chromosomal abnormalities at the stem cell level, resulting in deterioration of long-term fertility (Jégou et al, 1991). In the liver, the effects of these irradiation doses are minimal (Geraci et al, 1991).

In the present study, increased levels of diene conjugation were observed in the liver at 0.5 hours and in the testis at day 31 after 3.0-Gy irradiation. The acute increase after the exposure was expected. At day 31 the increase in conjugated dienes is suggested to be associated with the profound changes in cellular composition of the testis after depletion of certain germ cell types. Simultaneously, activities of lipid peroxide-metabolizing enzymes GSH-Tr and GSH-Px were increased. The changes in conjugated dienes are considered logical, but the changes in testicular TBARS do not follow the pattern. Measurement of diene conjugates is a direct method for detecting a class of intermediary peroxidation products (Corongiu et al, 1983). Thiobarbituric acid test is an indirect method for detection of a fraction of lipid peroxidation end products (Bird and Draper, 1984), and it has been criticized to possess considerable drawbacks (Janero, 1990). The TBA test is not specific for lipid peroxidation products: acidity and heat during the test can lead to generation of TBA-reactive compounds from many nonlipid molecules, and side products of enzymatic eicosanoid formation respond in the TBA test. In addition, under test conditions autoxidation of polyunsaturated fatty acids (PUFA) is possible. Butylated hydroxytoluene is used to prevent this, but despite this, variations in many biological constituents of the samples can influence the yield and distribution of peroxidation products during the test. These include variations in free transition metals, antioxidants, hydrogen atom donors, degree of PUFA unsaturation, and chemical interactions between aldehydes and simple sugars (Porter, 1984; Janero, 1990). Thus, in complex biological samples such as testis homogenate, the value of the TBA test as an indicator of lipid peroxidation is poor. In the present study, when there are changes in cellular composition of the samples, we draw the conclusion that the amount of TBARS in the testis does not reflect changes in lipid peroxidation. A clear explanation for the origin of the variations in testicular TBARS cannot be provided.

In the acute effects of irradiation to the function of antioxidant enzymes, the clearest observation was the decrease in SOD activity in the testis at 0.5 hours, which was not detected in the liver. The increase in the production of the superoxide anion radical, the substrate for SOD, is supposed to cause induction of this enzyme (see Fridovich, 1983). However, if the hydrogen peroxide produced is not removed effectively enough by catalase or GSH-Px, it is able to inactivate SOD (Bray et al, 1974; Sinet and Garber, 1981). Previously, SOD activities have been found to decrease in prooxidant states such as in cryptorchid rat testis (Ahotupa and Huhtaniemi, 1992) or in rat liver after 8 Gy whole-body γ-irradiation (Kergonou et al, 1981). In accordance, acute exposure to hyperoxia inactivates SOD in the rat lung (Januszkiewicz et al, 1986), whereas prolonged sublethal hyperoxia results in induction of pulmonary SOD and also other antioxidant enzymes (see Fridovich and Freeman, 1986). The inactivation of SOD in the testis could be suggested to be due to the low physiologic activities of catalase and GSH-Px in the testis compared to those in the liver, whereas the activity of SOD is in the rat testis near the level observed in the liver (Peltola et al, 1992). The present finding is in accordance with the proposed vulnerability of the testis to prooxidant states. However, whether SOD inactivation is a cause of increased oxidative injury or just a consequence of the prooxidant state cannot be judged. The different effect of 0.5 and 3.0 Gy in the testis, despite the similar reduction in SOD activities, could be associ-

ated with the different effect of these doses on other parts of the antioxidant defense system.

At 7 days postirradiation, induction of GSH-Px was observed in the liver. At the same time, a significant increase in the GSH-Tr activities and also slightly elevated activities of catalase were detected in the testis. These changes are suggested to be due to enzyme induction, as they were similar with both irradiation doses. GSH-Px, GSH-Tr, and HMS function in detoxification of the reactive products formed in lipid peroxidation. In the adult rat testis, GSH-Tr is a more important antioxidant enzyme than GSH-Px, compared to the activities of these enzymes in the liver (Peltola et al, 1992).

The level of oxidative stress and the function of the antioxidant enzymes were normalized in the liver by day 31 postirradiation, with the exception of reduced HMS activity in the 0.5-Gy group. The determinations made in the testis at this time-point after 0.5-Gy irradiation did not differ significantly from control levels. Thus, the findings observed in the testis at day 31 after 3.0-Gy irradiation seem to be mainly due to maturation-depletion process in the germ cells. In this group, the increase in diene conjugation level is suggested to reflect increased peroxidation of cellular lipids. Increased activities of the enzymes GSH-Px and GSH-Tr correspond to this suggestion and are in accordance with previously noted low activities of GSH-Px in pachytene spermatocytes and round spermatids (Yoganathan et al, 1989). SOD and catalase activities were both at the control level, although Yoganathan et al (1989) have reported high SOD but no catalase activity in pachytene spermatocytes and round spermatids. In the present results, the roles of different enzyme activities in testicular somatic cells, spermatogonia, and remaining late spermatids remain obscure. In addition, possible effects of the disappearance of interactions between germ cells and other testicular cells cannot be excluded in this model. However, with precaution it can be suggested that the physiological level of lipid peroxidation and activities of peroxide-metabolizing enzymes are lower in spermatocytes and early spermatids than in other testicular cells on the average.

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References

Ahotupa M, Huhtaniemi I. Impaired detoxification of reactive oxygen and consequent oxidative stress in experimentally cryptorchid rat testis. *Biol Reprod* 1992;46:1114–1118.

Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biol Reprod* 1989;40: 183–197.

Allen RG, Balin AK. Oxidative influence on development and differentiation: an overview of a free radical theory of development. *Free Radical Biol Med* 1989;6:631–661.

Alvarez JG, Touchstone JC, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa. Superoxide dismutase as major enzyme protectant against oxygen toxicity. *J Androl* 1987;8:338–348.

Beers RF, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J Biol Chem* 1952;195: 133–140.

Bird RP, Draper HH. Comparative studies on different methods of malonaldehyde determination. *Methods Enzymol* 1984;105:299–305.

Bray RC, Cockle SA, Fielden EM, Roberts PB, Rotilio G, Calabrese L. Reduction and inactivation of superoxide dismutase by hydrogen peroxide. *Biochem J* 1974;139:43–48.

Corongiu FP, Lai M, Milia A. Carbon tetrachloride, bromotrichloromethane and ethanol acute intoxication. *Biochem J* 1983;212:625–631.

Dym M, Clermont Y. Role of spermatogonia in the repair of the seminiferous epithelium following X-irradiation of the rat testis. *Am J Anat* 1970;128:265–282.

Ellis LC. Radiation effects. In: Johnson AD, Gomes WR, Vandemark NL, eds. *The Testis.* New York: Academic Press; 1970:333–376.

Fridovich I. Superoxide radical: an endogenous toxicant. *Annu Rev Pharmacol Toxicol* 1983;23:239–257.

Fridovich I, Freeman B. Antioxidant defenses in the lung. *Annu Rev Physiol* 1986;48:693–702.

Gee CA, Kittridge KJ, Willson RL. Peroxy free radicals, enzymes and radiation damage: sensitisation by oxygen and protection by superoxide dismutase and antioxidants. *Br J Radiol* 1985;58:251–256.

Georgellis A, Montelius J, Rydström J. Evidence for a free-radical-dependent metabolism of 7,12-dimethylbenz(a)anthracene in rat testis. *Toxicol Appl Pharmacol* 1987;87:141–154.

Geraci JP, Mariano MS, Jackson KL. Hepatic radiation injury in the rat. *Radiat Res* 1991;125:65–72.

Gerschman R, Gilbert DL, Nye SW, Dwyer P, Fenn WO. Oxygen poisoning and x-irradiation: a mechanism in common. *Science* 1954;119: 623–626.

Glock GE, McLean P. Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat liver. *Biochem J* 1953;55:400–408.

Gray BH, Stull RW. Radioprotection by polyethylene glycol–protein complexes in mice. *Radiat Res* 1983;93:581–587.

Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974;249: 7130–7139.

Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radical Biol Med* 1990;9:515–540.

Januszkiewicz AJ, Huntrakoon M, Wilson PK, Faiman MD. Isolated perfused lung histamine release, lipid peroxidation, and tissue superoxide dismutase from rats exposed to normobaric hyperoxia. *Toxicology* 1986;39:37–46.

Jégou B, Velez de la Calle JF, Bauché F. Protective effect of medroxyprogesterone acetate plus testosterone against radiation-induced damage to the reproductive function of male rats and their offspring. *Proc Natl Acad Sci USA* 1991;88:8710–8714.

Jones R, Mann T, Sherins R. Peroxidative breakdown of phospholipids in human spermatozoa, spermicidal properties of fatty acid peroxides, and protective action of seminal plasma. *Fertil Steril* 1979;31:531–537.

Kangasniemi M, Varomaa T, Kulmala J, Kaipia A, Parvinen M, Toppari

J. DNA-flow cytometry of defined stages of rat seminiferous epithelium: effects of 3 Gy of high-energy x-irradiation. *J Androl* 1990;11: 312–317.

Kergonou J-F, Braquet M, Rocquet G. Influence of whole-body γ irradiation upon rat liver mitochondrial fractions. *Radiat Res* 1981;88: 377–384.

Kojima S, Ishihara N, Hirukawa H, Kiyozumi M. Effect of *N*-benzyl-D-glucamine dithiocarbamate on lipid peroxidation in testes of rats treated with cadmium. *Res Commun Chem Pathol Pharmacol* 1990;67: 259–269.

Lähdetie J, Parvinen M. Meiotic micronuclei induced by x-rays in early spermatids of the rat. *Mutat Res* 1981;81:103–115.

Laihia JK, Jansen CT, Ahotupa M. Lucigenin and linoleate enhanced chemiluminescent assay for superoxide dismutase activity. *Free Radical Biol Med* 1993;14:457–461.

Layne E. Spectrophotometric and turbidimetric methods for measuring proteins. *Methods Enzymol* 1957;3:447–454.

Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972;247:3170–3175.

Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:158–169.

Peltola V, Huhtaniemi I, Ahotupa M. Antioxidant enzyme activity in the maturing rat testis. *J Androl* 1992;13:450–455.

Peng TX, Moya A, Ayala FJ. Irradiation-resistance conferred by superoxide dismutase: possible adaptive role of a natural polymorphism in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 1986;83:684–687.

Porter NA. Chemistry of lipid peroxidation. *Methods Enzymol* 1984;105: 273–282.

Rosenblum E, Gavaler JS, Van Thiel DH. Lipid peroxidation: a mechanism for ethanol-associated testicular injury in rats. *Endocrinology* 1985;116:311–318.

Sandeman TF. The effects of X irradiation on male human fertility. *Br J Radiol* 1966;39:901–907.

Scott MD, Meshnick SR, Eaton JW. Superoxide dismutase amplifies organismal sensitivity to ionizing radiation. *J Biol Chem* 1989;264: 2498–2501.

Sinet P-M, Garber P. Inactivation of the human CuZn superoxide dismutase during exposure to O_2^- and H_2O_2. *Arch Biochem Biophys* 1981;212:411–416.

Yoganathan T, Eskild W, Hansson V. Investigation of detoxification capacity of rat testicular germ cells and Sertoli cells. *Free Radical Biol Med* 1989;7:355–359.

19th Annual Meeting and Postgraduate Course

American Society of Andrology

1994—Springfield, Illinois, March 25–28. Contact: Dr. Armando Amador, Department of Obstetrics and Gynecology, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794 (Tel: 217/782-9306; Fax: 217/788-5561).

NOTE: There will be a change in the dates of the meeting to avoid a scheduling conflict with Passover.