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Protective effect of medroxyprogesterone acetate plus testosterone against radiation-induced damage to the reproductive function of male rats and their offspring

(prevention of gonadal damage)

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ABSTRACT **This study attempted to protect spermatogenesis and the reproductive performance of rats against the effects of acute scrotal exposure to x-rays. Daily subcutaneous injections of medroxyprogesterone acetate (8 mg/kg) plus testosterone (1 mg/kg) (MT group) were administered for 55 days (experiment A) or 15 days (experiment B). The rats were irradiated (3 grays) on the last day of MT pretreatment (MTX group). In both experiments, on days 1 and 130 posttreatment, rats from each of the four groups (control, x-irradiated, MT, and MTX groups) were killed to measure the weight of the reproductive organs and the number of epididymal spermatozoa. Breeding was started 3 days posttreatment by housing all males from the four groups each with two virgin females for six successive periods of 19 days, separated by a period of 2 days. The percentage of fertile males, the litter size, postimplantation losses, and dominant lethal mutations were calculated. In experiment A, in the last fertility trial, animals of both sexes were selected at random from the progeny of each group (F_1). When they were adults, their fertility was tested in a mating trial. A fertility trial was also performed with the F_2 males. Our data essentially reveal that (*i*) in addition to their adverse quantitative effects on spermatogenesis, x-rays also produce a significant increase in dominant lethal mutations in all germ cell classes, including stem spermatogonia; (*ii*) the F_1 and F_2 male descendants of irradiated male rats provoked abnormal rates of postimplantation losses in their female mates; (*iii*) the short as well as the long MT pretreatment protects testicular function of irradiated rats; and (*iv*) in experiment A, MT pretreatment totally prevented qualitative damage to spermatozoa and protected the descendants of the irradiated animals against altered spermatogenesis as well as against genetic damage in germ cells. In conclusion, pretreatment with MT, even for a short period of time, offers a method for potentially reducing the toxic and genotoxic effects of irradiation on the male reproductive system.**

Radiation has particularly severe adverse effects on spermatogenesis and therefore on fertility in both animals and man (1). Radiation therapy is nevertheless widely used because there is often no other choice for the treatment of a number of cancers in children and adults. The prevention of gonadal damage during radiotherapy is therefore a major concern. Since shielding the testes offers only poor protection to spermatogenesis, various other experimental approaches, aimed at preventing radiation-induced quantitative damage to the gonads, have been explored (2–8). Some of these have achieved protection of testicular function to various degrees (2–5, 8). However, a major limitation of these studies is that the reproductive performances of the experimental animals and of their progeny was not assessed. It is known that in addition to azoo- or oligozoospermia, radiation also produces germ-line mutations leading to inherited anomalies in rodents (9–13) and maybe in man (14, 15).

In a recent report, we have demonstrated that germ cells of male rats and of their progeny can be quantitatively and qualitatively protected against the antimitotic agent procarbazine by pretreatment with a mixture of medroxyprogesterone acetate (M) and testosterone (T) (16). In the present work we have investigated whether the same pretreatment could prevent radiation-induced cytotoxic and genotoxic damage. Furthermore, we have also studied the time factor in pretreatment with M plus T (MT).

MATERIALS AND METHODS

Animals. Adult Sprague–Dawley rats (90 days old) were provided by the Janvier Breeding Center (Le Genest, France). They were housed under normal laboratory conditions in a 12-h light/12-h dark cycle (7 a.m.–7 p.m.), fed standard commercial food, and given water ad libitum.

Experimental Protocols. Two consecutive experiments (A and B) conducted with four groups of animals each (n = 10 and 16 in experiments A and B, respectively) were performed. The control group received olive oil/benzilic alcohol, 95:5 (vol/vol) for 55 days in experiment A and for 15 days in experiment B; the steroid-only group (MT group) received s.c. injections of M plus T (Sigma) in olive oil/benzilic alcohol at 8 and 1 mg per kg per day, respectively, for 55 days in experiment A and for 15 days in experiment B. In the x-ray-only group (X group), on day 55 in experiment A and on day 15 in experiment B, the scrotum of each anesthetized rat (5% pentobarbital; 1 ml/kg i.p.) was irradiated for 10 min with 3 grays (Gy) of 0.25 MV x-rays from a conventional generator. The combined treatment group (MTX group) received M plus T plus x-rays, administered as described for the MT and X groups.

Collection of Tissue and Sperm Counts. In both experiments, at days 1 and 130 posttreatment, rats from each group were weighed and decapitated. The testes and epididymides were dissected and weighed. The cauda epididymides were stored at −20°C until sperm heads (sperm reserves) were counted according to a previously described method (17).

Mating Trials. In both experiments, starting 3 days posttreatment, 7–10 males from each group were individually housed with two sexually mature virgin females for six

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Abbreviations: M, medroxyprogesterone acetate; T, testosterone; PIL, postimplantation loss(es); DLM, dominant lethal mutation(s).

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successive periods of 19 days each, separated by a resting period of 2 days. Immediately after parturition, the size and weight of the litter were recorded, and the offspring were rapidly examined for external anomalies. The females were then killed, and the number of implantation sites was carefully counted. In this manner, a number of fertility parameters were determined: the number of fertile males, the number of postimplantation losses {PIL; [(number of implantation sites − number of offspring)/number of implantation sites] × 100, expressed per pregnant female}, and the number of dominant lethal mutations {DLM; [(1 − number of living offspring in treated group)/number of living offspring in control group] × 100} carried by spermatozoa derived from different germ cell types.

In experiment A, at the end of the last fertility trial, 15 males and 7 females (F_1 generation) were selected at random from the progeny of the four experimental groups. At the age of 80 days, their fertility was tested by housing them for 19 days with adult animals of the opposite sex whose parents had not been treated (1 F_1 male with 2 females; 1 F_1 female with 1 male). At the end of this trial, the F_1 male breeders descending from the four experimental groups were weighed and killed, and their tissue was processed; their mating partners and the F_1 females were kept until parturition. Fertility parameters were then determined as described above. Similarly, a last fertility trial was performed with the F_2 males ($n = 7$ per group).

Statistical Analysis. Analysis of variance in conjunction with Student's t test or the Wilcoxon test was used to examine differences between the groups.

RESULTS

Body and Organ Weight. The body weight of all rats from the four groups in experiment A did not show any significant changes, whereas in experiment B, the body weight of the animals of the two MT groups was slightly decreased by day 1 posttreatment but returned to normal thereafter (Table 1).

At the end of the treatment period, in both experiments, testis and epididymis weights had decreased in the two MT groups. At 130 days posttreatment, the organ weight of these animals had returned to normal (Table 1). In experiment A, on day 1 postirradiation, no change was seen in the weight of reproductive organs of the animals of the X group, whereas in the same group, by day 130, both testis and epididymis weights were significantly reduced in experiments A and B (Table 1).

Sperm Reserves. *Experiment A.* On day 1 posttreatment, sperm reserves were normal in the X group, whereas the cauda epididymides of the MT group contained virtually no sperm (Table 1). Conversely, 130 days posttreatment, while sperm reserves were not significantly different from controls in the two MT groups, they only represented 50% of the normal levels in the X group (Table 1).

Experiment B. One day after the end of MT treatment, sperm reserves had decreased by 43% (Table 1). As observed in experiment A at 130 days postirradiation, sperm reserves were within the normal range in the MT and MTX groups, whereas they had fallen significantly (by 65%) in the X group (Table 1).

Fertility Study. *Experiment A.* In the two MT groups, none of the rats were fertile at the end of the treatment period (Fig. 1*A*). However, fertility in the males progressively recovered thereafter. Conversely, fertility for the X group was within the normal range between the first and the third fertility trials but dropped to a nadir at the fourth trial. From then on, it progressively returned to normal. It is of note that fertility was higher between the fourth and the sixth trials, and sometimes much higher (fifth trial), in the MTX group than in the X group.

From the first fertility trial to the last, x-rays provoked a very significant increase in the PIL rate (X group; Fig. 1*B*). In sharp contrast, PIL were always within the normal range in the MT group and particularly in the MTX group, which clearly demonstrates that the steroid pretreatment had a highly protective effect on the quality of spermatozoa.

The size of the MT group litters was normal by the second fertility trial (Fig. 1*C*), whereas the return to normal was much slower in the MTX-treated animals. In contrast, from the second to the fifth fertility trial, the litter size of the X group was dramatically decreased (Fig. 1*C*). It is of note that the litter size was far smaller in the X group in the fourth and the fifth trials than in the MTX group.

At birth, none of the F_1 generation (males and females) or the F_2 male generation of any of the experimental animals presented any obvious gross external abnormalities. Body weights were also normal (data not shown). Moreover, at 100 days of age, all the rats were fertile (data not shown). Table 2 indicates that at this age body weight was normal and that, despite unaltered sperm reserves, the reproductive organ

Table 1. Effect of different treatments on body and reproductive organ weight and on sperm reserves of adult rats

Parameter	Days post-treatment	Control	MT	X	MTX
		Experiment A			
Body weight, g	1	390 ± 21 (6)	416 ± 12 (6)	380 ± 24 (6)	405 ± 10 (6)
	130	610 ± 11 (6)	584 ± 17 (6)	620 ± 13 (6)	580 ± 18 (6)
Testis weight, g	1	1.70 ± 0.04 (6)	0.82 ± 0.05^{c} (6)	1.61 ± 0.04 (6)	0.81 ± 0.04^{c,f} (6)
	130	1.72 ± 0.09 (6)	1.85 ± 0.03 (6)	1.34 ± 0.17^{b} (6)	1.64 ± 0.04^{e} (6)
Epididymis weight, g	1	0.57 ± 0.02 (6)	0.39 ± 0.01^{c} (6)	0.53 ± 0.02 (6)	0.36 ± 0.01^{c,f} (6)
	130	0.67 ± 0.02 (6)	0.68 ± 0.02 (6)	0.50 ± 0.05^{a} (6)	0.60 ± 0.03 (6)
Sperm reserves × 10^{-6}	1	93.8 ± 2.5 (6)	2.5 ± 0.3^{c} (6)	106.5 ± 1.4 (6)	1.5 ± 0.3^{c,f} (6)
	130	103.0 ± 7.8 (6)	124.9 ± 5.4 (6)	53.4 ± 13.5^{b} (6)	88.9 ± 3.9^{e} (6)
		Experiment B			
Body weight, g	1	430 ± 5 (7)	381 ± 6^{c} (7)	427 ± 6 (10)	385 ± 7^{c} (10)
	130	668 ± 7 (10)	657 ± 9 (10)	669 ± 11 (10)	671 ± 10 (10)
Testis weight, g	1	1.53 ± 0.06 (5)	0.96 ± 0.03^{c} (5)	—	—
	130	1.51 ± 0.07 (10)	1.52 ± 0.08 (10)	0.93 ± 0.11^{c} (10)	1.20 ± 0.09^{a} (10)
Epididymis weight, g	1	0.44 ± 0.01 (5)	0.31 ± 0.05^{c} (5)	—	—
	130	0.46 ± 0.05 (10)	0.46 ± 0.05 (10)	0.30 ± 0.04^{a} (10)	0.34 ± 0.04 (10)
Sperm reserves × 10^{-6}	1	116.9 ± 19.2 (5)	67.1 ± 15.2^{a} (5)	—	—
	130	167.2 ± 23.3 (10)	196.1 ± 15.9 (10)	57.9 ± 17.5^{c} (10)	114.7 ± 16.2^{e} (10)

Values are the means ± SEM; the number of rats per group is in parentheses. wt, Weight. a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$ compared with the control value. e, $P < 0.01$; f, $P < 0.001$ compared with the X group values (Student's t test).

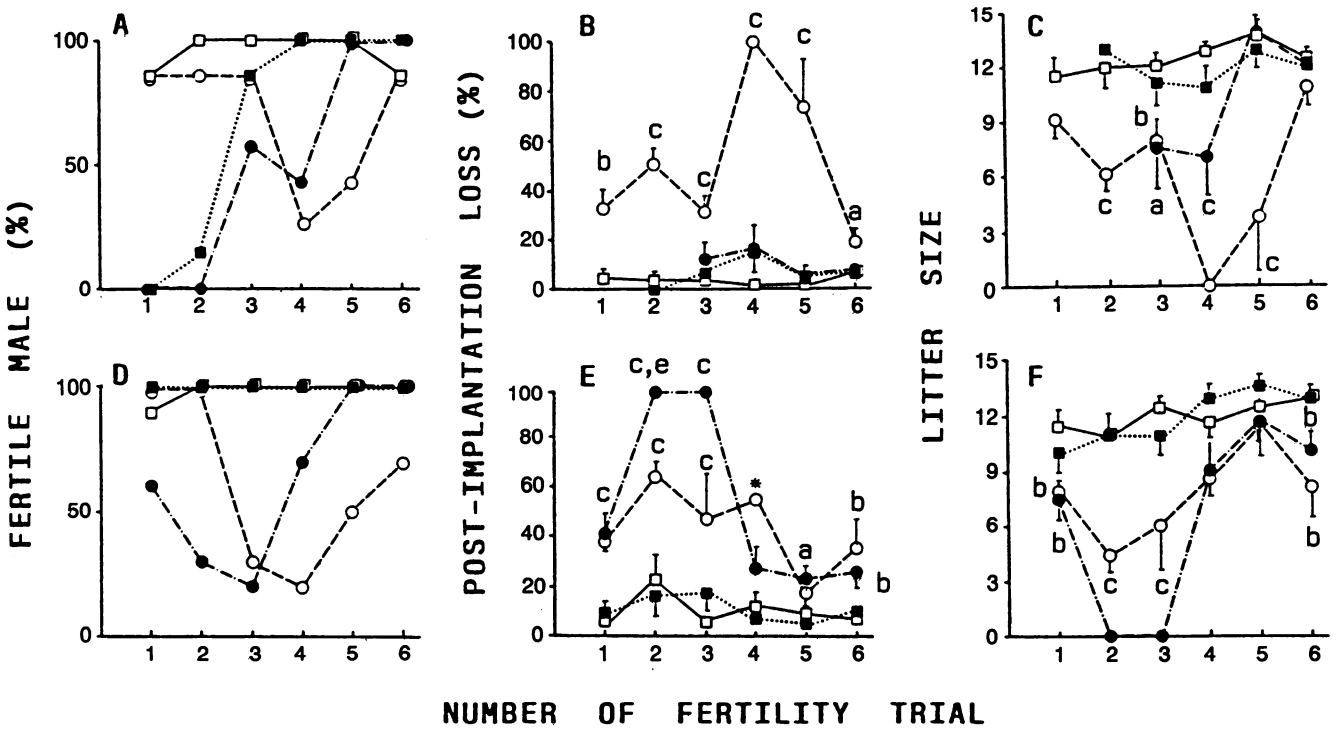

FIG. 1. Effect of the different treatments on the fertility parameters. (*A–C*) Results of experiment A. (*D–F*) Results of experiment B. Values are expressed as a percentage (*A*, *B*, *D*, and *E*) and/or per pregnant female (*B*, *C*, *E*, and *F*; mean ± SEM) for 7–10 males per group. The number of fertile females in each group for fertility trials 1–6, respectively, are 11, 10, 13, 14, 9, and 12 (control group, experiment A); 18, 18, 19, 20, 20, and 20 (control group, experiment B); 0, 1, 9, 14, 14, and 14 (MT group, experiment A); 13, 16, 20, 20, 20, and 20 (MT group, experiment B); 11, 12, 11, 2, 6, and 12 (group X, experiment A); 19, 18, 4, 2, 9, and 13 (group X, experiment B); 0, 0, 6, 6, 13, and 14 (MTX group, experiment A); 10, 3, 3, 10, 18, and 20 (MTX group, experiment B). a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$, compared with the control value. e, $P < 0.001$ compared with the X group. □, control; ■, MT group; ○, X group; ●, MTX group. The asterisk in *E* indicates that the error bar was omitted because there were only 2 fertile females in that group.

weight of the F_1 males descended from the X group animals was slightly but significantly reduced. It is worth noting that the rats of this latter group produced normal size litters, but the PIL had increased 2.6-fold (Table 2; $P < 0.052$). Similarly, a 2.1-fold increase in the PIL (Table 2; $P < 0.050$) was also observed with the F_2 males descended from the animals treated with x-rays alone. No change was observed in any of these parameters in the F_1 females descended from the X group or in the MTX and MT groups.

Experiment B. Throughout this experiment, the number of fertile males was almost identical in the control group and in the MT group (Fig. 1*D*). Fertility was normal in the X group through the second mating trial; then it fell to a minimum in the third and the fourth trials before progressively regaining a value that corresponded to 70% of the controls at the end of the experiment. In the MTX group, fertility dropped from 60% of the control value in the first trial to a nadir of 20% in the third trial. From then to the fifth trial, the number of fertile males returned to normal. Interestingly, as in experiment A, much higher rates of fertility were observed between the fourth and sixth trials in the steroid-treated rats than in the X group of animals.

The number of PIL for the MT group was normal (Fig. 1*E*). Conversely, for the X group, the PIL rate was significantly increased in all the trials except the fifth. In the MTX group, this parameter was also very high from the first to the third fertility trial. It then decreased but remained significantly elevated at the end of the experiment: a 3.4-fold increase in the sixth trial in this group versus a 4.8-fold increase in the same trial in the X group, when compared with control values

Table 2. Effect of the different paternal treatments on body and reproductive organ weights, sperm reserves, and fertility of F_1 and F_2 adult males and on fertility of F_1 females (♀)

Parameter	Offspring	Control	MT	X	MTX
Body weight, g	F_1	524 ± 19.2 (9)	521 ± 11.1 (9)	513.7 ± 14.1 (9)	519.3 ± 14.1 (9)
	F_2	561 ± 42.2 (5)	517.5 ± 14.5 (5)	538.3 ± 42.2 (5)	526.7 ± 46.0 (5)
Testis weight, g	F_1	1.79 ± 0.03 (9)	1.79 ± 0.04 (9)	1.63 ± 0.04[a] (9)	1.87 ± 0.03[d] (9)
	F_2	1.65 ± 0.10 (5)	1.82 ± 0.08 (5)	1.55 ± 0.08 (5)	1.66 ± 0.08 (5)
Epididymis weight, g	F_1	0.68 ± 0.01 (9)	0.68 ± 0.01 (9)	0.62 ± 0.02[a] (9)	0.67 ± 0.02[d] (9)
	F_2	0.66 ± 0.04 (5)	0.66 ± 0.02 (5)	0.60 ± 0.01 (5)	0.60 ± 0.01 (5)
Sperm reserves × 10^{-6}	F_1	113.2 ± 7.5 (6)	—	126.2 ± 6.7 (6)	—
PIL/pregnant female, %	F_1	4.6 ± 1.9	4.0 ± 1.7	12.2 ± 3[g]	8.2 ± 1.9
	F_1 ♀	7.9 ± 3.8	8.6 ± 4.1	7.0 ± 3.9	6.9 ± 2.8
	F_2	7.0 ± 2.0	7.8 ± 1.2	14.8 ± 2.0[a]	6.9 ± 2.3[d]

Values are the mean ± SEM; the number of rats per group is given in parentheses. The numbers of animals used for the fertility trials (PIL/pregnant female) are 15 F_1 males, 7 F_1 females, and 7 F_2 males. The numbers of fertile females are 22 (control), 26 (MT), 28 (X), and 27 (MTX), for F_1 males and 11 (control), 14 (MT), 13 (X), and 14 (MTX) for F_2 males. wt, Weight. a, $P < 0.05$; g, $P < 0.052$ compared with the control value. d, $P < 0.05$ compared with the X group value (Wilcoxon test).

(Fig. 1E). The litter size of the MT group of animals was not significantly different from the controls at any time point. From the first to the third trial, a highly significant decrease of the litter size was observed in the MTX and X groups (Fig. 1F). In the two subsequent trials, the litter size was not significantly reduced in these groups, but it was again significantly decreased in the last fertility trial.

DISCUSSION

It is well known that exposure of the rat testis to 3 Gy of x-rays kills most cycling spermatogonia (18–20). The disappearance of these cells causes a maturation-depletion process resulting in a progressive and sequential decrease in the number of the subsequent germ cell types from spermatocytes to spermatids and therefore eventually of spermatozoa. Since all spermatogonia are not destroyed by irradiation, repopulation of the seminiferous tubules and therefore recovery of fertility eventually occurs. Fifty-two to 54 days are required for rat spermatozoa to develop following division of A1 spermatogonia (21, 22). Transit through the epididymis takes 7–11 days (23). It can therefore be calculated that when cycling spermatogonia are destroyed by irradiation, the number of gametes in the ejaculate is reduced at 59–65 days postirradiation. This is compatible with the peak of the subfertile/sterile period we observed in the X groups. Survival of spermatozoa and the continued development of spermatids and of most spermatocytes explain why relatively high rates of fertility were initially maintained in the irradiated animals. Subfertility occurred earlier (45–64 days posttreatment) in experiment B than in experiment A (66–85 days). One possible explanation is that the sedation period in experiment B was shorter than in experiment A due, perhaps, to differences in the pentobarbital batches (pentobarbital is known to protect against x-ray damage; ref. 24).

The total duration of the spermatogenetic process (65 days from As stem spermatogonia to spermatozoa; ref. 25), plus the time for spermatozoa to pass from the testis to the cauda epididymis (23), is 72–76 days. Therefore, the fact that at 130 days postirradiation sperm reserves in both experiments were still significantly reduced in the X group of rats indicates that some stem cells had survived irradiation and others had died. Our fertility trials clearly show that, in conjunction with the fall in the number of germ cells and therefore of gametes, the quality of spermatozoa produced by the irradiated animals was modified and largely contributed to the subfertility/sterility period. An increase in the frequency of miscarriages (PIL) in the females mated with the rats from the X group was observed as early as the first mating trial in both experiments. This suggests that DLM had affected both spermatozoa and elongated spermatids (20–30%). Very high rates of PIL persisted from the second to the fourth trial and remained significantly elevated during the last trial: an acute dose of 3 Gy of x-rays therefore also causes DLM successively to spermatocytes and differentiating spermatogonia (27–100%) and to stem cells (14–39%). Such mutagenic x-ray effects have previously been observed in spermatids and spermatocytes in mammals other than rats (11, 13, 26–28) and have also recently been described in mice spermatogonia, for an x-ray dose double the one used here (10). That radiation-induced chromosomal abnormalities occurred at the stem cell level is particularly important since these cells are implicated in long-term fertility. This is illustrated by the fact that although irradiated paternal stem cells did not induce any quantitative change in the spermatogenic process of the progeny, PIL increased in females mated with F_1 and F_2 males.

The results of experiment A confirm our previous findings: the administration of MT for 55 days can be successfully used for contraceptive purposes in the adult rat (16). The MT-induced inhibition of the spermatogenic process appears to result essentially from a blockage of spermiogenesis (16). Moreover, 15 days of treatment with these steroids (experiment B), resulting in a 43% reduction of sperm reserves, had no immediate effect on fertility parameters. This suggests that contraception in the rat is secured somewhere between days 15 and 55 of MT administration.

One of the main purposes of this study was to determine if MT treatment would protect the spermatogenesis and fertility of male rats against x-rays. Our results show that a 55-day pretreatment with these steroids affords major quantitative and qualitative protection of the male reproductive function. Furthermore, this study also clearly establishes that short MT pretreatment (experiment B) is as effective as the long one (experiment A) at preventing the long-term effects of x-rays on sperm reserves and fertility. This is particularly important since therapy in men is begun shortly after cancer is diagnosed and spermatogenesis must therefore be rapidly and effectively protected. However, it must be noted that in the last fertility trial of experiment B, although PIL were 28% lower for the MTX rats in comparison to the X group of animals, they remained higher than the controls, in contrast to the results in experiment A. This may be due to the shorter pretreatment period of 15 days or more simply to the fact that the effects of irradiation were more severe in experiment B than in experiment A. Further investigation is needed to ascertain if this partial protection of stem cell chromosomes against x-ray damage would be sufficient to prevent transmission of anomalies to offspring as observed in experiment A. In experiment B, although the proportion of PIL was not significantly affected by MT alone, it was much higher in the second and third fertility trials in the MTX group than in the X group. This indicates that, for an unknown reason, spermatids and spermatocytes are more vulnerable to x-ray-induced chromosomal damage after a short MT pretreatment.

Importantly, in experiment A, our results also demonstrate that MT treatment can prevent x-ray-induced alteration of the reproductive abilities in the F_1 and F_2 male offspring. A similar observation was made on the F_1 male offspring of procarbazine-treated rats (16). This strongly suggests that these steroids protect spermatogenesis against different cytotoxic treatments and that, most probably, a similar mechanism, which remains to be elucidated, underlies this protection.

In conclusion, there is no doubt that a method of protecting male reproductive function against anticancer treatment would be of great clinical utility. The data presented herein demonstrate that MT, even when administered for a short period of time, can protect rat germ cells and most importantly stem cells against the toxic and genotoxic effects of x-rays. The clinical potential is evident. It remains to be shown that the protective effect of MT is selective to normal cells and not to cancerous cells.

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