Short Term Pretreatment with Medroxyprogesterone and Testosterone May Potentiate Irradiation Damage to Spermatogenesis in Rats

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Supported by the Ministère de l'Environnement–Contrat Toxicité des Espèces Activées de l'Oxygène. During this work, S. E. was a recipient of scholarships from la Ligue Nationale contre le Cancer (Val de Marne) and l'Association Dialogue Biologistes-Cliniciens (Paris, France).

The authors thank Professor D. Schwartz for his invaluable advice regarding statistical analysis of our data and Dr. M. L. Meistrich for scientific advice.

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Received May 15, 1998; revision received October 26, 1998; accepted October 26, 1998.

BACKGROUND. Hormonal treatments lasting 2–6 months inhibit spermatogenesis in men and have been proposed as germ cell protection against anticancer therapy. Because it is unthinkable to delay anticancer treatments, the authors investigated the protection afforded against irradiation of rats by 22 days of hormonal pretreatment.

METHODS. Adult Sprague–Dawley rats were assigned to an untreated control group (C) or to one of 5 treatments: medroxyprogesterone acetate plus testosterone only (M), 3 or 5 gray of irradiation (R3 and R5), or hormonal treatment prior to 3 or 5 gray of irradiation (MR3 and MR5). Mating trials were conducted 1, 24, 45, 65, 86, and 109 days after treatment. At 122 days, genital organ weights, testis histology, and epididymal spermatozoa were evaluated.

RESULTS. Irradiation reduced sperm production and had a clastogenic effect on postmeiotic germ cells. No protective effect of steroid treatment was observed. Moreover, testis weight, tubule diameter, the repopulating index, and the sperm head count decreased more in the MR5 group than in the R5 group. Mating tests showed decreases in positive vaginal smears and fertility at both 45 and 65 days, and an increase in resorption at 109 days.

CONCLUSIONS. These results indicate that hormonal pretreatment potentiates irradiation damage to germ cells, especially stem cells, as regards survival and genomic alterations, probably because of increased lipoperoxidation of late spermatids. ***Cancer* 1999;85:1313–22.** © *1999 American Cancer Society.*

KEYWORDS: cancer therapy, fertility, irradiation, medroxyprogesterone and testosterone, testis.

To improve the prognoses of some young men with cancer, the side effects of antineoplasic treatments on testis function must be taken into account. Most of these treatments are toxic to germ cells and result in long term sterility, or they may have mutagenic effects in fertile patients.[1,2]

To reduce the gonadal toxicity of this antineoplasic therapy, hormonal treatments that inhibit spermatogenesis before and during this therapy have been proposed.[3] However, conflicting experimental results with rats, mice, dogs, and monkeys were obtained with gonadotropin-releasing hormone (GnRH) analogs or sex steroids (reviewed by Morris and Shalet[4]): in some cases, they increased the gonadotoxicity of anticancer drugs[4–6]; in others, they did not affect it[4,7–9]; and in many investigations, they were reported to reduce it.[4,10–22] The positive results reported for the latter investigations led to clinical attempts to protect fertility in patients treated for testicular carcinoma or Hodgkin disease; however, these attempts proved unsuccessful.[4,23]

These failures were thought to result from allowing too short a period between the initiation of the treatment inhibiting spermatogenesis and the start of the anticancer treatment.[11] In this connection, it has been reported that the protective effect of steroids against the effects of procarbazine only begins after 42 days of administration, with an optimum duration of 56–70 days.[24] Thus, in rats treated with testosterone and estradiol, the protection rate, as assessed by the sperm head count per testis, was about 1% after 2 weeks of hormonal pretreatment but rose to 20% after 6 weeks.[22] Nevertheless, two other reports demonstrated that a protective effect could be obtained with only 2 weeks of hormonal pretreatment. In the first, the combination of medroxyprogesterone and testosterone (MP-T) was used to counter the effects of 3 gray (Gy) irradiation to the testis and provided 34% protection, as estimated from the sperm reserves; this percentage is similar to that obtained with 55 days of hormonal treatment.[13] In the second study,[18] a combination of the GnRH antagonist Nal-Glu and the antiandrogen flutamide was used and provided protection of 5%, as assessed by the sperm head count per testis.

These results have important implications. They raise the question of whether or not spermatogenesis must be inhibited to obtain a protective effect. They also call for clinical trials with short time lags between the hormonal treatment and the anticancer therapy.

In the current study, we attempted to evaluate precisely the rate of gonadal protection obtained after a short hormonal pretreatment, by 1) pretreating rats for slightly longer than 2 weeks, to increase the protective effect; and 2) using irradiation doses of either 3 Gy or 5 Gy, because 5 Gy are supposed to smooth the large variations in the mating trial data reported for irradiation with 3 Gy.[13]

Pretreatment with MP-T was chosen because 1) GnRH analogs did not protect against the effects of irradiation initiated 5 days after hormonal treatment; 2) MP-T seemed very effective, as a 55-day course preserved the fertility of rats against the effects of irradiation with either 3 or 9 Gy[13,14] and also reduced the mutagenic effects of cyclophosphamide and procarbazine on germ cells[10,12]; and 3) we considered using the MP-T combination in a clinical assay, because both the oral and the trancutaneous forms were reported to be safe.[25]

MATERIALS AND METHODS

Animals

Sprague–Dawley rats age 3 months (IFFA CREDO, France) were randomly assigned to one of the following groups: untreated controls (C, n = 20); a 3-week course of steroids only (M, n = 10); irradiation only with 3 or 5 Gy (R3, n = 10, and R5, n = 10); and steroid treatment prior to 3 or 5 Gy irradiation (MR3, n = 15, and MR5, n = 15.

Untreated virgin female rats age 90 days were selected for the mating trials. All rats were kept under controlled temperature and lighting conditions (12 hours dark, 12 hours light). They were fed standard dry pellets and given water ad libitum.

Hormonal Treatment

MP and T (Sigma) were dissolved in olive oil/benzilic alcohol (95:5, volume/volume) and injected subcutaneously into the M and MR groups at 8 and 1 mg per kg per day, respectively, for 22 days. Groups R3, R5, and C were injected with solvent only.

Irradiation Procedure

On Day 23, the rats in groups R3, R5, MR3, and MR5 were restrained in cylindric boxes and positioned so that only the testis and the surrounding organs were exposed to a collimated gamma-ray beam. Groups C and M were sham irradiated. Total doses of 3 or 5 Gy from a cobalt-60 source were delivered in 104 and 307 seconds, respectively. Doses were measured using a tissue-equivalent chamber of the Victoreen 415 dosimeter type.

Mating Trials

Trials were performed during 6 posttreatment periods, between Days 1 and 9, 24 and 32, 45 and 53, 65 and 73, 86 and 94, and 109 and 117. Each rat was allowed to mate with two virgin females. A vaginal smear was sampled every day to determine the date of mating. All females were killed on postmating Day 19.

The number of fetuses per female was recorded. After soaking the oviducts in 4% ammonium sulfide, implantation sites were counted and the number of resorption sites was then calculated. The mating of two females with one male resulted in one of the following situations:

1. Neither female was pregnant (no implantation site): the male was considered infertile.
2. One female was pregnant: the numbers of fetuses, implantation sites, and resorption sites were recorded.
3. Both females were pregnant: the counts obtained for each one were averaged. This meant that the true experimental variable was the male.

Sampling of Tissue, Epididymal Sperm Counts, and Testis Histology

Male rats were killed 122 days after irradiation with 60 mg/kg sodium pentobarbital. The testis and epididymis were dissected out and weighed. The right testis was fixed for histology and the right epididymis was immediately frozen and stored at −30 °C until the epididymal spermatozoa were counted according to a previously reported method.[26] The testes were fixed in Bouin–Hollande solution and embedded in paraffin. Each testis was sliced into 5-μm sections, which were stained with periodic acid–Schiff stain and hematoxylin. Germ cells at the different stages of differentiation were classified according to Leblond and Clermont[27] and underwent qualitative evaluation. The diameters of the seminiferous tubules were measured in 20 cross-sections per testis, using an ocular micrometer. The repopulating index (RI) was obtained by scoring 400 seminiferous tubules in 4 different sections from each testis; a tubule was scored as repopulating if it contained 3 or more spermatogonia that had reached the type B stage or later.[28]

Statistical Analysis

Results were expressed as mean values ± standard error. The chi-square test or, by default, the Fisher exact test was used to compare the percentages of fertile males and sperm positive vaginal smears. To evaluate the differences between groups, the Mann–Whitney *U* test was used for the numbers of fetuses and resorption sites and for spermatozoa with an abnormal morphology, and the Student *t* test was used for genital organ weights, sperm cell counts, hormone concentrations, greatest seminiferous tubule dimension, and the RI. Differences were considered significant at $P < 0.05$.

For dead implants at the sixth mating test, R3 was compared with MR3 and R5 with MR5 using the Mann–Whitney *U* test. *U* was calculated from the epsilon mean for each of these two comparisons and was used to compare groups R3 and R5 with groups MR3 and MR5.

RESULTS

Genital Data for Rats 122 Days after Irradiation

Testis and epididymis weights

The weight of these organs in group M was not very different from control weight (Table 1). Epididymis weights decreased by 10% in groups R3 and R5 (irradiation only), whereas in groups MR3 and MR5 (hormonal pretreatment) they fell more markedly, were dose-dependent, and were associated with a decrease in testis weights (compared with group M, MR3: epididymis weight −16% and testis weight −7%; MR5: epididymis weight −30% and testis weight −27%). Comparison of groups MR5 and R5 (epididymis weight −21%, $P < 0.01$ and testis weight −30%, MR5 vs. R5, $P < 0.05$) showed that pretreatment with MP-T enhanced these weight reductions.

TABLE 1
Testis and Epididymis Weights (g) 122 Days after 22 Days of MP-T Treatment Only (M), Testicular Irradiation with 3 or 5 Gray (R3 and R5), or MP-T Treatment Prior to 3 or 5 Gray Irradiation (MR3 and MR5)

	Weights	
Group	Testis[a]	Epididymis[a]
C (n = 20)	2.06 ± 0.03	0.77 ± 0.02
M (n = 10)	2.01 ± 0.03	0.79 ± 0.02
R3 (n = 10)	1.95 ± 0.09	0.68 ± 0.03*
R5 (n = 10)	1.83 ± 0.09	0.70 ± 0.03*
MR3 (n = 15)	1.86 ± 0.05***°	0.66 ± 0.02***°°°
MR5 (n = 15)	1.46 ± 0.10***°°°†	0.55 ± 0.03***°°°††

MP-T: medroxyprogesterone and testosterone.
[a] Mean weight of right and left organs; C: controls.
Values are mean ± standard error, n: no. of rats. Significantly different from controls: $^{*}P < 0.05$, $^{***}P < 0.001$; from M: $^{\circ}P < 0.05$, $^{\circ\circ\circ}P < 0.001$; from R: $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ (Student *t* test).

TABLE 2
Seminiferous Tubule Diameter, Repopulating Index, Epididymal Sperm Count, and Abnormal Spermatozoa 122 Days after 22 Days of MP-T Treatment Only (M), Testicular Irradiation with 3 or 5 Gy (R3 and R5), or MP-T Treatment Prior to Testicular Irradiation with 3 or 5 Gy (MR3 and MR5)

Group	ST diameter (μm)	RI (%)	ES number ($\times 10^6$)	AS (%)
C	308 ± 6	100.0 ± 0.0	466 ± 18	4.6 ± 0.8
M	305 ± 8	99.7 ± 0.2	414 ± 15	4.5 ± 0.7
R3	250 ± 7***	97.2 ± 2.3	370 ± 25**	5.3 ± 0.6
R5	288 ± 8*	95.9 ± 3.1	286 ± 38***	7.4 ± 1.0
MR3	251 ± 11***°°	95.7 ± 2.5*	344 ± 21***°	7.5 ± 1.7*
MR5	222 ± 24***°°††††	71.8 ± 9.2***†	171 ± 31***°°°†	9.6 ± 0.7***°°°

MP-T: medroxyprogesterone and testosterone; ST: seminiferous tubules; RI: repopulating index; ES: epididymal spermatozoa; AS: abnormal spermatozoa; C: controls.
Values are mean ± standard error. Significantly different from C: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; from M: $^{\circ}P < 0.05$, $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$; from R: $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ (Student *t* test and Mann–Whitney *U* test for abnormal spermatozoa).

Sperm production

Sperm production is represented in Table 2.

Seminiferous tubule diameter. Tubule diameters were identical in the M and C groups. Irradiation shrinks the seminiferous tubules. Thus, although no difference was evident between the R3 and MR3 groups,

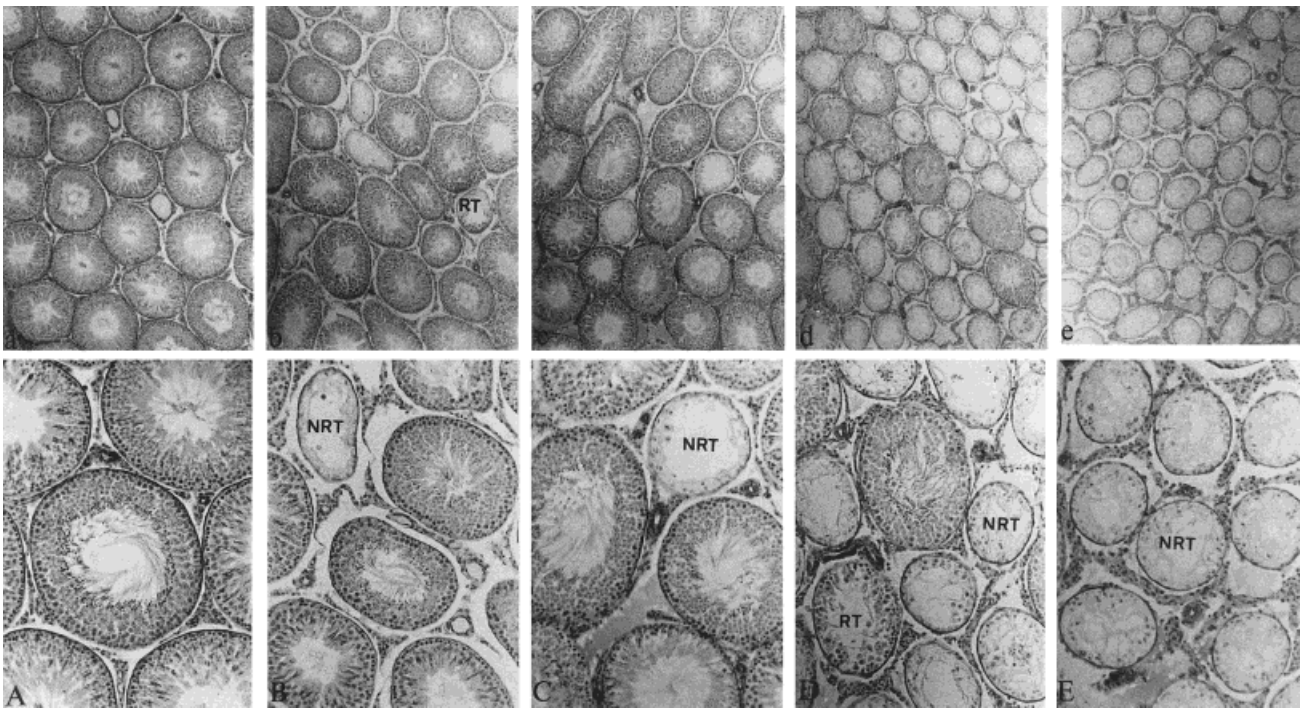

FIGURE 1. Photomicrographs (original magnification a–e ×50, A–E ×125) of sections of rat testes. (a–A) All tubules exhibit active spermatogenesis, as seen in control and hormone-treated rats 122 days after the end of treatment. (b–B) Rat irradiated with 3 gray (Gy) 122 days after irradiation. Most tubules display normal spermatogenesis, but a few contain Sertoli cells with a small number of germ cells (RT: repopulating tubule) and others contain Sertoli cells only (NRT: nonrepopulating tubule). (c–C, d–D, and e–E) Effects of 5 Gy irradiation on rats pretreated with medroxyprogesterone and testosterone: in c–C and d–D, partial recovery of spermatogenesis was observed (c–C 70%, d–D 30%); in e–E, none of the tubules displayed recovery of spermatogenesis.

tubule diameters in group MR5 were significantly smaller than in group R5 ($P < 0.001$).

Testis histology: repopulating index. In both the C and M groups, all seminiferous tubules exhibited normal spermatogenesis at all stages (Figs. 1a and 1A). In groups R3, R5, and MR3, all tubules showed a complete spermatogenic process except for a few, which contained only Sertoli cells with a few germ cells (Figs. 1b and 1B).

The RI of group MR3 was significantly lower than in the controls. In group MR5, damage to testes was markedly greater, as evidenced by the great variability of the RIs (Fig. 2); thus, 8 of the 15 rats in this group had an RI of 100%, but this rate decreased slightly to 70–78% in 3 rats (Fig. 1c and 1C) and was very low (0–33%) in 4 others (Table 2, Figs. 1d and 1D, 1e and 1E).

Number and morphology of epididymal spermatozoa. Groups R3 and R5 exhibited a dose-dependent decrease in sperm cell counts compared with the controls (R3: −20% and R5: −38%); these counts fell dras-

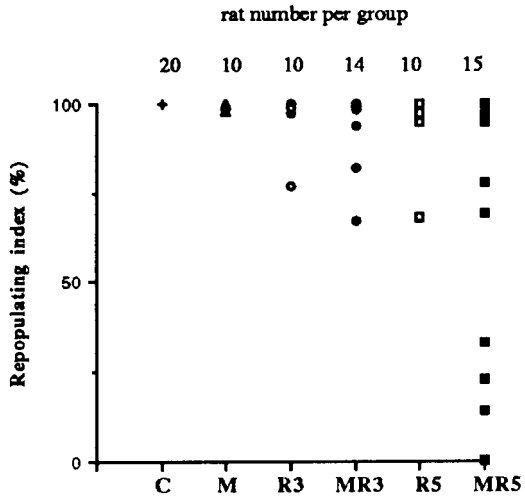

FIGURE 2. Distribution of the RI in rats 122 days after treatment. C: controls; M: daily treatment with MP-T for 22 days; R3: irradiated with 3 gray (Gy); R5: irradiated with 5 Gy; MR3: MP-T treatment before 3 Gy irradiation; MR5: MP-T before 5 Gy irradiation; RI: repopulating index; MP-T: medroxyprogesterone and testosterone.

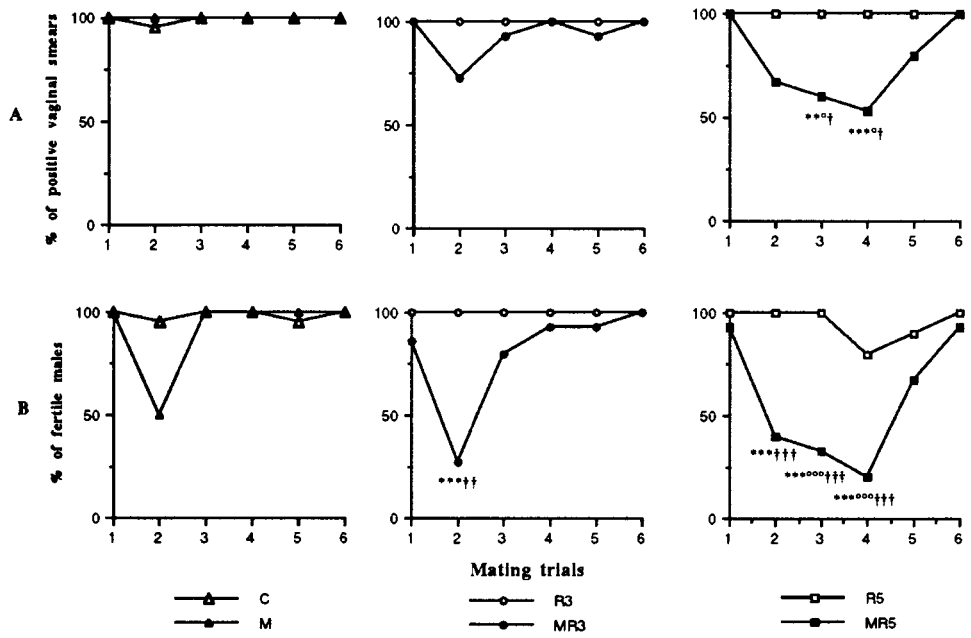

FIGURE 3. From serial mating trials, percentages of positive vaginal smears after mating per fertile male rat (A) and percentages of fertile male rats (B) 22 days after (MP-T) treatment alone (M), 3 gray (Gy) or 5 Gy testicular irradiation (R3 and R5), or MP-T treatment prior to 3 or 5 Gy irradiation (MR3 and MR5). C: controls. Significantly different from C $^{*}P < 0.01$, $^{***}P < 0.001$; from M $^{\circ}P < 0.05$, $^{\circ\circ\circ}P < 0.001$; and from R $^{\dagger}P < 0.05$, $^{\dagger\dagger\dagger}P < 0.001$ (Mann–Whitney *U* test). MP-T: medroxyprogesterone and testosterone.

tically compared with the M group (MR3: −17% and MR5: −58%). The worsening effect of hormonal pretreatment was confirmed by the decrease in the number of sperm cells observed in the MR5 group compared to the R5 group (−40%, $P < 0.05$). The decrease in epididymal weight (Table 1) might be partly due to the drop in the number of sperm cells.

Both the MR groups exhibited a moderate rise of the number of spermatozoa that were abnormal, essentially because of head–tail dissociation.

Mating Trials

Sperm positive vaginal smears

These smears are represented in Figure 3A. All the females that mated with C, M, R3, and R5 male rats had sperm positive vaginal smears. In both the MR3 and MR5 groups, the number of positive smears fell by 30% as early as the second mating test; in group MR5, this decrease persisted and even rose in the subsequent tests (MR5 vs. R5, third mating trial: −40%, and fourth mating trial: −47%, $P < 0.05$).

Fertility

Fertility is represented in Figure 3B. The fertility of the irradiated groups was not affected, except for a moderate, nonsignificant decrease at the fourth mating trial in group R5. In all three groups treated with hormones (M, MR3, and MR5), fertility fell steeply by the second mating trial, clearly as a result of the hormonal treatment. However, whereas in the M and MR3 groups this drop was transitory, in the MR5 group it persisted and continued to fall during the third and fourth trials (respectively, by −67 and −80% vs. group M). Comparison of the MR5 and R5 groups strongly suggested that the large, significant decrease in the fertility of MR5 rats evident at the third and fourth mating trials ($P < 0.001$) probably resulted from hormone-induced potentiation of the gonadal toxicity of irradiation.

Strikingly, the decrease in positive vaginal smears cannot alone account for the magnitude of the drop in the fertility of MR5 rats (third mating trial: positive smears 60% and fertility 33%; 4th mating trial: positive smears 53% and fertility 20%), but it might indicate that the spermatozoa of MR5 rats was not able to achieve fertilization or normal implantations.

Number of live fetuses and numbers of implantation and resorption sites per fertile male

The very small number of fertile rats in the MR3 group at the second mating trial and in the MR5 group at the fourth mating trial did not allow any statistical comparisons with the other groups for these variables.

Live fetuses. Live fetuses are represented in Figure 4. As early as the first mating trial, irradiation induced a decrease in the number of live fetuses of the same order of magnitude in all the irradiated groups, whether treated with hormones or not. Compared with the controls, the decreases in these groups amounted to −28% in R3 and −37% in R5; and compared with group M rats, −21% in MR3 and −45% in MR5. At the second mating test, group M, already characterized by reduced fertility, also showed a 30% reduction in the number of live fetuses. This reduction

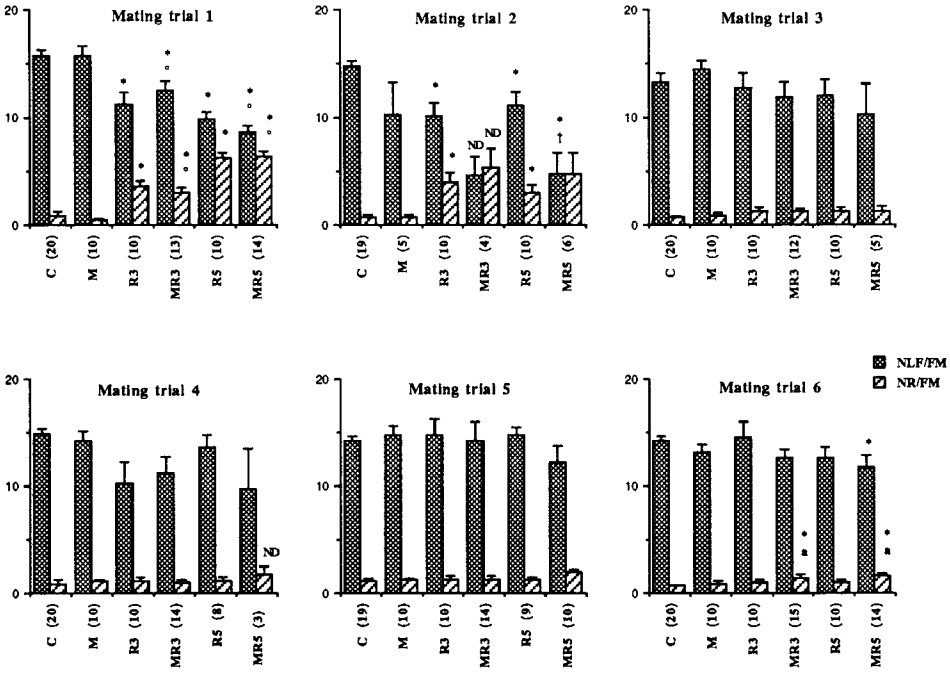

FIGURE 4. From serial mating trials, number of live fetuses (NLF) and resorptions (NR) per fertile male (FM) 22 days after MP-T treatment alone (M), 3 gray (Gy) or 5 Gy testicular irradiation (R3 and R5), or MP-T treatment prior to 3 or 5 Gy irradiation (MR3 and MR5). C: controls. Mean values ± standard error, ( ): number of fertile rats. Significantly different from C: $^{*}P < 0.05$; from M: $^{\circ}P < 0.05$; and from R: $^{\dagger}P < 0.05$ (Mann–Whitney *U* test). $^{a}P < 0.05$ (*U* was calculated from the epsilon mean for each of two comparisons: R3 vs. MR3 and R5 vs. MR5. It was used to compare groups R3 and R5 with groups MR3 and MR5). ND: not determined; the very small number of fertile rats in this test did not allow valid comparison with other groups.

was larger in groups MR3 and MR5 than in the controls or in groups R3 and R5 (vs. controls R3: −32%, MR3: −69%, R5: −25%, and MR5: −69%). The difference between the R and MR groups probably resulted from the cumulative effects of hormonal treatment and irradiation. Note that in group MR5, the fetus count versus the controls was still low at the sixth mating trial (−17%, $P < 0.05$).

Implantations. These results are not shown. The number of implantation sites remained almost unchanged in all groups at all the mating trials. Compared with the controls, it had only decreased by the second trial in the 3 hormone-treated groups M, MR3, and MR5 (−30, −27, and −54%). This probably explains the decrease in fetus counts observed in these groups at the second mating trial.

Resorptions. Resorptions are represented in Figure 4. In the irradiated groups, the number of resorption sites was very large at the first and second mating trials (first trial vs. controls, R3: ×4.2 and R5: ×7.2; vs. M, MR3: ×6 and MR5: ×13; second trial vs. controls, R3: ×5 and R5: ×3.8; vs. M, MR3: ×7.7 and MR5: ×6.7). However, it decreased thereafter. There was no signifcant difference between the MR groups and the corresponding R groups. Note that, at the sixth mating trial, there were significantly more dead implants in the MR3 and MR5 groups than in the R3 and R5 groups ($P < 0.05$).

DISCUSSION

Validity of the Model

These results emphasize one particular feature of the method: that it is essential to determine the extent of irradiation-induced damage before considering whether an associated treatment might afford protection against the adverse effects of irradiation, or whether it might enhance them. In fact, when damage to the testes is moderate and they recover rapidly, it may be difficult to demonstrate the protective effect of an associated treatment. Conversely, when the damage is serious and permanent, it may be difficult to prove its potentiation by such treatment.

Germ cells of Wistar rats and $LBNF_1$ rats are very sensitive to radiation.[29,30] Thus, in $LBNF_1$ rats, 5 Gy irradiation induced an 80% fall in testis weight and reduced the RI to 0%.[30] Testes of Sprague–Dawley rats are known to resist irradiation better: after 5 Gy, their weight only decreased by 11%, and the RI was 72%.[29] Irradiated Sprague–Dawley rats may therefore be considered valid models for demonstrating potentiation effects.

Potentiation

Organ weights and histology: variability of damage

No differences were found between group M and the controls regarding testis weight or histology, thus confirming that gonad function had recovered 122 days after hormonal treatment that was designed to inhibit spermatogenesis.

Analysis of the results for group MR3 suggested

that hormonal pretreatment had a detrimental effect, because the decreases in testis and epididymis weights and in the epididymal sperm count were greater than in group R3. However, it was in the MR5 group that the damaging effect of the hormonal pretreatment became obvious: thus, the decreases in testis and epididymal weights, seminiferous tubule diameters, RI, and the sperm count, all of which were clearly greater in MR5 than in R5 rats, indicated that the stem cells had been damaged.

However, the finding that LH, plasma testosterone, and androgen-dependent sexual glands were unchanged (data not shown) proves that the Leydig cells were not damaged and that the potentiation of irradiation damage was confined to the seminiferous tubules.

The great variability in germinal impairment between rats (observed, for instance, in the MR5 group) (Fig. 1) is probably a characteristic of the Sprague–Dawley strain. We had already observed such variability in a previous work,[14] in which 55 days of treatment with MP-T prior to 9 Gy irradiation only enabled 4 of 10 rats to recover normal fertility. The same variability has been observed in Sprague–Dawley rats after procarbazine injection: at doses as high as 125 mg/kg[15] and 750 mg/kg (personal unpublished data), the effects of this treatment on the testis were very different from one rat to another, and ranged from total or limited tubule atrophy to the persistence of normal spermatogenesis.

Serial mating trials

The mating trials were conducted every 20–23 days for 117 days. Under these conditions, it is possible to determine the sensitive stage of spermatogenesis by measuring the interval between the time of exposure and the occurrence of anomalies,[31] as it is known that epididymal transit requires 8 days, that differentiated spermatogonia become testicular spermatozoa in about 53 days, and that 13 days are necessary for the transition from As to A1 spermatogonia. In the current investigation, drops in fertility were due to the specific type of germ cells damaged: thus, the Day 1 trial evidenced a toxic effect on epididymal sperm, the Day 24 trial on spermatids, the day 45 trial on spermatocytes, the Day 65 trial on differentiated spermatogonia, and the Day 86 and 109 trials on undifferentiated spermatogonia.

Within group M, which was treated with steroids only, a sharp decrease in the number of fertile rats was observed at the second mating trial. This was probably because MP-T treatment perturbs spermiogenesis and therefore reduces the number of epididymal spermatozoa by approximately 20% (see "Mechanism of Potentiation") as well as their fertilizing ability; the smear tests were all positive in group M females.

In irradiated rats, the main abnormality, observed at the first and second mating trials, consisted of an increase in conceptus lethality. This is evidence of the clastogenic effects of irradiation on epididymal spermatozoa and spermatids.

In both the MR3 and MR5 groups, it seems obvious that at the second mating trial, the effects of hormonal treatment and irradiation were cumulative, which would account for both the decrease in fertility and the increase in dead implants. In addition, three anomalies that did not occur in any other group were reported in the MR groups, namely:

1. A significant decrease in the number of sperm positive vaginal smears at the third and fourth mating trials (Days 45 and 65) in the MR5 group, suggesting exacerbation of the cytotoxic effects on germ cells, especially spermatocytes and differentiated spermatogonia;
2. A large drop in fertility at the third and fourth mating trials in MR5 rats (probably due to the sperm decrease and, in the animals with positive smear tests, a loss of fertilizing ability of the remaining spermatozoa); and
3. A slight but significant increase in the number of dead implants at the sixth trial (Day 109), suggesting chromosomal damage to As spermatogonia.

To conclude, the data provided by the serial mating trials indicate that the hormonal pretreatment failed to correct the clastogenic effect of irradiation on postmeiotic germ cells observed in the first and second trials. Moreover, they are evidence of the potentiation of the radiation toxicity in spermatocytes, differentiated spermatogonia, and stem spermatogonia.

Data from the Literature

Our results confirm the lack of protection observed in rats injected with estrogen for 3 weeks prior to local irradiation of the testis with 4 or 15 Gy.[7] However, contrary to the results of another study in which rats were treated for 2 weeks with MP-T before 3 Gy irradiation,[13] we failed to demonstrate the existence of any protection against genetic or tubular damage, even though we used a more active hormonal treatment, which lasted for 22 days, to increase the protective effect.

The reasons for this discrepancy are not easy to discern. We can only stress the following differences between the methodologies used in the the two studies:

1. The rats did not come from the same supplier. The IFFA-CREDO (IC) rats used in our experiments may have had more radio resistant testes than the Janvier (J) rats used in the previous study[13]; thus, after 3 Gy irradiation, the decreases in testis weights and the spermatozoa count were smaller in IC than J rats (IC: −3% and −20% vs. J: −17% and −50%).
2. In our protocol, rats were not anesthetized before irradiation, because pentobarbital is thought to protect against irradiation damage.[32]
3. For greater accuracy, a) we evaluated the implantation sites, not by direct observation after delivery but by killing the females almost at the end of pregnancy and by soaking the oviducts in ammonium sulfide; b) mating trials included two females for one male, and we reported the data per fertile male instead of per pregnant female because the latter mode would lead to overestimation of the statistical significance of the results.[33]

The potentiation by the hormonal treatment was more markedly dose-dependent for 5 Gy than 3 Gy irradiation. It is noteworthy that the authors who reported protection at this dose indeed demonstrated a larger paradoxical increase in postimplantation losses 40 days after irradiation in the pretreated-irradiated group than in the group exposed to irradiation only.[13]

Thus, after 5 Gy irradiation, we observed an increase in gonadal toxicity, as did other researchers. Pretreatment with a GnRH agonist increased the gonadal toxicity of cyclophosphamide in dogs[34] and of cisplatin in rats[35]; the same observation was made in rats when estrogen was given before procarbazine.[5] Note that in one experiment, which demonstrated clearly potentiated toxicity in rats, the antimitotic treatment was administered after a short hormonal treatment of 14 days.[35]

Mechanism of Potentiation

How can 22 days of pretreatment with MP-T be toxic when a pretreatment of 55 days is protective?[10,12,14] A short hormonal treatment probably induces biologic phenomena capable of amplifying the effects of radiation. The kinetics of endocrine and cytologic modifications in the testis might provide comprehensive information about this apparent paradox. Our previous comparative data[14] for control rats versus rats treated with MP-T for 10, 20, 30, 40, or 55 days demonstrated the following:

1. Intratesticular testosterone fell to 2% in the controls from the 10th day and remained low throughout the treatment period;
2. The data obtained after 20 days of MP-T treatment showed that at Stage VII, the numbers of pachyten spermatocytes, round spermatids, and epididymal spermatozoa had dropped by 40%, and that at 55 days the numbers of pachyten spermatocytes and round spermatids had respectively dropped by 40% and 50% and the numbers of late spermatids and epididymal spermatozoa had dropped by 93%.

It has been suggested that a rise in intratesticular testosterone impairs protection by GnRH agonists.[21] However, as intratesticular testosterone remained at the same level at 22 days as at 55 days, this hypothesis cannot explain the potentiation observed in the current study. The main difference between the testes of the rats respectively treated for 22 and 55 days consisted of the presence, at 22 days, of a large number of elongated spermatids. The Sertoli cells, which at that stage are no longer controlled by androgens, might not then fulfill their function in relation to the germ cells. As a result, the latter may be in an abnormal hormonal and metabolic environment, possibly explaining why some of them degenerate and others, especially late spermatids that are metabolically dependent on Sertoli cells, become more vulnerable to aggression by radiation.[36] Late spermatids possess abundant membrane material. Their biologic environment contains more oxygen than that of the other germ cells, making them more radiosensitive.[37] Under these conditions, spermatids may, after irradiation, generate an excess of lipid peroxides and free radicals responsible for potentiation.

Some of the results published in the literature substantiate this hypothesis. It has been demonstrated that radiation causes iron to increase in the testis, and iron activates oxidoreduction activator.[38] Radiation reduces the activity of superoxide dismutase and then increases that of conjugated dienes, a product of lipid peroxidation degradation.[39] Moreover, it was recently demonstrated that the testes of Sprague–Dawley rats had a lower capacity for resistance to oxidative stress than those of other species.[40] In a previous work, we showed that immature human spermatozoa with high levels of cytoplasmic material in their middle piece were more sensitive than other types of spermatozoa to lipid peroxidation reactions.[41]

Clinical Trials

The shortness of the pretreatment period might be one of the factors accounting for the failure to protect fertility in the clinical trials in which anticancer treatment was initiated soon after a treatment inhibiting spermatogenesis.[4,23] One should bear in mind that in men, complete inhibition of spermatogenesis takes longer than in rats (72–120 days[24,42] vs. 55 days[14]). This increases the risk that in men, a short inhibitory pre-

treatment might be ineffective or even toxic. However, one may hope that new treatments combining an antiandrogen drug with a GnRH antagonist will prove more effective, as this combination entails more drastic suppression of spermatogenesis than that achieved with androgens or progestogens.[18] The combination of a GnRH antagonist and flutamide indeed seems to reduce spermatogenesis faster than MP-T. Thus, after 14 days of treatment with 600 μg/kg/day of Nal-Glu and 20 mg/kg/day of flutamide, rat testicular weight diminished by 70% and the testis sperm head count by 98%.[18] For the same period of treatment, MP-T only reduced testicular weight by 30% and the caput epididymal sperm head count by 12% (Es-slami, unpublished data). Moreover, clinical trials in man have shown that a faster and more profound suppression of spermatogenesis could be obtained with the antiandrogenic progestin cyproterone acetate.[43]

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