Protection from Radiation-induced Damage of Spermatogenesis in the Rhesus Monkey (Macaca mulatta) by Follicle-stimulating Hormone

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ABSTRACT

In adult rhesus monkeys a two- to threefold increase in the number of spermatogonia was found at Day 75 after 1 Gy of X-irradiation when the animals were pretreated with two intramuscular injections of follicle-stimulating hormone (FSH) each day. Also the percentage of sections of seminiferous tubules showing spermatogonia (repopulation index) was much higher when FSH was given before irradiation. At 75 days postirradiation the repopulation index was 39 ± 10% after irradiation alone and 81 ± 11% when FSH pretreatment was applied.

The pretreatment with two injections of FSH each day during 16 days caused an increase in the number of proliferating A spermatogonia. In view of earlier results in the mouse, where proliferating spermatogonial stem cells appeared more radioresistant than quiescent ones, it is suggested that the protective effects of FSH treatment are caused by the increase in the proliferative activity of the A spermatogonia and consequently of the spermatogonial stem cells. The results indicate that in the rhesus monkey the maximal protective effect of FSH is reached after a period of treatment between 7 and 16 days.

INTRODUCTION

One of the consequences of radiotherapy and/or chemotherapy for cancer treatment is testicular damage (1, 2). In patients with early stages of Hodgkin’s disease long-term remission or a complete cure can be achieved by radiotherapy and chemotherapy in about 80% of the patients (3). Because most of these patients are of reproductive age, loss of fertility has become a major concern.

Since the first study by Glode et al. (4) many attempts have been made to protect spermatogenesis from radio- and chemotherapy by way of pretreatment with hormones (5–14). It was found that a short pretreatment with LHRH-A (luteinizing hormone-releasing hormone) analogues, estradiol or testosterone had no protective effect on spermatogenesis in the human (7), the mouse (8) and the rat (11–13). However, beneficial effects have been observed after a prolonged pretreatment with testosterone for 6–12 weeks (11–13) or LHRH-analogues (17). Ad, Ap spermatogonia, and Sertoli cells were described before (17). Ad, Ap spermatogonia, and Sertoli cells were counted and spermatogonial numbers were expressed per 1000 Sertoli cells. Also the RI, the percentage of tubular cross-sections showing A spermatogonia, was determined. For statistical analysis Student’s t test was used.

RESULTS

In the monkeys treated with FSH twice daily during a period of 16 days, the total number of A spermatogonia was found to be significantly higher than before treatment (Table 1). This increase in total numbers was due to an increase in the number of proliferating A spermatogonia. In the mouse it was found that the spermatogonial stem cells are more radioresistant when they are proliferating than when they are quiescent (15). Second, in the monkeys Macaca mulatta and Macaca fascicularis treatment with FSH caused an increase in the number of proliferatively active Ap spermatogonia within 16 days (16). The number of quiescent Aa spermatogonia remained the same. As spermatogenesis in the monkey is very similar to that in the human and assuming that in primates too, proliferating stem cells are more radioresistant, we have tried to protect spermatogenesis in the rhesus monkey against radiation damage by a 16-day pretreatment with FSH. The results indicate a substantial protective effect.

MATERIALS AND METHODS

Animals. Seven adult male rhesus monkeys (Macaca mulatta) obtained from the Primate Center of TNO, Rijswijk, The Netherlands were used.

Experimental Protocol. A group of four monkeys received two (16) daily intramuscular injections (at 0830 h and 1700 h) of 15 IU FSH (Metrodin, Serono, Switzerland) during 16 days. After this 16-day period, the FSH treatment was continued for 7 days during which the monkeys received one injection of 15 IU FSH daily in order to maintain the stimulating effects of the FSH. During this period the effects of FSH on spermatogenesis were evaluated and the biopsy wound could heal. FSH administration was stopped after irradiation. Another group of three monkeys were not treated with FSH prior to the irradiation.

Local irradiation of the testes was performed at the Radiobiological Institute TNO, Rijswijk, The Netherlands, using a Philips-4 Muller X-ray generator (type MG 300) as described before (17). All monkeys received a single dose of 1.0 Gy of X-rays. The FSH treatment and the irradiations were carried out during the months November and December which is in the fertile season (18).

Testicular biopsies were taken 2 weeks before the start of the FSH administration (control biopsy), immediately after the 16-day period of twice-daily FSH administration and at 75 days after irradiation. The biopsies were fixed in Bouin’s fluid, embedded in hydroxyethylmethacrylate (Technovit 7100; Kulzer, Germany) and 5-μm sections were made. The sections were stained with the periodic acid-Schiff reaction and hematoxylin (19).

Cell Counts. The Aa and the Ap spermatogonia were identified as described before (17). Aa, Ap spermatogonia, and Sertoli cells were counted and spermatogonial numbers were expressed per 1000 Sertoli cells. Also the RI, the percentage of tubular cross-sections showing A spermatogonia (20–22), was determined. For statistical analysis Student’s t test was used.

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1 The abbreviations used are: LHRH, luteinizing hormone-releasing hormone; FSH, follicle-stimulating hormone; Ap, Ap.i« spermatogonia; Aa, Aa.s spermatogonias spermatogonia; RI, repopulation index.

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4 The percentages used are: LHRH, luteinizing hormone-releasing hormone; FSH, follicle-stimulating hormone; Ap, Ap.i« spermatogonia; Aa, Aa.s spermatogonia; RI, repopulation index.
Table 1 The numbers of A\(_d\), A\(_p\), and the total number of A spermatogonia (A\(_\text{total}\)) present in the seminiferous epithelium of the rhesus monkey (Macaca mulatta) after treatment with two daily doses of 15 IU FSH during a period of 16 days. Numbers are expressed as a percentage of the numbers that were present in the control biopsies. Mean ± SEM, n = 4.

<table>
<thead>
<tr>
<th>Spermatogonial type</th>
<th>Number (% of control present after FSH treatment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(_d)</td>
<td>79 ± 8*</td>
</tr>
<tr>
<td>A(_p)</td>
<td>153 ± 6*</td>
</tr>
<tr>
<td>A(_\text{total})</td>
<td>117 ± 5*</td>
</tr>
</tbody>
</table>

\*P < 0.01.  
\^P < 0.001.

of the control number, respectively (Fig. 1). In the FSH-pretreated monkeys the total number of A spermatogonia (A\(_d\) and A\(_p\)), and the numbers of A\(_d\) spermatogonia and of A\(_p\) spermatogonia were found to be 22 ± 6%, 18 ± 5%, and 26 ± 7% of the numbers present before FSH treatment and irradiation, respectively (Fig. 1).

The numbers of spermatogonia present after irradiation can also be expressed as a percentage of the numbers that were present after 16 days of FSH treatment. At 75 days after irradiation the total number of A spermatogonia was 19 ± 5% of that present after 16 days of FSH treatment. The numbers of A\(_d\) and A\(_p\) spermatogonia were 12 ± 4% and 34 ± 9% of the numbers present after FSH treatment, respectively.

After irradiation alone the RI was found to be 39 ± 10% (Fig. 1). When the irradiation was preceded by FSH treatment the RI was found to be 81 ± 11%.

**DISCUSSION**

Administration of FSH to adult rhesus monkeys during 16 days caused an increase in the number of A\(_d\) spermatogonia while the number of A\(_p\) spermatogonia decreased. However, the total number of A spermatogonia increased significantly as a result of the FSH administration (Table 1). For the increase in the number of A\(_p\) spermatogonia two possible mechanisms have been proposed (16). First, FSH may cause an activation of the resting A\(_d\) spermatogonia into proliferating A\(_p\) spermatogonia followed by a restoration of the number of A\(_d\) spermatogonia. Second, FSH may cause an enhanced proliferation and/or self-renewal within the population of A\(_p\) spermatogonia. Irrespective of the mechanisms involved, it can be concluded that FSH treatment leads to an increase in the proliferative activity of the total population of A spermatogonia and consequently of the spermatogonial stem cells. The mechanism by which FSH stimulates the proliferative activity of the A spermatogonia is not yet clear. Probably, as suggested earlier (16), FSH acts on the spermatogonia by an indirect action via the Sertoli cells which are known to have receptors for FSH and to secrete mitogenic factors (23).

The cell counts at Day 75 after irradiation revealed that pretreatment with twice daily administration of FSH during 16 days causes a two- to threefold increase in the number of A spermatogonia present in the RI compared to the non-treated X-irradiated controls (Fig. 1).

The FSH treatment was stopped after irradiation since the FSH injected will disappear from the plasma within a few hours (24). Hence, it can be excluded that the exogenous FSH enhanced the growth of the repopulating colonies after irradiation. However, there are two other ways to explain the protective effect of FSH observed. Firstly, with the 17% increase in the total number of A spermatogonia after FSH treatment there could be a concomitant rise in the number of spermatogonial stem cells. At present it is not known whether or not, all A\(_d\) and A\(_p\) spermatogonia are stem cells or just part of them (25).

Secondly, in the mouse proliferating spermatogonial stem cells are more resistant to ionizing radiation than quiescent ones (15). In the monkey, FSH increased the number of proliferating A\(_p\) spermatogonia. Regardless whether all, or just part of the A spermatogonia are stem cells, this indicates an increase in the proliferative activity of the stem cells. Consequently, the protective effect of FSH pretreatment may well be caused by an increase in the radioresistance of the spermatogonial stem cells concomitant with their increased proliferative activity. In line with this suggestion, at 75 days after irradiation, the numbers of spermatogonia are also higher after FSH treatment when expressed as a percentage of the numbers present after the 16-day pretreatment period. This indicates a more resistant population of stem cells.

In the other studies on the protective effect of hormone pretreatment LHRH-analogues or -antagonists, estradiol or testosterone were administered before irradiation in order to inhibit the secretion of gonadotropins by the pituitary gland and consequently to suppress spermatogenesis (4–14). The rationale behind these studies was that quiescent spermatogonial stem cells would be more resistant towards irradiation and harmful drugs such as Adriamycin and procarbazine than proliferating stem cells. However, quiescent stem cells may well be more radiosensitive (15), and although the production of spermatooza stops during treatment with these hormones, there are no data that show an arrest of spermatogonial proliferation and consequently of stem cell proliferation. In contrast, Clermont and Harvey (26) concluded that in hypophysectomized rats after an initial drop in the number of spermatogonial stem cells, the remaining stem cells proliferated and renewed in the same
manner as in the normal rat. In mice, spermatogonial kinetics did not change during the administration of an gonadotropin-releasing hormone-analogue for 3 weeks (8). Furthermore, stem cell activity could not be suppressed with testosterone and, after estradiol or testosterone are likely caused by enhancing stem cell proliferation.

The experimental protocol used in this study may not be the most simple and best possible. First, the monkeys were given two injections of FSH per day, to ensure that at least during a considerable part of the day, FSH levels would be supranormal. However, less frequent injections of FSH may suffice, although the effect on spermatogonia was very small when only three injections per week were given (16). Second, higher or lower doses of FSH may give still better or similar results, respectively. Finally, the minimal duration of FSH pretreatment required to obtain a maximal protective effect may be less than 16 days. In the cynomolgus monkey (Macaca fascicularis) no further increase in the number of A spermatogonia occurred when the FSH treatment was continued for more than 16 days. Hence, the maximal effect of the FSH treatment was already established on Day 16 and probably before that time. On the other hand no effect was seen after 7 days of FSH treatment (16). This indicates that the minimal duration of the FSH pretreatment required to obtain a maximal effect on the A spermatogonia and accordingly a maximal protection against radiation damage of the testis is more than 7 days and possibly less than 16 days. Further studies will have to be carried out to establish whether or not the present protocol can be shortened, simplified, and/or improved with respect to the protective effect.

It is difficult to say whether in the human the optimal period of FSH pretreatment would be similar to that in the monkey. The time course of the increase in the number of A spermatogonia could be related to the cycle of the seminiferous epithelium. In that case a longer pretreatment period will be necessary as the epithelial cycle in the human takes 16 days (29) versus 10.5 days in the rhesus monkey (30).

In conclusion, pretreatment with FSH causes an increase in the proliferative activity of the spermatogonial stem cells, and possibly a small increase in the number of stem cells. These two phenomena make spermatogenesis less vulnerable towards irradiation. Probably because it acts more directly, the necessary duration of the pretreatment is shorter with FSH than with LHHRH or androgens which have to be given for more than 7 weeks to achieve beneficial effects (4, 9, 11-13).

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REFERENCES

RADIATION PROTECTION OF MONKEY SPERMATOGENESIS


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