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 cross-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 LHRRH-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 LHRRH-analogues or -antagonists for more than 7 weeks (4, 9) in the mouse and the rat. No protection was observed in the dog after long-term LHRRH-agonist pretreatment (6). As protection of testicular function was only found after long-term LHRRH or T-pretreatment, these approaches are less suitable for clinical use as the cancer treatment cannot be postponed very long.

The present study into the possible protection of spermatogenesis by hormonal treatment is based upon two recent find-
of the control number, respectively (Fig. 1). In the FSH-pre-
treated monkeys the total number of A spermatogonia (Aa and
Ap), and the numbers of Ap spermatogonia and of Aa sperma-
togonia 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
Aa and Ap 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 Aa spermatogonia
while the number of Ap 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 Ap spermatogonia two possible mechanisms have
been proposed (16). First, FSH may cause an activation of the
resting Aa spermatogonia into proliferating Ap spermatogonia
followed by a restoration of the number of Aa spermatogonia.
Second, FSH may cause an enhanced proliferation and/or self-
renewal within the population of Ap spermatogonia. Irrespec-
tive 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 and 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 en-
hanced 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 Aa
and Ap spermatogonia are stem cells or just part of them (25).

Anyhow, in view of the two- to threefold increase in the number
of A spermatogonia present at Day 75 postirradiation after
FSH pretreatment, a more than 17% increase in the numbers
of spermatogonial stem cells would have to occur.

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 prolif-
erating Aa 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 spermatog-
nial stem cells would be more resistant towards irradiation and
harmful drugs such as Adriamycin and procarbazine than prolif-
erating stem cells. However, quiescent stem cells may well be
more radiosensitive (15), and although the production of sper-
matozoa 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

Table 1 The numbers of Aa, Ap, and the total number of A spermatogonia (Aa+Ap) 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>
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<tbody>
<tr>
<td>Aa</td>
<td>79 ± 8*</td>
</tr>
<tr>
<td>Ap</td>
<td>153 ± 6*</td>
</tr>
<tr>
<td>Aa+Ap</td>
<td>117 ± 5*</td>
</tr>
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* P < 0.01.  b P < 0.001.
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 severe impairment of spermatogenesis by treatment with estradiol for 4 weeks, the proliferative activity of the undifferentiated spermatogonia even increased (27). The latter phenomenon was explained by the enhanced degeneration of differentiating spermatogonia that occurred probably as a result of the tubular shrinkage. Consequently, the negative feedback that the differentiating spermatogonia exert on the proliferative activity of the undifferentiated spermatogonia (28), including the stem cells, was alleviated allowing enhanced proliferation. In view of this the beneficial effects of long-term pretreatment with compounds that decrease plasma gonadotropin levels may have been caused by activation of the spermatogonial stem cells instead of arresting them as was assumed by the investigators. As the numbers of differentiating spermatogonia decrease only gradually during this kind of treatment the radioprotective effect will develop only slowly. In conclusion, both the protective effect of the latter compounds may be a result of an alleviation of the negative feedback control while FSH, probably via Sertoli cells, stimulates stem cell proliferation. However, the protective effect of the latter compounds may be a result of an alleviation of the negative feedback control while FSH, probably via Sertoli cells, stimulates 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 spermatogenensis less vulnerable towards irradiation. Probably because it acts more directly, the necessary duration of the pretreatment is shorter with FSH than with LHRH 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


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