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LHRH analogues do not protect the germinal epithelium during chemotherapy

An experimental animal investigation

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Summary. The protective effect of Decapeptyl, a D-Trp^6-LHRH analogue, on the germinal epithelium of rats during chemotherapy was examined in an experimental animal study. Four groups were formed consisting of 15 (groups A and B) and 20 (groups C and D) Sprague-Dawley rats, respectively. The animals were treated as follows: group A (control group) was given 0.9% NaCl i.m.; group B (LHRH group) D-Trp^6-LHRH analogue (Decapeptyl); group C (LHRH + chemotherapy group) Decapeptyl + cyclophosphamide; group D (chemotherapy group) cyclophosphamide. The gonads were removed before therapy was initiated and on days 13, 52, and 182 after treatment and examined histologically. In addition, follicle-stimulating hormone (FSH) levels in the serum, a sensitive indicator of tubular damage, were determined. The most damaging effects of chemotherapy on histology and hormone levels were observed in the animals in group C, which were additionally treated with LHRH analogues. Therefore, it is not possible to protect the germinal epithelium of the rat with LHRH analogues during chemotherapy.

Key words: LHRH analogues – Gonadal chemoprotector

After chemotherapy 70%–80% of male patients have infertility problems, which can be of great clinical and social importance [12]. Preventive measures for the protection of reproductive functions therefore seem advisable for young patients with good prognoses who still want to have children. To date there are no substances that can protect the germinal epithelium during chemotherapy.

Spermatogonia are known to have a high regenerative capacity following exposure to various noxious effects [6]. This knowledge led to the question whether the germinal epithelium could be protected during chemotherapy with the newly developed LHRH analogues. Because LHRH analogues show *reversible* inhibition of spermatogenesis and because they have few side effects, they have been investigated as protective substances for the germinal epithelium in animal experiments, with varying results [3, 5, 11]. These studies were, however, strictly histological, with inevitably subjective interpretations. In recent times LHRH analogues have been applied more and more frequently in humans to prevent postcytotoxic infertility [8, 9], although their protective effect is not all clear to date. Therefore, it is imperative to investigate this question using quantitative parameters. In this paper we present the results of studies of the rat testis during experimental LHRH and chemotherapy, combining a histological examination with a quantitative evaluation and an analysis of follicle-stimulating hormone (FSH) levels.

Materials and methods

The experimental animals consisted of 75 male Sprague-Dawley rats weighing between 250 and 350 g and which were between 86 and 92 days of age. They received standard feed and water ad libitum. The animals were divided into four groups (A, B, C, D). Groups A and B consisted of 15 animals each; because of the expected losses due to chemotherapy, groups C and D consisted of 20 animals each.

Treatment plan

The animals in group A served as control and remained untreated. At the different times at which treatments were given in groups B, C and D, the animals in group A were given injections of 0.9% NaCl to ensure that they were subjected to the same stress factor.

The animals in group B received 650 μg/kg body weight of the depot form of D-Trp^6-LHRH analogue (Decapeptyl). Four weeks later they received a second and last LHRH dose at the same rate.

The animals in group C were treated with a combination of the LHRH analogue and chemotherapy. The depot form of Decapeptyl was administered first, as described for group B. Four weeks after the second LHRH dose chemotherapy was begun. Cyclophosphamide (Endoxan) was chosen as the cytotoxic agent. The animals received three injections of cyclophosphamide at 3-day intervals intraperitoneally. A single dose amounted to 100 mg/kg, the total dose being 300 mg/kg body weight. The animals in group D received cyclophosphamide only at the same dosage and schedule as in group C.

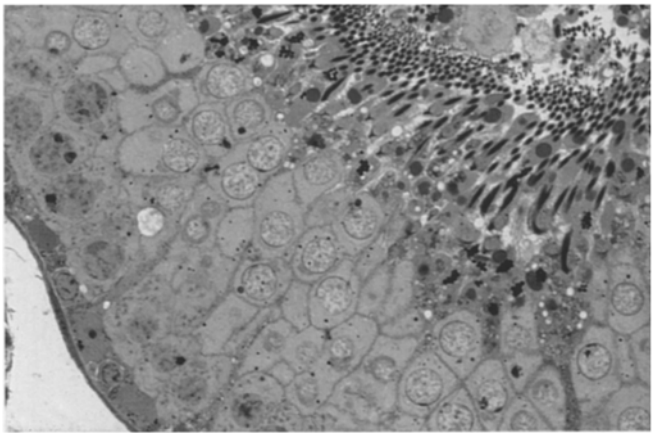

Fig. 1. Normal structure of germinal epithelium (T1). Semithin section, ×600

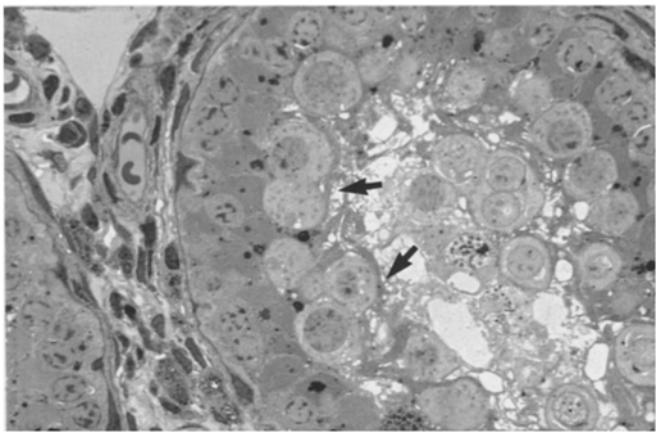

Fig. 3. Distinct flattening of the germinal epithelium (*arrows*) without evidence of spermatids and spermatozoa (T3). Semithin section, ×600

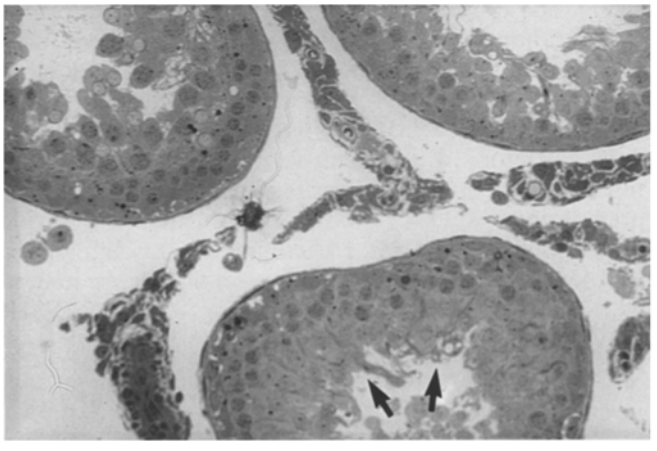

Fig. 2. Regeneration of germinal epithelium with evidence of spermatocytes and spermatids (T2; *arrows*). Semithin section, ×250

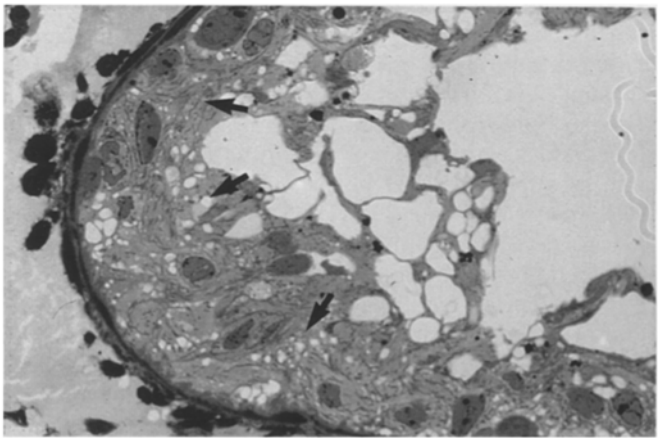

Fig. 4. Shriveled seminiferous tubule (*arrows*); extensive loss of germinal cells (T4). Semithin section, ×600

In order to obtain baseline values five animals were killed and examined before the study began. Since the spermatogenic cycle of the rat takes 13 days [7], the animals were investigated on days 13, 52, and 182 after treatment began. Five animals in each of the groups were killed and examined. Gonads were removed for histological examination and blood samples taken to determine FSH levels from anesthetized living animals.

FSH serum levels were evaluated with the rat-FSH determination kit by NIADDK. The measured levels were expressed in nanograms per milliliter, related to the NIADDK standard rat-FSH Rp2. The gonads were fixed in phosphate-buffered 5% glutaraldehyde solution and counterfixed in 1% OsO_4. Then semithin sections with a thickness of 0.5 μm were made. The histological changes were registered systematically using cross sections of 20 seminiferous tubules per animal. To evaluate the histological sections quantitatively, the degree of histologically demonstrable damage to the germinal epithelium was subdivided into four types [14]:

Type 1 (T1; Fig. 1): Normal germinal epithelium (corresponds to findings in healthy animals that have not been operated on).

Type 2 (T2; Fig. 2): Regeneration of germinal epithelium with isolated evidence of spermatocytes, spermatids, and spermatozoa.

Type 3 (T3; Fig. 3): Flattening of the germinal epithelium with no evidence of spermatids or spermatozoa.

Type 4 (T4; Fig. 4): Shriveled testicular tubule with no evidence of germinal epithelium. Only Sertoli cells and atypical cells are evident.

The means and standard deviations were calculated for each of the different investigation periods. The *t*-test for independent samples was used for comparison between the groups. A P value of 0.05 was regarded as statistically significant.

Results

Histological findings (Table 1)

Group A (control group): The germinal epithelium of the animals in the control group did not show any pathological changes during the observation period of 6 months (T1 = 100%).

Group B (LHRH group): Following 2 months of treatment with LHRH analogues regeneration of the germinal epithelium (T2) on day 13 was seen in 22% of the tubules

Table 1. Degree of damage (T1, T2, T3, T4) to testicular tubular epithelium at the three examination dates following treatment (see also Figs. 1–4)

Group	Day 13	Day 52	Day 182
A	T1 = 100%	T1 = 100%	T1 = 100%
B	T1 = 78% T2 = 22%	T1 = 44% T2 = 48% T3 = 1% T4 = 7%	T1 = 34% T2 = 45% T3 = 14% T4 = 7%
C	T1 = 27% T2 = 28% T3 = 32% T4 = 13%	T1 = 41% T2 = 44% T3 = 6% T4 = 7%	T1 = 32% T2 = 48% T3 = 12% T4 = 8%
D	T1 = 51% T2 = 45% T3 = 2% T4 = 2%	T1 = 46% T2 = 50% T3 = 3% T4 = 1%	T1 = 41% T2 = 57% T3 = 2%

A, Control group; B, LHRH group; C, LHRH + chemotherapy group; D, chemotherapy group

of animals in group B. On day 52, 44% of the testicular tubules were still intact (T1), while 7% were irreversibly damaged (T4). At the 6-month follow-up only 34% normal tubules (T1) were evident, whereas regeneration of tubular epithelium (T2) was observed in 45% and irreversible destruction of germinal epithelium in 14% (T3) and 7% (T4).

Group C (LHRH + chemotherapy): In contrast to control group A, on day 13 the structure of the tubular epithelium of the testicular tubules was inconspicuous (T1) in only 27% of the animals in group C. On day 52 after the last administration of the cytotoxic agent, regeneration of germinal epithelium (T2) was seen in 44%, and in 6% the germinal epithelium was flattened without evidence of spermatids or spermatozoa (T3). In 7% the testicular tubules were irreversibly damaged (T4). Only 32% of the testicular tubules of group C animals had normal tubular epithelium (T1) 6 months after treatment. In 48% the germinal epithelium was regenerated (T2), however in 12% the germinal epithelium was flattened (T3), and in 8% there was irreversible damage (T4).

Group D (chemotherapy): On day 13 following the last administration of the cytotoxic agent, 51% of the animals in group D had normal tubular epithelium (T1) and 45% regenerated tubular epithelium (T2). In a small percentage (2%), the germinal epithelium was either flattened (T3) or was irreversibly damaged (T4). On day 52, 46% of these testicular tubules had normal, unaltered tubular epithelium (T1). As a sign of regeneration, sperm cells were demonstrated histologically in 50% of the testicular tubules (T2). In 3% of the testicular tubules the germinal epithelium was flattened (T3), while only 1% of the tubules were irreversibly damaged (T4). At the 6-month follow-up pretty much the same picture was seen in group D animals, though there were fewer normal testicular tubules than regenerated testicular tubules. Irreversibly damaged tubules (T4) were not evident in group D animals 6 months after treatment.

Follicle-stimulating hormone (Table 2)

The FSH levels measured in the serum of all animals and at all investigation times are listed in Table 2. As this table shows, FSH levels are significantly higher 6 months after treatment in the animals treated with LHRH (groups B and C) than in those in control group A ($P = 0.039$; $P = 0.005$).

Table 2. Means and standard deviations (first line), minima and maxima (second line) of serum FSH levels (in ng/ml)

Group[a]	Before treatment	After treatment		
		Day 13	Day 52	Day 182
BV	798 ± 170 (605–1,018)			
A		765 ± 145 (556–917)	691 ± 53 (630–769)	547 ± 138 (438–781)
B		649 ± 167 (101–187)	693 ± 101 (562–845)	788 ± 169* (537–999)
C		497 ± 212 (241–779)	727 ± 172 (527–992)	800 ± 137* (577–978)
D		801 ± 146 (660–1,127)	792 ± 164 (545–1,041)	605 ± 188 (338–999)

[a] $n = 5$

* Significantly different from the control group

BV, Baseline values; A, control group; B, LHRH group; C, LHRH + chemotherapy group; D, chemotherapy group

Discussion

Some 80%–90% of all young men suffering from malignant diseases such as testicular cancer, Hodgkin's and non-Hodgkin's lymphoma, and acute leukemia can be cured thanks to chemotherapy [12]. The goal of chemotherapy is to kill off tumor cells. In the process, however, normal cells are also damaged, and tissues with high rates of cell division, such as the germinal epithelium, are affected. Some 70%–80% of the male patients are found to be azoospermic or severely oligospermic following chemotherapy [1, 4]. Recently calls to protect the germinal epithelium during chemotherapy have been made more and more frequently [2]. Isolated reports in the literature [5, 10] have given rise to the hope that the newly developed LHRH analogues could protect the germinal epithelium before and during chemotherapy by suppressing spermatogenesis. After the LHRH analogues are discontinued, spermatogenesis would set in again. The contradictory results of the few studies in the literature using animal experiments are based on the usual histological evaluations. Our experiments therefore included objective quan-

titative parameters for analyzing the protective effect of LHRH analogues on the germinal epithelium.

The testes of different species show a varying degree of sensitivity to LHRH analogues, not only primarily but also correlated with dosage and duration of treatment [17]. Histological examinations of the testes of rats after treatment with LHRH have shown that some sectors of the testis can be found in which the seminiferous tubules are completely free of germinal epithelium while other sectors with unaltered spermatogenesis are observed at the same time [13]. For a more thorough evaluation of the degree of damage to the germinal epithelium in rats following the influence of chemical agents, a histological evaluation involving quantitative criteria is therefore necessary.

In the present study rats were chosen as experimental animals. The seminal epithelium of rats is ideal for investigating proliferation and proliferative disorders because of its distinct structure [7]. The substance cyclophosphamide was chosen for experimental chemotherapy because of its well-known damaging effect on the germinal epithelium [7, 15] and its clinical relevance. The depot form of D-Trp^6-LHRH analogue, Decapeptyl, was used as an LHRH analogue because it is easier to administer (monthly injection).

If we compare the histological results of all treatment groups for each killing date, we can conclude that the most damaging effects are seen in group C (LHRH + chemotherapy) and group B (LHRH). Six months after treatment 20% of the testicular tubules are still badly damaged (T3 and T4). In group D animals only 2% of the testicular tubules show flattening of germinal epithelium (T3), and there are no irreversibly damaged tubules (T4) 6 months after treatment. Thus, in contrast to the results of Glode et al. [5] no protective effect of LHRH analogues on the germinal epithelium can be proven histologically.

FSH secretion is regulated by a peptide (inhibine?) that is produced by spermatozoa [16]. Severe destruction of germinal epithelium, for example, following radiotherapy or chemotherapy, and germinal cell aplasia in the Sertoli cell only syndrome (SCOS) are associated with elevated FSH levels. The assumption is that a shortage of spermatozoa leads to an inhibine deficiency and as a result FSH secretion is not adequately suppressed, hence the elevated FSH levels.

In our investigations we found significantly elevated FSH levels, compared with the animals in the control group, 6 months after treatment in group C animals, which were treated with LHRH and a cytotoxic agent. Group D animals (cytotoxic agent only) did not show significantly elevated FSH levels, compared with the animals in the control group. Thus the elevated FSH levels in group C animals 6 months after treatment should be interpreted as a result of more severe tubular damage.

This study shows that it is not possible to protect the germinal epithelium with LHRH analogues during chemotherapy. On the contrary, the damage to the germinal epithelium is even greater. The clinical application of LHRH analogues in humans therefore appears to be not only unjustified but contraindicated. The guidelines adhered to thus far in cancer treatment – precautionary cryopreservation of sperm and long-term contraception following chemotherapy – still hold.

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