Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 22 (1965)
Heft: 2

Artikel: Miscellanea : Effect of CIBA 32,644-Ba on spermatogenesis in laboratory animals : preliminary communication
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DOI: https://doi.org/10.5169/seals-311265

Effect of CIBA 32,644-Ba on Spermatogenesis in Laboratory Animals.

Preliminary communication.

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(with the technical collaboration of P. STAUFFER *).

Chemically, CIBA 32,644-Ba is 1-(5-nitro-2-thiazolyl)-2-imidazolidinone; it was synthesized by WILHELM & SCHMIDT (22) and selected by LAMBERT, in preference to the other derivatives of the group, on account of its better schistosomicidal activity and lower toxicity (11, 12).

During investigations into the schistosomicidal action of CIBA 32,644-Ba, histological examination of the gonads of *Schistosoma mansoni* males obtained from previously treated mice showed that mature spermatozoa had disappeared and that the germinal cells had undergone coarse fragmentation (21).

Moreover, reports in the literature indicate that certain derivatives of nitrofurazone, nitrothiazole, dinitropyrole, and bis-(dichloroacetyl)-diamine, as well as sulphonic esters, may provoke prolonged sterility in rodents without affecting their sexual activity. The inhibition of spermatogenesis is transitory and reversible (1, 2, 3, 4, 5, 7, 8, 9, 10, 13, 14, 15, 16, 17, 18, 19).

These facts have prompted us to carry out detailed histological studies in an attempt to assess the inhibitory effect exerted by CIBA 32,644-Ba on spermatogenesis in various mammalian species, and to discover whether it is functionally reversible in mice treated with 100% effective schistosomicidal doses.

1. Methods.

Two types of study were undertaken:

a) A study of the *histological* appearance of the testes in various species of animal following treatment.

b) A study of the *fertility* of treated animals.

a) *Histological studies.*

In the *rat,* the testes of a group of 5 non-treated rats were compared with those of groups of 5 animals treated with 100 mg/kg daily for 5 consecutive days. Autopsies were performed 0, 10, 20, and 30 days after completion of the treatment.

In one *dog,* one testis was removed prior to treatment and the other after 4 weeks' continuous treatment with a daily dose of 20 mg/kg; in a second dog, one testis was removed following completion of an identical course of treatment, and the other 22 days afterwards.

In one *cynocephalus,* one testis was removed prior to treatment and the other on the day following completion of a 10-day course of continuous treatment with a daily dose of 32 mg/kg; in a second cynocephalus, one

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testis was removed on the day following completion of an identical course of treatment and the other testis one month afterwards.

b) The *"fertility"* of treated animals was assessed by comparing fertility in various groups of mice, each group containing 20 pairs. The controls and the treated animals were mated 3 days after completion of the treatment (see below), each pair being kept in a separate cage; the criteria employed were: date of parturition, and number and quality of the newborn.

Seven groups, each containing 20 pairs of mice, were made up as follows:

Group 1: 20 non-treated pairs as controls.
Group 2: 20 pairs, in which the females only were treated with a daily dose of 50 mg/kg for 10 days.
Group 3: 20 pairs, in which the females only were treated with a daily dose of 100 mg/kg for 10 days.
Group 4: 20 pairs, in which the males only were treated with a daily dose of 50 mg/kg for 10 days.
Group 5: 20 pairs, in which the males only were treated with a daily dose of 100 mg/kg for 10 days.
Group 6: 20 pairs, in which the males and the females were treated with a daily dose of 50 mg/kg for 10 days.
Group 7: 20 pairs, in which the males and the females were treated with a daily dose of 100 mg/kg for 10 days.

2. Results.

a) *Histological studies.*

In the *rat,* spermatogenesis was found to be inhibited in treated animals submitted to autopsy *at day 0;* mature spermatozoa were still present in the seminiferous tubules in 3 out of 5 animals.

At day 10, marked inhibition of spermatogenesis was observed in 2 out of 5 animals; there was atrophy of the entire germinal epithelium.

At day 20, a certain degree of restoration to normal was noted, atypical spermatids appearing side by side with spermatocytes.

At day 30, only about 20% of the tubules still displayed inhibition; a few scarred tubules were observed in 2 out of 5 animals.

In the first *dog,* treated uninterruptedly for 4 weeks, a clear-cut inhibition of spermatogenesis was found in 9.6% of a total of 400 tubules examined; the inhibition was partial, consisting of a reduction in the number of secondary spermatocytes and of an almost complete disappearance of mature spermatozoa. In the second dog, on the other hand, complete spermatogenic arrest was observed in the testis removed 22 days after completion of the 4-week course of treatment.

In the *cynocephalus,* the control testis (removed prior to treatment) displayed a regular succession of cellular elements of the germinal series, culminating in the formation of spermatozoa, which were numerous in the majority of the seminiferous tubules.

In the *testes removed on the day following completion of a 10-day course of treatment,* no elements of the germinal series could be observed any longer in the majority of seminiferous tubules. In the tubules still containing such elements, the latter were less numerous and exhibited certain changes in their morphological and staining characteristics—e.g. formation of giant multi-

nucleated elements owing to the fusion of spermatozoa. No changes were observed in the interstitial tissue or in Leydig's cells.

27 days after completion of the treatment, germinal epithelial cells appeared to recover in some of the seminiferous tubules, and the cellular elements developed again into mature spermatozoa.

In *sheep,* histological examinations showed that spermatogenesis was inhibited to some extent only in a few males, autopsied 10 and 11 days after the end of the treatment; in the animals autopsied later after treatment, the testes were histologically normal (6).

b) *Fertility study.*

Table 1 summarized the results obtained in each group of 20 pairs of mice on the basis of the following criteria: number of parturitions, average number of days elapsing between mating and parturition, average number of newborn per pair.

The same production batch of CIBA 32,644-Ba was used to test the product's schistosomicidal activity in mice infected with *S. mansoni.* These tests showed that:

50 mg/kg daily, admin. orally for 7 consecutive days, had an activity of 40%.
50 mg/kg daily, admin. orally for 9 consecutive days, had an activity of 75%.
50 mg/kg daily, admin. orally for 12 consecutive days, had an activity of 100%.
75 mg/kg daily, admin. orally for 5 consecutive days, had an activity of 80%.
75 mg/kg daily, admin. orally for 7 consecutive days, had an activity of 100%.
100 mg/kg daily, admin. orally for 5 consecutive days, had an activity of 85%.
100 mg/kg daily, admin. orally for 7 consecutive days, had an activity of 100%.

As far as could be seen, the sexual activity of the treated groups did not show any change in comparison with that of the controls.

The table clearly shows that:

—The number of parturitions per group of 20 pairs was not significantly influenced by the treatment. In fact, considering that virgin female mice were used, variations of the order of 25% could still have been regarded as normal. The variations actually found between the different groups in our experiments ranged from 0 to 15%.

—The average number of newborn per pair in the different groups did not show any significant variation.

—The average number of days elapsing between mating and parturition indicates that there was a delay in fertilization in the groups in which the males had been treated. The differences between the average numbers of days recorded in the treated groups and the controls were as follows:

13.6 days, when the males were treated for 10 days with 50 mg/kg daily.
11.8 days, when the males and females were treated for 10 days with 50 mg/kg daily.
27.8 days, when the males were treated for 10 days with 100 mg/kg daily.
16.4 days, when the males and females were treated for 10 days with 100 mg/kg daily.

—In the groups in which only the females were treated the average number of days elapsing between mating and parturition was not much different from that recorded in non-treated controls; similarly, there was little variation

TABLE 1.

Groups (20 pairs each)	Dose (mg./kg. daily by mouth)	Duration of treatment (days)	No. of parturitions per group of 20 pairs	Average no. of days elapsing between mating and parturition	Average no. of newborn per pair	Comments
Group 1 Controls	–	–	20	25.6	11.1	1 female died after parturition
Group 2 Females only treated	50	10	20	32.9	13.0	–
Group 3 Females only treated	100	10	19	33.5	10.0	1 female died before parturition. 3 non-treated males died
Group 4 Males only treated	50	10	20	39.2	12.2	1 male died
Group 5 Males only treated	100	10	19	53.4	12.4	1 non-treated female died before parturition
Group 6 Males and females treated	50	10	20	37.4	11.6	1 male died
Group 7 Males and females treated	100	10	17	42.0	10.5	1 female died before parturition. 1 male died before fertilization was possible *

* The remaining pair was the only unproductive one observed over a period of 3 months.

between the groups in which only the males were treated and those in which both males and females were treated.

—Qualitatively speaking, the newborn in the treated groups did not differ from those in the non-treated groups.

—The mortality rate among the 280 adult mice employed (140 males and 140 females) was as follows:

2/60 males and 2/60 females = 4/120 = 3.3% of the *non-treated animals.*
2/80 males and 2/80 females = 4/160 = 2.5% of the *treated animals.*

In order to discover *whether the milk of females treated during lactation might have a toxic effect on the young,* we isolated non-treated dams together with their newborn.

We then formed 4 groups, each containing 5 dams with their young, and recorded the mortality rate among the young when the dams were treated as follows:

Group 1: Untreated controls.
Group 2: 50 mg/kg by mouth daily for 10 consecutive days.
Group 3: 75 mg/kg by mouth daily for 7 consecutive days.
Group 4: 100 mg/kg by mouth daily for 7 consecutive days.

Results.

(Cf. Table 2.)

TABLE 2.

Groups of 5 female mice + young	Dose (mg./kg. daily)	Duration of treatment (days)	Total no. of young before treatment	Total no. of young after treatment	Mortality rate among the young
Group 1 controls			67	62	7.5%
Group 2	50	10	53	52	1.9%
Group 3	75	7	40	38	5 %
Group 4	100	7	71	69	2.8%

We did not observe any difference between the young in the groups in which the dams were treated and the young in the control group.

It may be *concluded* that in the mouse the treatment of lactating dams with 100% schistosomicidal doses does not have any effect on the mortality or quality of the young.

Discussion and conclusions.

Our studies have demonstrated that spermatogenesis is inhibited by treatment with CIBA 32,644-Ba. This inhibition appears to be reversible from the functional point of view in mice treated with doses even higher than the fully effective schistosomicidal dose. The drug has no effect on the fertility of the females, although in males reproductive capacity was delayed to a certain extent. Treatment of lactating dams had no effect on the young.

Preliminary experiments seem to indicate that the drug acts directly on the germinal epithelium of the testes. There is no evidence whatever to suggest that the change in the testes is mediated by any disturbance in the production of gonadotrophins by the pituitary (20).

It appears that antiparasitic treatment with CIBA 32,644-Ba does not alter sexual function and has little if any effect on reproductive capacity. Temporary impairment of spermatogenesis does occur, but has been shown to be reversible. Incidentally, this effect explains why nitrofurazone derivatives have been investigated with the aim of finding a substance which, administered to males on an intermittent basis, could be used to control fertility.

Acknowledgement.

We wish to thank Dr. R. Hess for performing the histological studies.

Summary.

The paper reports preliminary results on inhibition of spermatogenesis in different species of animals treated with a nitrothiazole derivative, CIBA 32,644-Ba. Histological studies, as well as a functional study in mice are described; the reversibility of the phenomenon and the effect of the milk of treated dams are assessed.

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