Original Article

Tolnidamine-Induced Changes in the Testis, Sperm Count, Fertility and Accessory Sex Glands of the Laboratory Mouse

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Abstract

Background: Tolnidamine-induced changes have been reported earlier on spermatogenesis, fertility and sperm count in rat, rabbit and langur monkey. The aim of this study is to assess the response of these aspects to tolnidamine in the laboratory mouse.

Materials and Methods: Adult male mice (12-14 weeks old) of Parkes (P) strain were used in the present study. All the animals were divided into five groups. Groups I, II and V were taken as untreated, vehicle-treated initial and vehicle-treated terminal controls, respectively. Meanwhile, animals of Group III were administered with tolnidamine (100mg/kg BW, twice a week) orally for 3, 5 and 7 weeks and killed 24 hrs. after the last injection. Animals of Group IV were administered with the same dose of the tolnidamine for 7 weeks and then sacrificed 5 and 7 weeks after withdrawal of the drug. Tolnidamine-induced changes were evaluated on spermatogenesis, motility and count of epididymal spermatozoa, fertility and accessory sex glands and compared with the untreated and vehicle-treated controls.

Results: Tolnidamine treatment induced significant decrease in the weights of the testis and epididymis; however, the weights of the accessory sex glands remained unaltered following the treatment. Duration-dependent degenerative changes were noticed in the testicular germinal epithelium showing vacuolization and loosening of the germ cells and Sertoli cells. Percentage motility and count of epididymal spermatozoa declined significantly following administration of tolnidamine. Likewise fertility of the treated males as well as number of the live blastocysts in females impregnated with such males also exhibited a significant decrease when compared with the controls. However, no change was noticed in the mating ability of the mice treated with tolnidamine. The level of seminal vesicular fructose also remained unaltered after the treatment. Withdrawal studies revealed duration-dependent recovery in spermatogenesis, percentage motility and count of spermatozoa and fertility.

Conclusion: The findings of the present study, therefore, reveal that tonidamine administration in P mice induces reversible inhibition of spermatogenesis, motility and count of spermatozoa and fertility without affecting the androgen-dependent parameters.

Keywords: Fertility, Mouse, Permatogenesis, Testis, Tolnidamine

Introduction

There are number of Indazole Carboxylic Acid derivatives which have been reported to possess antispermatogenic activity in several mammalian species (1-16). Some recently developed analogues of Indazole Carboxylic Acid derivatives have proved their efficacy and reversibility of spermatogenesis in rat (10, 11, 16) and have demonstrated their potential use as oral contraceptive for men. Tolnidamine (AF 1923), 1-(4-chloro-2-methyl-benzyl)-1H-indazole-3 carboxylic acid, one of the analogues of such derivative has been reported to induce marked impairment of seminiferous epithelium in rat (17), rabbit (18) and langur monkey (19). In some species, for example, in rabbit (18) administration of tolnidamine induces reversible inhibition of spermatogenic activity while the treatment in rat (17) and langur monkey (19) fails to induce reversibility of spermatogenesis. Administration of tolnidamine causes significant reductions in the testicular weight and epididymal sperm count in rat (17, 20, 21) and rabbit (18). Fertility in rat (17) and rabbit (18) is also impaired following treatment with tolnidamine. However, the treatment is failed to alter the functions of accessory sex glands in langur monkey (19).

From the foregoing, it is noticed that the effects of tolnidamine on the male reproductive organs have been studied in various mammalian species. However, in view of conflicting reports regarding reversibility of spermatogenesis and scanty reports on the effects of

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this derivative in the mouse, in the present study an attempt has been made to investigate the response of the male reproductive organs and fertility of the laboratory mouse to tolnidamine.

Materials and Methods

Forty (12-14 weeks old) male mice of the Parkes (P) strain were used in the present investigation. The animals were housed under standard laboratory conditions and maintained on the pelleted diet (Hindustan Lever Limited, Ghaziabad) and water ad libitum. After recording the initial body weights, the animals were divided into five groups. Group I, II and V comprised of 5 animals each while group III and IV contained 15 and 10 animals, respectively. The animals were treated as following:

Group I: Untreated controls

Group II: Vehicle-treated controls (initial)

Group III: Administration of tolnidamine (100 mg/kg B.W., twice a week) for 3 (Group III a), 5 (Group III b) and 7 (Group III c) weeks. Animals were killed 24hrs. after the last injection.

Group IV: Administration of tolnidamine (100 mg/kg B.W., twice a week) for 7 weeks followed by killing of the animals 5 (Group IV a) and 7 (Group IV b) weeks after withdrawal of the drug

Group V: Vehicle-treated controls (terminal)

Tolnidamine was dissolved in 0.25% methyl cellulose and administered orally. Animals of Group II and V were injected with solvent alone. Mice of Groups I and II were killed with that of Group III c while those of Group V were killed with that of Group IV b. After recording the final body weights, the animals were sacrificed under ether anaesthesia. The testes, epididymes and accessory sex glands viz., seminal vesicle, preputial gland and Cowper's gland were dissected out, blotted free of blood and weighed. The testes of one side of 5 animals were fixed in Bouin's fluid for histological studies while the seminal vesicles of the same side were frozen for estimation of fructose (22).

Bouin's fixed testes were dehydrated, embedded in paraffin, cut the sections at 6µ and stained with Periodic Acid Schiff's reagent followed by counterstaining with Harris Haematoxylin.

Cauda epididymides from the other side of the control and treated animals were minced in 0.9% normal saline and observed the sperm motility immediately under the light microscope. Sperm count in the cauda epididymides was measured according to WHO laboratory manual (23).

Fertility of the mice was tested in each group. Each male was caged with two proestrus females for overnight and according to the presence of vaginal plug and implantation sites in females, the mating ability and fertility of the males were assessed, respectively. The mated females were killed at mid- pregnancy and counted the number of live and resorbed blastocysts.

All the data were analyzed statistically by one way ANOVA followed by Newman- Keul's Test except that the fertility of the males and pregnancies in the females for which Chi- square test was used. Student's T test was used for analysis of the body weight and number of live and resorbed blastocysts. Values were considered significant at $p<0.05$.

Results

Body weight

No significant change was noticed in the body weight of the treated animals as compared with the controls (Table 1).

Organs Weight

Weights of the testis, epididymis and accessory sex glands of the vehicle-treated controls were comparable with that of the untreated controls. In the animals killed 3, 5 and 7 weeks after treatment with tolnidamine, significant decrease was noticed in the weights of the testis and epididymis as compared with the controls. However, by 5 to 7 weeks after withdrawal of the drug treatment, a significant increase was noticed in the weights of the testis and epididymis reaching to the values of terminal vehicle-treated control.

Table 1: Effect of Tolnidamine on body weight and weights of testis, epididymis and accessory sex glands

Group and Duration of the treatment	Body weight (g)		Sex Organs Weight (mg/100 gm Body Weight)				
Group	Initial	Final	Testis	Epididymis	Seminal Vesicle	Preputial Gland	Cowper's Gland
I, Untreated Controls	35.32±0.4	38.31±0.6	245.88±3.62	89.44±1.40	237.10±6.00	38.8±1.47	25.62±1.75
II, Vehicle- treated Controls (Initial)	34.22±0.41	38.22±0.61	242.10±4.05	87.40±3.35	237.44±6.11	37.52±2.92	25.94±1.57
III[a], 3 weeks	31.05±0.43	33.31±0.83	160.64±6.25[a]	72.22±2.59[a]	241.81±3.39	40.44±o.46	27.18±0.34
III[b], 5 weeks	33.83±1.12	38.05±1.51	156.18±7.27[a]	61.60±4.90[a]	236.06±8.78	39.32±2.58	26.18±2.23
III[c], 7 weeks	34.11±1.82	37.82±1.81	124.22±6.72[a]	64.92±3.49[a]	238.3±10.58	39.78±4.71	25.0±2.24
IV[a*], 5 weeks	34.05±0.44	37.63±0.74	210.32±3.09[a]	80.56±1.33[a]	243.88±2.84	40.7±1.32	25.74±1.63
IV[b,*] 7 weeks	33.08±0.45	38.11±0.52	253.74±5.01	93.48±4.05	249.00±2.51	40.76±0.76	26.8±2.18
V Vehicle- treated Controls (Terminal)	34.26±0.42	39.41±0.92	254.12±3.22	92.52±4.24	248.06±6.06	41.60±0.73	28.00±1.11

Values are Mean±SD of five animals.

[*]Animals were treated with the drug for 7 weeks and then killed 5 and 7 weeks after withdrawal of the treatment

[a]: significantly different from controls ($p < 0.05$) by ANOVA followed by Newman-Keul's multiple range test.

Table 2: Effect of Tolnidamine on the concentration of seminal vesicular fructose

Group and duration of the treatment	*Concentration of seminal vesicular fructose (µg/100mg tissue wt.)*
I, Untreated Controls	239.86 ±7.68
II, Vehicle-treated Controls (Initial)	234.00 ± 3.73
III [a], 3 weeks	234.18 ± 8.93
III [b], 5 weeks	232.64 ± 8.09
III[c], 7 weeks	233.68 ± 6.98
IV [a],* 5 weeks	231.90 ± 8.53
IV [b],* 7 weeks	242.28±10.27
V, Vehicle-treated Controls (Terminal)	240.16± 7.73

Values are Mean ±SD of five animals
****Animals were treated with the drug for 7 weeks and then killed 5 and 7 weeks after withdrawal of the treatment***

Table 3: Effect of Tolnidamine on the Motility and Count of Spermatozoa in the Cauda epididymidis

Group and Duration of the treatment	*Sperm Motility (%)*	*Sperm Count (06)*
I, Untreated Controls	*77.00 ± 2.60*	*10.10 ±0 .99*
II, Vehicle- treated Controls (Initial)	*76.40 ± 2.57*	*11.22 ± 1.36*
III a 3 weeks	*15.61 ± 1.85a*	*6.28±1.43a*
III b 5 weeks	*6.02 ± 1.41a*	*3.78±0.88a*
III c 7 weeks	*4.40 ± 1.01a*	*3.72±1.60a*
IV a 5 weeks*	*50.81 ± 2.13a*	*8.14±2.16a*
IV b 7 weeks*	*61.81± 4.62a*	*10.22±1.68*
V, Vehicle- treated Controls (Terminal)	*71.01± 1.09*	*10.78±0.51*

Values are Mean ± SD of five animals
**** Animals were treated with the drug for 7 weeks and then killed 5 and 7 weeks after withdrawal of the treatment***
a Significantly different from controls (p<0.05) by ANOVA followed by Newman-Keul's test

Table 4: Effect of Tolnidamine on Fertility of Male Mice and on the Number of Live and Resorbed Blastocysts in impregnated Females

Group and Duration of the treatment	*Number of Males*			*Number of females*			*Number of live blasto-cysts observed in uteri of females*	*Number of resorbed blastocysts*
	Tested	*Mated*	*Fertile*	*Tested*	*Mated*	*Pregn*		
I, Untreated Controls	*5*	*5*	*5*	*10*	*10*	*10*	*8.91±0.53*	*Nil*
II, Vehicle- treated Controls (Initial)	*5*	*5*	*5*	*10*	*09*	*09*	*7.92±0.52*	*0.08±0.08*
III a, 3 weeks	*5*	*5*	*4*	*10*	*10*	*08*	*5.72 ±1.42`*	*0.35±0.20*
III b, 5 weeks	*5*	*5*	*3*	*10*	*09*	*05*	*2.48±0.81 b*	*0.14±0.09*
III c, 7 weeks	*5*	*4*	*1a*	*10*	*08*	*02a*	*1.17±0.64 b*	*0.16±0.12*
*IV a *5 weeks*	*5*	*5*	*4*	*10*	*10*	*07*	*6.49±1.11*	*0.58±0.32*
*IV b *7weeks*	*5*	*5*	*4*	*10*	*10*	*08*	*6.25±1.32*	*1.08±0.51*
V Vehicle- treated Controls (terminal)	*5*	*5*	*5*	*10*	*10*	*10*	*8.21±0.51*	*Nil*

Values are Mean ± SD of five animals
**** Animals were treated with the drug for 7 weeks and then killed 5 and 7 weeks after withdrawal of the treatment a Significantly different from controls by using Chi square Test.***
b Significantly different from controls by the Student's T Test

However, no significant alterations were noticed in the weights of accessory sex glands viz. seminal vesicle, preputial gland and Cowper's gland in the treated or in drug-withdrawal groups (Table 1).

Chemical Analysis

The concentration of fructose in the seminal vesicle of the drug-treated mice did not exhibit significant alterations as compared with the controls (Table 2).

Sperm Analyses

Sperm motility and count remained unaffected in the vehicle-treated controls when compared with the untreated controls. However significant reductions were noticed in the motility and count of spermatozoa in the cauda epididymides of all the tolnidamine-treated mice compared to the controls. By 5 to 7 weeks after withdrawal of the treatment, these parameters returned to control values (Table 3).

Mating performance

Administration of tolnidamine did not induce any alteration in the mating ability of the treated males (Table 4) as evidenced by presence of vaginal plugs in all the co-

habited females.

Fertility performance
Fertility of the vehicle-treated control was comparable to that of the untreated control. However a significant decrease was noticed in the fertility of the males which were treated with tolnidamine for 7 weeks. In this group only one male was found fertile out of five tested. Withdrawal of the treatment induced recovery in fertility of all the males (Table 4).

Number of live blastocysts
Number of live blastocysts in females impregnated by 3 and 5 weeks drug- treated males did not show significant change compared to the controls. However, in the females impregnated by 7 weeks treated males the number of live blastocysts declined significantly. Duration-dependent increase was noticed in the number of live blastocysts in females impregnated with the drug-withdrawal males (Table 4).

Number of resorbed blastocysts
No significant changes could be noticed in the number of resorbed blastocysts in the females impregnated by the drug treated males as compared with the controls. However the females in which impregnations were by 5 to 7 weeks drug-withdrawal males, exhibited a slight increase in the number of resorbed blastocysts but the values were not significantly different from the control (Table 4).

Histological studies
Histological examination of the testis in the vehicle-treated controls revealed normal features comparable with that of the untreated controls (Fig 1).

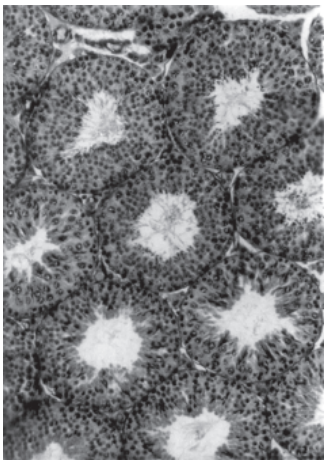

Fig 1: Testis of an untreated control (Group I) to show full spermatogenic activity in the seminiferous tubules×108

Administration of tolnidamine induced duration-dependent regressive changes in the seminiferous tubules. Three weeks after treatment, focal degeneration of seminiferous tubules was noticed (Fig 2).

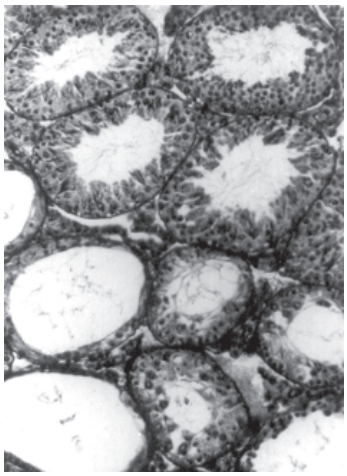

Fig 2: Testis of an animal killed 3 weeks after treatment with tolnidamine (Group III a 100 mg/kg BW, twice a week, for 3 weeks). Note focal degeneration of seminiferous tubules. Some of the seminiferous tubules appear completely degenerated while others appear unaffected cf. Fig 1×108

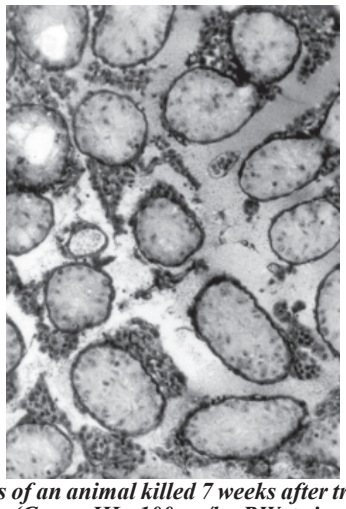

Fig 3: Testis of an animal killed 7 weeks after treatment with tolnidamine (Group IIIc-100mg/kg BW, twice a week, for 7 weeks). Note severe regressive changes in majority of the seminiferous tubules with thinning of the tunica propria, loss of germ cells and loosening of the germ and Sertoli cells. cf. Figs. 1 and 2×108

In general, three categories of the seminiferous tubules could be noticed in sections of the testes of the treated animals. The tubules of category I exhibited normal histological features while the tubules of category II and III exhibited partial and severe regressive changes, respectively. The normal tubules exhibited successive stages of transformation from spermatogonia to spermatozoa while the partially regressed tubules were devoid of spermatids and spermatozoa and consisted mainly of spermatogonial cells, primary and secondary spermatocytes and Sertoli cells. Sertoli cells and most of the germ cells appeared vacuolated and detached from each other. Exfoliation of germ cells was also evident in the seminiferous tubules in the testis of the treated mice. In severely degenerated tubules, all the germ cells were lost except

that the presence of spermatogonial cells, few primary spermatocytes and vacuolated Sertoli cells. Intraepithelial vacuolization and loosening of the germ cells were frequently observed. Severity of regressive changes in the seminiferous tubules in the testis was progressed markedly with increased duration of the treatment as noticed in the mice killed 7 weeks after the treatment (Fig 3). In mice individual difference in response of the testis to administration of tolnidamine were noticed remarkably. In contrast to severe regressive changes in the seminiferous tubules, morphology of the Leydig cells appeared to be unaffected in the treated mice. Withdrawal of the drug treatment induced duration-dependent recovery in the affected seminiferous tubules. In mice killed 7 weeks after withdrawal of the treatment, majority of the tubules exhibited recovery in spermatogenic activity (Fig 4).

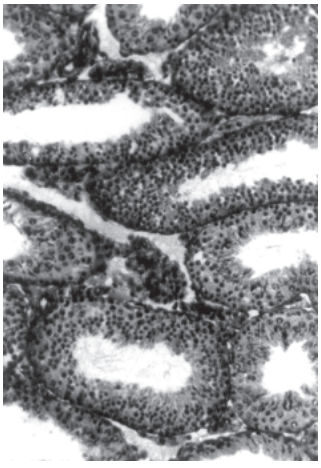

Fig 4: Testis of an animal treated with tolnidamine (Group IV b -100 mg/kg BW, twice a week, for 7 weeks) followed by sacrificing 7 weeks after withdrawal of the drug. Note recovery in spermatogenic activity in majority of the seminiferous tubules cf. Fig1 and 3×108

Discussion

The result reported in the present study indicates that administration of tolnidamine in P mice does not alter the body weight till the end of the treatment. This is consistent with the finding reported in the rat (6), rabbit (18) and langur monkey (19). A significant decrease in the testicular weight after treatment with tolnidamine in P mice, as noticed in the present study has also been reported in the rat (6, 16, 20). In the present study, marked regressive changes were noticed in the seminiferous tubules resulting in the suppression of spermatogenic activity. Consistent findings are reported in the rat (6, 17, 21) and rabbit (18). In contrast to reversible inhibitory effects of tolnidamine on spermatogenic activity as noticed in P mice in the present study, it is reported that daily administration of tolnidamine (30 or 120 mg/kg BW) up to 8 weeks induced irreversible suppression of spermatogenic activity in rat and even by 25 weeks after withdrawal of the drug, no sign of recovery could be noticed in the affected seminiferous tubules (17). Likewise tolnidamine administration in the rabbit (18) at the dose of 50 mg/kg BW/day induced irreversible inhibition of sperm production and fertility was reduced to zero after 150 days of the treatment and at 90 and 150 days after cessation of the treatment. However, in the present study, recovery in spermatogenic activity was evident only 7 weeks after withdrawal of the drug. The discrepancy between the present study and that of the above may be due to daily administration of the drug by later authors (17, 18) while in the present study, the drug was given only twice/week. However, reversible inhibition of spermatogenic activity has been reported in the rat following administration of few recently developed analogues of indazole carboxylic acid derivatives (10, 11, 15).

In the present study, a significant decrease has been noticed in the weight of the epididymis following treatment with tolnidamine. Consistent finding is reported in the rat (17, 21, 24). The result of the present study further indicates a significant decrease in the epididymal sperm count in tolnidamine-treated mice. This is consistent with the finding reported in the rabbit (18) and langur monkey (19). It is well known that weights of the testis and epididymis are associated with spermatozoa content (25, 26). In the present study significant decrease in the weights of the testis and epididymis induced by administration of tolnidamine; therefore, it is due to spermatogenic inhibition leading to a decrease in sperm count.

Tolnidamine administration in the P mice fails to induce significant alteration in the weights of the accessory sex glands. This is consistent with the findings reported in the rat (6) and langur monkey (19). Similar to the findings reported in the rabbit (18) and langur monkey (19), the mating ability of tolnidamine-treated mice in the present study also remained unaltered when allowed to mate with virgin female mice. However, fertility of the treated males and the number of live blastocysts in females mated with such males, both declined markedly. This may be due to significant decrease in the count and or /in the motility of epididymal spermatozoa following administration of the drug. This possibility is supported in the present finding that an increase in the sperm count and motility following withdrawal of the drug led to recovery in the fertility of the treated males and in the number of live blastocysts in females derived from such males. Recovery in fertility following administration of some new analogs of Indazole Carboxylic acid derivatives have also been reported in the rat (11).

Some recently developed analogs of Indazole carboxylic acid derivative i.e.1-(2,4-dichlorobenzyl)-1H-indazole-3-carbohydrazide and 1-(2,4-dichlorobenzyl)-indazole-3-acrylic acid have been reported to exert antispermatogenic activity in rat by depleting germ cells prematurely from the seminiferous epithelium (9, 10) without affecting the hypothalamo-pituitary-testicular axis. These analogs have been reported to induce germ cells loss from the seminiferous epithelium by disrupting cell adhesion between Sertoli and germ cells particularly spermatids and spermatocytes (15). In the present study,

loss of germ cells, loosening of the remaining germ cells and vacuolization of Sertoli cells in the regressed seminiferous tubules indicate the possibility of direct action of the tolnidamine on the spermatogenic activity. In the present study no significant changes could be noticed in the androgen-dependent parameters such as the weights of the accessory sex glands and the level of seminal vesicular fructose; further morphology of the Leydig cells and mating ability also appeared unaffected supporting the possibility of an unaltered hypothalamo-pituitary-testicular axis and circulating androgen in the drug-treated mice.

Conclusion

The present study, therefore, reveals that oral administration of tolnidamine in P mice induces reversible inhibition of spermatogenic activity and fertility without affecting the androgen status and the body weight.

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