HISTOLOGICAL DETERMINATION OF SITE OF ANTIFERTILITY ACTION OF SULFASALAZINE IN MALE ALBINO RATS

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Summary

In the present study effect of sulfasalazine on reproductive functions and fertility of male rats was investigated. Oral administration of sulfasalazine (500mg./kg.b.wt. /day) for 60 days did not cause any effect on body weight. The weight of testes and epididymides were reduced slight significantly ($P<0.05$), while the weight of seminal vesicle and ventral prostate remained statistically unchanged. A marked decrease ($P<0.001$), in sperm count and motility was observed in sperm collected from the cauda epididymis of treated animals. The histological picture of testis is almost normal where as we see lesser sperms in the epididymal lumen,and the epithelial lining also shows degenerative changes. The fertility rate also declined significantly. These effects were reversible after 60 days of recovery period. Thus, the above mentioned results indicate that sulfasalazine induces reversible antifertilty effects in male rats and the possible site of action is epididymis.

Keywords:sulfasalazine,epididymis histology, infertility, male rats

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Introduction

Sulfasalazine is a sulfonamide drug, which belongs to the class of drugs called the sulfa drugs. It inhibits bacterial growth by interfering with metabolic process that require p-aminobenzoic acid (PABA). Therefore it has been used for many years for the treatment of ulcerative colitis. (1). Ever since a positive correlation was established between sulfasalazine and antifertility in male, a lot of studies have been undertaken to comprehend the exact mechanism by which it does so (2,3,4,5). However, no precise mechanism has been suggested till date and therefore the drug continues to be enigmatic. Sulfasalazine given orally is metabolized into 5-aminosalicylate and sulfapyridine (6). It is the sulfapyridine moiety and not 5-aminosalicylate which is responsible for suppressed fertility. (4,7) .The present study is an extension of earlier works where we have tried to study the long term (60 days) effect of sulfasalazine at higher dose level (500 mg/kg.b.wt.) especially on testes and epididymis histology so as to obtain a histological insight into exact site of sulfasalazine action.

Materials and Methods

Test Chemical

Sulfasalazine was procured from the market. (Brand name Sazo marketed by Wallace,India.)

Animals

Colony bred adult healthy male albino rats of Wistar strain, weighing 160-200 g were used in the study. The rats were housed in polypropylene cages under standard husbandry conditions (12 hrs light/dark cycle: 25 ± 3°C). Rats were provided water and pellet diet *ad libitum*. The Institutional Ethical Committee for animal care approved the study.

Experimental Design

Male rats of proven fertility were divided into three groups of 5 rats each.

Group I: Rats served as control and received the vehicle (0.5 ml corn oil /day/rat) for 60 days.

Group II: Rats were administered orally Sulfasalazine (500mg/kg b.wt. /day) suspended in corn oil for 60 days.

Group III: Rats were administered orally Sulfasalazine (500mg/kg b.wt. /day) suspended in corn oil for 60 days. They were then left for 60 days of recovery.

On day 61,all the animals of group I and II were sacrificed, however animals of group III were left for another sixty days of recovery and sacrificed at the end of it. The testes and accessory sex organs were dissected out, cleared and weighed. The bodyweight of each animal was recorded initially and then at the time of autopsy.

Fertility Test

The mating test was performed during last five days of the treatment. The male rats were cohabited with pro-estrus females in the ratio of 1: 2 respectively. The presence of vaginal plug and sperm in the vaginal smear in the next morning were considered the index for positive mating. The mated females were separated and allowed to deliver after full term.

Sperm Analysis

Sperm density and motility of cauda epididymal spermatozoa were evaluated by the method of Prasad et al. (1972)(8).

Histological Analysis

Testes and epididymides were fixed in Bouin's fluid. Paraffin sections were cut (5 µm) and stained with hematoxylin and eosin. Mean tubular diameter was determined by measuring 100 round sections of seminiferous tubules with the help of ocular micrometer. Diameters of Leydig cells nuclei were measured at X 800.

Statistical Analysis: - Data were expressed as mean ±SEM and the significance of difference was analyzed by the Student's 't'-test.

Results

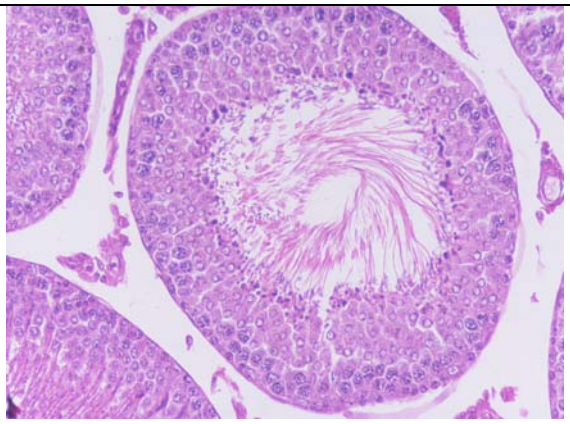

Fig (1a)

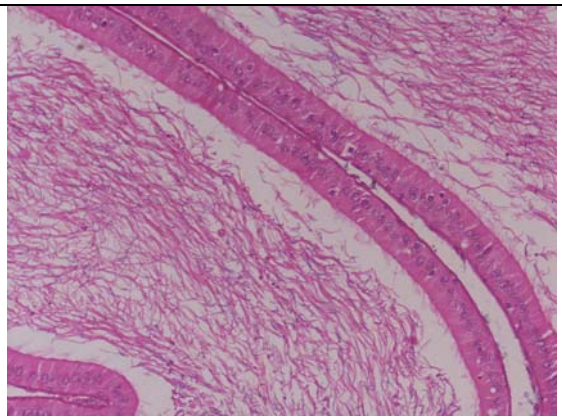

Fig (2a)

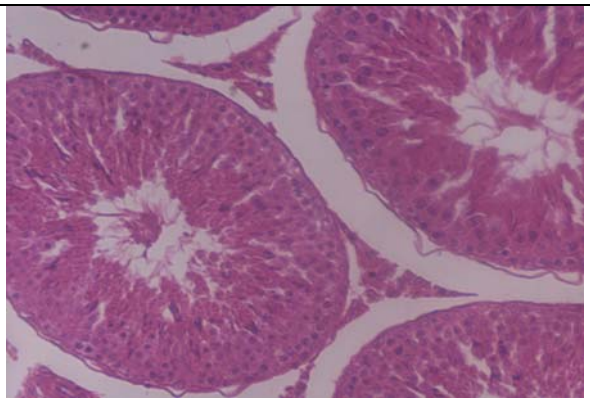

Fig (1b)

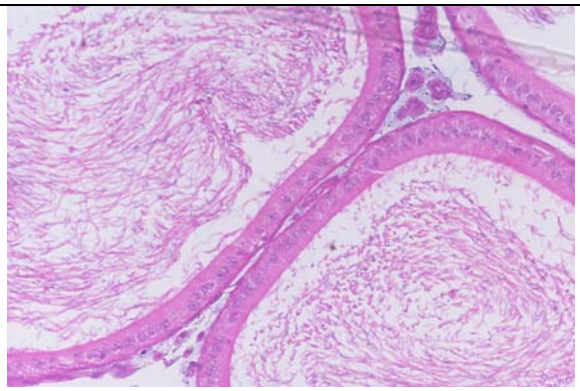

Fig (2b)

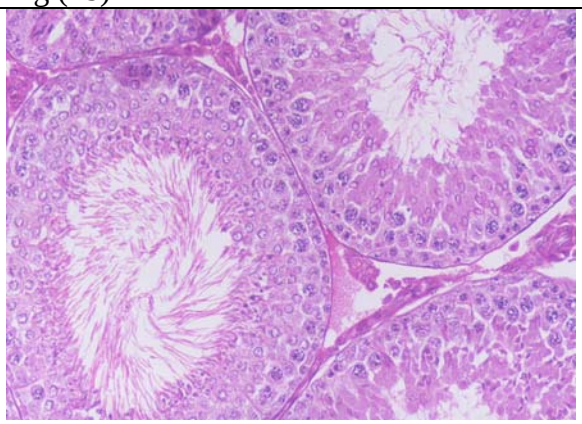

Fig (1c)

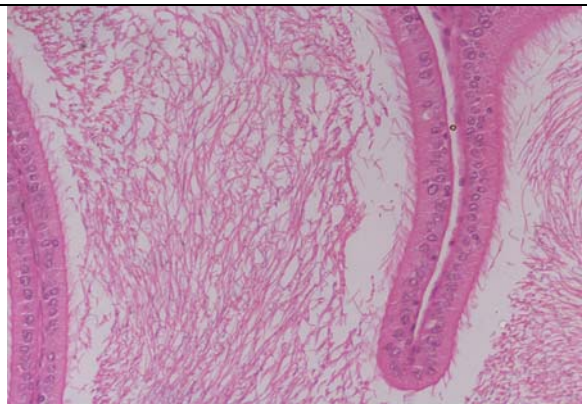

Fig (2c)

Fig (1) Cross section of testes (a), control rat (b) Sulfasalazine treated rat (c), recovery group (after 60 days of drug withdrawl)
Fig (2) Cross section of cauda epididymis (a), control rat, (b) Sulfasalazine treated rat (c), recovery group (after 60 days of drug withdrawl)

Table I:Effect of sulfasalazine treatment on body and organ

Group (n= 5)	Body weight (gm)		Organ weight (mg/100 g b.wt.)			
	Initial	Final	Testis	Epididymis	Seminal vesicle	Ventral prostate
GroupI Control, Vehicle treated)	195± 4.47	209±5.09	1238± 56.54	540±10.61	680± 39.62	325±18.18
Group II (Sulfasalazine treated)	166± 2.71	176.8±2.60	1067± 38.67*****	468 ± 20.98*****	587± 54.67	296± 8.91
Group III (recovery group)	176± 2.91	194.6±4.38$^{@}$	1162± 27.0	508± 30.2	635± 33.24	316±19.64

@ P<0.01 compared to initial body weight **Value are mean ±SEM (n=5)**

- **P<0.05 compared to control**

Table II:Effect of Sulfasalazine treatment on cauda epididymal spermatozoa number and motility and fertility test in rat

Treatment	**Cauda epididymis**		**Fertility test(%)**
	Sperm count (million/ml)	**Motility (%)**	
Group I Control (vehicle treated)	47.6±2.29	77.0±2.72	100
Group II Sulfasalazine treated)	26.0±2.44***	43.0±4.44***	50
Group III (recovery group)	42.4±3.62	69.45±3.16	90

Levels of significance

*** P<0.001 when compared with control

Table III: Seminiferous tubule & Leydig cell nuclear diameter

Treatment	Seminiferous tubule diameter (μm)	Leydig cell nuclear diameter (μm)
Group I (Contol,vehicle treated	285±13.19	6.94±0.17
GroupII(sulfasalazine treated}	255.8±8.65	6.52±0.26
GroupIII(recovery group)	264.0±8.57	6.78±0.16

Discussion

The present study reinforces the view that sulfasalazine suppresses fertility in male rats. (3,4,9,10). Sulfasalazine treatment did not cause any significant change in the body weight as has also been reported by the previous workers. (3,9). (Table 1) There was a mild significant decrease in the testicular weight. (Table 1) Histological picture of the testis (fig.1b) reveals an almost normal architecture with only a few seminiferous tubules showing fewer numbers of spermatozoa, indicating a mild, if at all, impairment of spermatogenesis. An earlier study suggests that kinetics of spermatogenesis is not disturbed by sulfasalazine treatment, however alterations in sperm release were observed. (11) A recent finding also suggest that a few genes controlling spermatogenesis are inhibited by sulfasalazine (12) No significant changes were observed in either the seminiferous tubule or the leydig cell nuclear diameter (Table 3) which suggests that testosterone synthesis is not hampered by sulfasalazine. (9) Besides, the weight of seminal vesicles and ventral prostate, which is testosterone dependent (13), was also comparable with their normal counterparts, (Table 1) further lending support to our view that testosterone synthesis is not inhibited by sulfasalazine. Previous workers also held a similar opinion. (14)

The most significant changes were observed in the epididymides. There was a significant decline ($p<0.05$) in the epididymal weight), (Table 1) which is consistent with the previous findings. (10,11) We observed highly significant decline ($p<0.001$) in the epididymal sperm counts, motility, and fertility. (Table 2) Many previous workers also observed a similar decline in these parameters. (4,9,10,11,14) Histological study of the epididymis of sulfasalazine treated rat can explain all these effects (fig.2b) as we can see that not only has the epithelial cell height been reduced but it also exhibits degenerative changes and the lumen contains sperm debris. This seems to have reduced the secretory activity of the epididymal epithelium, thereby altering the milieu of the epididymal lumen, preventing maturation of spermatozoa. Earlier finding observed alteration in the normal profile and lesions on the sperm head. (9) In a recent study it was found that acrosome related genes CD59,membrane cofactor protein(MCP) and decay accelerating factor (DAF) in the epididymides of the sulfasalazine treated rats were significantly decreased which led to the hypothesis that the suppression of epididymal acrosome membrane proteins synthesis, with their consequent reduced incorporation to the sperm membrane leads to a depressed sperm motility and acrosome

reaction, leading to infertility in sulfasalazine treated rats. (15) These workers observed attenuated epididymal gene expression of CD59 and DAF as early as day 1. (16) Some earlier workers are of the view that sulfasalazine induces antifertility by inhibition of prostaglandin metabolism. (17)

All the above mentioned changes in the sperm count, motility and fertility were reversed after a recovery period of sixty days. (Table 2) Normal histological picture of the testis and epididymis (fig.1c, 2c) of the recovery group further supports this. Earlier workers have also reported such reversibility. (7,18,19,20)

We can therefore conclude that epididymis is the main site of antifertility action of sulfasalazine, where it seems to disrupt the secretory functions of epithelium, besides acting directly on the sperms leading to abnormal spermatozoa and depressed sperm counts resulting in impaired fertility.

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