

GENTAMICIN INDUCED INHIBITION OF STEROIDOGENIC ENZYMES IN RAT TESTIS

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Abstract : Gentamicin is an aminoglycoside antibiotic, widely used for treating many gram negative bacterial infections. Though nephrotoxicity is the most highlighted side effect, it has also been found to cause an alteration in the phosphatase activities of testes and accessory sex organs and a decline in the sperm count. This study was designed to assess the effects of gentamicin on testicular steroidogenesis and to ascertain whether such alterations are reversible. Laboratory inbred adult, male, 'Wistar' strain rats were chosen as the experimental animal. A significant dose-dependant reduction in the activities of the two steroidogenic enzymes, accompanied with a significant decrease in ascorbic acid and elevation of level of cholesterol was observed. The effects were maximum at a dose of 100 mg/kg, b.wt. After 15 days of withdrawal of the drug therapy the biochemical parameters namely ascorbic acid and cholesterol returned to normal levels whereas the activities of the two dehydrogenases showed a compensatory increase. This indicates that gentamicin affects the steroidogenic enzymes, causing an alteration in the formation of testosterone, which was manifested in the elevated cholesterol in the adult rat testes. However, these alterations were reversible.

Key words : gentamicin steroidogenesis ascorbic acid
hydroxysteroid dehydrogenase sperm count
cholesterol

INTRODUCTION

Gentamicin, has been reported to cause various side effects in the recipient (1). The most prevalent adverse effect is nephrotoxicity followed by ototoxicity (2).

Aminoglycosides also affect the arachidonic acid metabolism in rats (3) which, in turn, is known to be involved in the hypothalamic control of ovulation (4). An alteration of testicular functions in patients with type II hyperlipoproteinemia, treated with

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neomycin, another aminoglycoside antibiotic, has been reported (5). In male rats, gentamicin influences the phosphatase activities of the reproductive organs and affects sperm count (6). This suggests that this antibiotic may cause adverse effects on testes and other accessory reproductive organs.

The present study was designed to assess the possible effects of different doses of gentamicin on testicular steroidogenesis and to ascertain whether these changes are permanent or reversible.

METHODS

Adult laboratory inbred male Wistar rats, of body weight 180–200 gms, were used. Animals were maintained in well ventilated room with 12 hours light/dark cycle and diet, prepared in the laboratory (7). After 7 days of acclimatization, they were divided into groups, of 10 animals each.

Group I served as control and received an appropriate volume of the vehicle (sterile water) injected intramuscularly. Group II–IV animals were administered intramuscularly with gentamicin sulphate, dissolved in sterile water at doses of 40, 60, 80 and 100 mg/kg respectively for 7 days, everyday between 10.30 am and 11.30 am. Group VI received a similar treatment as group V, ie gentamicin 100 mg/kg but were given an additional recovery period of 15 days after the 7 day drug treatment. At the end of the drug treatment, animals of all groups excepting those of group VI were fasted overnight and sacrificed by decapitation from 7-00 to 11-00 hours to

avoid any possible diurnal variation. At the end of a 15-day recovery period, animals of group VI were also sacrificed in a similar fashion.

Subsequently testes were dissected out, adhering blood and tissue fluid blotted clean and a portion homogenised in cold, 0.2 M Sucrose buffer at 4°C, to assay the activities of 3 β hydroxysteroid dehydrogenase and 17 β hydroxysteroid dehydrogenase (8). Rest of the tissue was frozen immediately at -20°C for analysis of ascorbic acid (9) and cholesterol (10), spectrophotometrically.

All results were expressed as Means \pm SEM and were statistically analyzed using student's 't' test. $P < 0.05$ was taken to be statistically significant.

RESULTS

Gentamicin treatment significantly reduced the activities of the two steroidogenic enzymes namely 3 β hydroxysteroid dehydrogenase (3 β HSD) and 17 β hydroxy steroid dehydrogenase (17 β HSD) in a dose dependant fashion (Table I). A significant increase in cholesterol level [$P < 0.05$] was also observed in the group IV & V animals receiving 80 & 100 mg/kg gentamicin, respectively while, concentration of ascorbic acid decreased significantly [$P < 0.05$].

On the other hand, 15 days after withdrawing the drug, in the group V animals, the two steroidogenic enzymes showed a significant elevation, (Table II) as compared to the normal control as well as drug treated rats, receiving 100 mg/kg b.wt [$P < 0.05$]. The ascorbic acid and cholesterol levels, however, reverted to a near normal

TABLE I : Effect of gentamicin sulphate at different doses on 17 β hydroxysteroid dehydrogenase, 3 β hydroxysteroid dehydrogenase activities, ascorbic acid and cholesterol contents in the adult male rats [Means \pm SEM; n=10/group]

Treatment mg/kg b.wt.	17 β hydroxysteroid dehydrogenase (U/gm tissue)	3 β hydroxysteroid dehydrogenase (U/gm tissue)	Ascorbic acid (mg/gm tissue)	Cholesterol (mg/gm tissue)
Control	151.71 \pm 8.21	167.35 \pm 6.50	185.3 \pm 6.64	2.44 \pm 0.16
Gentamicin 40 mg/kg b.wt.	150.5 \pm 7.35 [†]	167.0 \pm 6.0 [†]	180.5 \pm 7.60 [†]	2.43 \pm 0.16 [†]
60 mg/kg b.wt.	145.75 \pm 5.65 [†]	165.2 \pm 7.50 [†]	175.5 \pm 5.50 [†]	2.40 \pm 0.15 [†]
80 mg/kg b.wt.	100.5 \pm 7.5 [*]	90.50 \pm 6.75 [*]	110.35 \pm 7.45 [*]	2.55 \pm 0.17 [*]
100 mg/kg b.wt.	95.49 \pm 6.50 [#]	88.22 \pm 5.76 [#]	90.23 \pm 8.21 [#]	2.82 \pm 0.18 [#]

*P<0.05 experimental group as compared to the control

†P = NS

#P<0.001 experimental group as compared to the control

TABLE II : Effect of gentamicin sulphate (100 mg kg⁻¹ f.wt.im) on 17 β hydroxysteroid dehydrogenase, 3 β hydroxysteroid dehydrogenase activities, ascorbic acid and cholesterol contents in the adult male rats and the withdrawal effect after 15 days [Means \pm SEM; n=10/group]

Parameters	Control Group	Gentamicin Group	Withdrawal Group
17 β hydroxysteroid dehydrogenase	151.71 \pm 8.21	95.49 \pm 6.50 [*]	249.09 \pm 8.86 ^{**@}
3 β hydroxysteroid dehydrogenase	167.35 \pm 4.07	88.22 \pm 5.76 [*]	206.9 \pm 2.97 ^{**@}
Ascorbic acid (mg./gm tissue)	185.32 \pm 6.64	90.23 \pm 8.21 [*]	180.21 \pm 6.51 ^{**}
Cholesterol (mg./gm tissue)	2.44 \pm 0.16	2.82 \pm 0.18 [*]	2.48 \pm 0.21 ^{**}

*P<0.05 gentamicin group compared to the control group

**P<0.05 withdrawal group compared to the gentamicin group

@P<0.05 withdrawal group compared to the control group

value, after 15 days of recovery from the drug therapy.

DISCUSSION

The steroidogenesis in testes is under physiological control of two dehydrogenases. A constant supply of ascorbic acid and cholesterol is also required in the testes for the formation of the steroid hormones. Both the enzymes are directly involved in biosynthesis of testosterone from pregnenolone. Any alteration in the activity of these two enzymes reflects on the androgen production. Reduced activities of these steroidogenic enzymes in mature testes of adult rats indicate reduced steroidogenesis (11).

Role of cholesterol (12) as precursor molecule in the synthesis of steroid hormones is well established. In this study, cholesterol content in the testes of experimental group of rats showed significant increase as compared to the normal control. This high accumulation of cholesterol may suggest the non utilization of lipid towards testosterone biosynthesis and thereby corroborates gentamicin induced reduced steroidogenesis.

Ascorbic acid, an easily diffusible water soluble biological reductant, is found in abundance in testes (13), where it plays an important role in testicular hormonogenesis (14). Induction of stress on a living body alters ascorbic acid concentration in

metabolically active tissues as well as in tissues with steroidogenic potentiality. Its deficiency leads to aspermiogenesis along with elevation of cholesterol. In the present study, it is evident that gentamicin causes elevation of testicular cholesterol alongwith impairment of spermatogenesis accompanied by a significant reduction in ascorbic acid. Gentamicin induced mobilization of ascorbic acid from the testis may also lead to decreased reproductive potentiality.

All the parameters showed alteration in a dose dependant fashion, 40 and 60 mg/kg being the least effective in causing an alteration of the steroidogenic enzymes as well as the levels of cholesterol and ascorbic acid whereas 80 mg/kg gentamicin caused significant alteration and 100 mg/kg caused highly significant alteration.

After 15 days interval for recovery from the drug treatment at a dose of 100 mg/kg, ascorbic acid and cholesterol content reverted to normal or near normal levels. However, the activities of the two steroidogenic enzymes showed a significant elevations compared to the normal control as well as compared to the drug treated group of rats. This may probably be explained on the basis of fact that withdrawal from gentamicin causes a compensatory rise in the activities of these two enzymes.

Therefore it can be concluded that a short term treatment with gentamicin for 7 days at different doses of 40, 60, 80 and 100 mg/kg tends to lower the activities of the two steroidogenic enzymes in a dose dependant fashion. However, all these alterations are reversible.

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