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REVERSIBLE PLASMA TESTOSTERONE LEVELS REDUCTION AFTER GENTAMICIN ADMINISTRATION AND FREUND'S ADJUVANT ARTHRITIS IN RATS

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Abstract : The present study was undertaken in order to investigate the influence of gentamicin on plasma testosterone levels of healthy and with Freund's adjuvant arthritis rats. Gentamicin (40 mg/day for 4 days) induced significant decrease of testosterone levels in comparison with the control group ($P<0.025$). Intraperitoneal calcium administration (30 mg/kg bw) prevented gentamicin effect and maintained testosterone levels to that of the control. Decreased testosterone levels were also observed in gentamicin received Freund's adjuvant arthritic rats, in the acute stage of the inflammatory disease ($P<0.025$), and in the acute stage of Freund's adjuvant arthritis ($P<0.001$). It is concluded that the administration of gentamicin decreases plasma testosterone levels without any effect on body and seminal vesicles weight. Calcium loading counteracts gentamicin reducing effect on plasma testosterone levels. Freund's adjuvant arthritis influences the function of body and seminal vesicles as it was shown by the reduction of testosterone levels, body and seminal vesicles weight during the acute phase of the inflammatory disease. In any case the effect was reversible.

Key words : gentamicin calcium rats testosterone Freund's adjuvant arthritis seminal vesicles weight

INTRODUCTION

Gentamicin (G), an aminoglycoside antibiotic, besides its well-known side effects, has been implicated in inhibition of steroidogenic enzymes in rat testis, a decline in the sperm count of Wistar rats (1) and the activity of calcium dependent phosphatidylinositol phospho-diesterase (2). Gentamicin as a cationic substance seems

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to interact with Ca^{++} binding to lipid monolayers and to biomembranes (3, 4). Several *in vitro* and *in vivo* with different experimental models studies show the capacity of gentamicin and other aminoglycosides to block various calcium channels (5–7) and the reversal of the aminoglycosides toxic effects by calcium coadministration (8, 9).

On the other hand Freund's adjuvant arthritis (FAA) has been connected with reduced testosterone levels in rats (10–12). Reduced testosterone levels were also reported in males with rheumatoid arthritis (13–14).

The target of our study was firstly, to detect and evaluate any possible interaction of gentamicin on plasma testosterone levels of healthy and with FAA rats, secondly to investigate the role of calcium ions in simultaneous with gentamicin administration on rat testosterone levels.

METHODS

Adult male Wistar rats (300–350, bw/g) were used. Animals were maintained in well ventilated room (12 h light/dark cycle-food and water ad libitum), and acclimated for one week before use. (Guide to the Care and Use of experimental Animals) (15). Two series of experiments were taken place. In the first series rats into groups of 10 animals were treated as follows: Group (I) the control one received 0.1 ml intradermally of the Complete Freund's Adjuvant vehicle (paraffin oil) in the paw of the right back foot (day 0). The same group received on the 20th day of the experiment till the 24th an injection/12 h of 0.5 ml of sterile water i.m. (vehicle of gentamicin). Group (II) received an i.m. injection of gentamicin sulfate (20 mg/12 h) for 4 consecutive days between 20th–24th day of the experiment. Group (III) received an injection of 0.1 ml Complete Freund's Adjuvant (CFA) (M. butyricum in paraffin oil, 5 mg/ml) in the paw of the right back foot and the same treatment of gentamicin as group (II). Group (IV) received only the CFA on the day 0. Group (V) received 20 mg/12 h of gentamicin i.m. only on the 34th day of the experiment, and Group (VI) was treated as group (IV) but was sacrificed on the same day as Group (V), on the 35th day of the experiment.

Freund's adjuvant arthritis (FAA) was provoked by the injection of CFA in the paw of the right back foot of the rats. The immune response (clinical evaluation) was started to manifest 24–48 h after the CFA injection (day 0) reaching its peak at the end of the 3rd week (acute phase of FAA) where signs of inflammation of all the extremities, testes tail, hyperalgesia, lack of mobility and decreased body weight were observed. Thymus and spleen were also affected. The inflammatory reaction started to fade away (or progressively decreasing) in the mid of the 4th week (regression phase).

In the second series of the experiment, 30 rats in 3 groups (10 rats/group) were treated as follows: Group A received an i.m. injection of NaCl 0.9% for 7 days (control group), group B an i.m. injection of 80 mg/kg of gentamicin sulphate for the same period and group C an intraperitoneal (i.p) injection of calcium chloride (30 mg/kg) just before the i.m. injection of gentamicin for 7 days as well. For the optimum dose of calcium chloride a preliminary study was

performed. According to Niemczyk S et al (1991) the i.p. administration of 45 mg/kg of calcium prevented the nephrotoxicity of gentamicin in the rat (16). We observed that 45 mg/ kg was toxic for our strain and 15 mg/kg too low to counteract the gentamicin effect. The dose of 30 mg/kg was the right one.

Animals were weighed the first day of the experiment, during the procedure and few minutes before sacrifice. Sacrifice of the rats was taken place by decapitation 12 h after the last injection from 8 am – 11 am to avoid any possible diurnal variation (12). Blood was collected and the seminal vesicle weight was also measured. Testosterone (T, whole plasma T) determinations were performed by specific radioimmunassay kit of Serono Diagnostics SA. The results of the first series were expressed as means±SEM and were statistically analysed using student's t-Test, where $P<0.05$ was considered to be statistically significant. The results of the second series were expressed by the statistical method analysis of variance.

RESULTS

First series of experiments:

Gentamicin administration for 4 days (20–24th, group II) induced a statistically significant decrease of plasma testosterone levels in comparison with the control (group I) ($P<0.025$), while the administration of gentamicin in 2 doses one day before sacrifice does not seem to change substantially plasma testosterone levels ($P>0.1$) although there is a tendency to decrease (group V) (Table I).

TABLE I

Group	*Body weight*	*Testosterone levels (ng/ml)*	*SVW (mg)*
I (control)	364±3.1	4.50±0.75	1445.7±78.3
II	364.5±5.3	1.42±0.42*	1450±81.0
III	283±5.3	1.51±0.26*	728.2±67.9**
IV	271.2±10.8	1.22±0.31*	713.7±91.0**
V	328.9±7.9	2.86±1.07	1400.1±85.6
VI	243.0±6.9	4.14±1.42	1360.0±95.0

*P<0.05; **P<0.001.

Decreased testosterone levels were also observed in the case of gentamicin administration (20–24th day) in healthy rats and in rats with FAA in the acute stage of the inflammatory disease ($P<0.025$) (group III). The lower plasma testosterone levels were observed in the group of FAA in the acute stage of the inflammatory disease (group IV) in comparison with the control (group I) ($P<0.001$). Testosterone levels reached again normal values in the regression phase of FAA (35th day of the experiment) in group VI.

Reduced body and seminal vesicles weight was observed only in the groups with FAA (III, IV & VI) in comparison with the controls (Table I, figure 1) ($P<0.0001$).

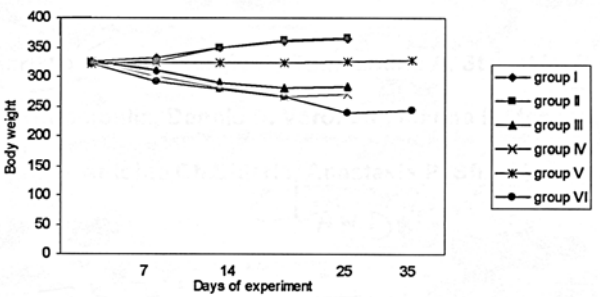

Fig. 1: The effects of gentamicin administration on the body weight of control and FAA Wistar rats.

Second series of the experiments:

In this case gentamicin (group B) significantly reduced rat testosterone levels in comparison with the control (group A) P<0.001. The i.p. calcium coadministration prevented gentamicin lowering effect on rat testosterone levels (group C) P<0.002 (comparison between group B and C) (Table II).

TABLE II

Group	*Body weight*	*Testosterone levels (ng/ml)*	*SVW (mg)*
1 (control)	350±39	3.23±0.814	1400±58.4
2	353±37	1.33±0.57**	1410±45.3
3	351±45	3.09±0.13**	1395±48.5

**P<0.001; **P<0.002.

DISCUSSION

We have examined the effect of gentamicin on testosterone levels in: healthy, with FAA rats and the role of calcium as an antagonistic agent on testosterone reducing effect of gentamicin. The overall analysis of our data revealed that gentamicin significantly reduces rat testosterone levels. In addition, the effect of gentamicin on plasma testosterone levels also appears dose-dependent and reversible as it was shown by the lower gentamicin administration (in dose and duration) in our study (group V) (Table I). Gentamicin as a cationic substance seems to interact with Ca^{++} binding to lipid monolayers and to biomembranes (3). A number of studies show the capacity of gentamicin and other aminoglycosides to block various calcium channels (6, 7, 17). Calcium plays a major role in the excitation-secretion coupling of neurotransmitters and hormones (18). It is possible that gentamicin blocks testosterone synthesis either interfering with the activity of the relative steroidogenic enzymes (1), or inhibiting testosterone release in the blood as it happens with the acetylcholine release, via calcium antagonism in the aminoglycosides induced neuromuscular blochade (19, 20, 21). The impregnation of gentamicin in testosterone pellet implants, in humans, although not significant resulted in 20% reduction of the testosterone extrusion rate (Kelleher S et al) (22). The chronic use of calcium channel blockers (diltiazem and cinnarizine) caused a marked decrease in circulating blood levels of testosterone in male rats (23). Furthermore, calcium in simultaneous with gentamicin administration reduces aminoglycosides nephrotoxicity (9, 16) or ototoxicity (8, 24), while calcium channel antagonists exacerbate gentamicin nephrotoxicity (25).

We proved that calcium loading also prevented gentamicin reducing effect and left unaffected testosterone synthesis or release as it was manifested by the normal testosterone levels in the group of the combined treatment (calcium chloride+ gentamicin) (Table II).

Freund's adjuvant arthritis provoked a more significant decrease of rat testosterone levels in the acute phase of this inflammatory disease (14–24 days from day 0). Similar observations are reported by Barbier et al (1985) and Bruot & Clements (1987) (10, 11) although there are some differences in the time of major testosterone reduction, possibly due to different rat strain and methodological procedures. Reduced

serum testosterone levels were also reported by some investigators in patients with rheumatoid arthritis (26, 13, 14, 27). According to Gordon et al (1986) data (in man), Bruot and Clemens (1987) (FAA in rats) an impaired hypothalamic-pituitary axis is not responsible for the reduced testosterone levels found in the FA arthritic rats. Freund's adjuvant arthritis is a good model of experimental arthritis in the rat. The fact that there is a parallel reduction of testosterone levels and seminal vesicles weight only in the FAA rats in the acute phase of the inflammation, suggests that the inflammatory process is responsible for both reductions. The above is supported by the recovery of testosterone levels in the regression phase of the FAA by an increase of the seminal vesicles weight.

In conclusion, gentamicin induces a dose dependent reduction of plasma testosterone levels in healthy rats. This reduction is reversible and could be prevented by simultaneous calcium administration, proving that gentamicin effect on rat testosterone levels is due to calcium antagonism. Our study also indicates that FAA (during the acute phase) in the rat provokes a parallel and reversible reduction of plasma testosterone levels, body and seminal vesicles weight. Gentamicin administration does not seem to additionally influence any of the above examined parameters in rats with FAA.

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