EFFECT OF GENTAMICIN ON DEVELOPMENT OF RABBIT TESTICULAR TISSUE IN NEW BORN AND ADULTS

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ABSTRACT

Objective*: Aminoglycosides comprisea family of antibiotics which are used frequently to treat gram- negative bacterial infections. Among them gentamicin is the most common choice for treatment of urinary tract infections. The present study aimed to investigate histological and histomorphometrical changes induced by gentamicin administration during different stages of pregnancy in newborn and adult rabbits.*

Materials and methods*: Forty Albino rabbits around 5- months old (32 female, 8 male) weighing 1.8-2 kg were randomly divided into control (n=10) and experimental (n=30) groups. The experimental groups were subdivided into three groups which received 1.7 mg/kg (Intramuscular injection) gentamicin every 8 hours in (group P) Pre differentiation stage, (group E) Embryonic stage and (group F) Fetal stage. Three day-old newborns and adult rabbits were sacrificed and the testis was expelled out. After sample preparing and staining, morphological changes were assessed. The diameter of the seminiferous tubules and the thickness of germinal epithelium was evaluated using mage tools III Microsoft software.*

Statistical analyzes *was made by One-Way ANOVA followed by Turkey post hoc test to evaluate the statistical significance between different groups. A value of $p<0.05$ was considered statistically significant.*

Results*: The results revealed that gentamicin administration in the pre-differentiation(P) and embryonic(E) stages induced early lumination of testicular cords in 3day- old newborns. A significant increase in diameter of seminiferous tubules and germinal epithelium thickness in groups P and E was also observed ($p<0.05$). In adult rabbits, diameter of seminiferous tubules significantly increased in groups E and F and decreased in group P ($p<0.05$). Germinal epithelium thickness in groups P and E reduced significantly whereas in group F this reduction was not significant.*

Conclusion*: Gentamicin administration in pregnancy induces adverse effects on testicular development and results in early lumination of testicular cords in newborns and reduction in germinal epithelium thickness in adult rabbits.*

Key words*: Gentamicin, Seminiferous tubules, Germinal epithelium, newborn and adult rabbits.*

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Introduction

It has been proved that antibiotics can damage developing fetal organs during pregnancy. Placenta is permeable to the most of the antibiotics. For example gentamicin and cephalotin preserve in amniotic fluid and embryonic tissues in high concentration. It is worthy to note that permeability of placenta to various antibiotics varies during pregnancy. As it is reduced to Gentamicin in the last days of the pregnancy but increased to Cephalotin[(1)].

Gentamicin is an aminoglycoside derived from Micomonosporapurpurea[(2)]. It has a bactericidal effect on gram-negative microorganisms[(3)]. It is quickly absorbed after intra muscular injection and mainly diffused in extracellular fluid (peak serum levels usually reached within 30 to 90 minutes)[(4)] and it accumulates in the renal proximal convoluted

tubules (50 to 100 times more than that in serum)[5]. Although gentamicin passes through the placenta and is excreted in breast milk, it is not absorbed from the gastrointestinal tract.Gentamicin concentration in umbilical cord blood and the placenta reaches 50% and 8% of mother's blood serum respectively, after 1-2 hours of intra muscular injection[6, 7].

Nowadays gentamicin is commonly used as a choice drug for treatment of kidney and urinary tract infections in pregnant women because of its bactericidal efficacy and low price as well as low bacterial resistance[8].

On the other hand, aminoglycosides such as streptomycin sulfate are predominantly used in embryonic culture, sperm wash and cryopreservation media in order to reduce bacterial infections[9]. Various studies have evaluated the safety of these drugs for therapeutic use[10] but some other studies have reported that they can considerably damage testicular structures and impair their function and result in apoptosis in testicular cells[11-13].

Most of investigations concerning the reproductive toxicity of gentamicin focused on the biochemical semen analysis[13-17] and to our knowledge, structural and histological studies of the testis are insufficient[18].

Since gentamicin passes the placenta during pregnancy, it may cause adverse effects on different organs such as the testis in the developing embryo. Therefore, the present study was designed to evaluate the effects of gentamicin on development of rabbit testicular tissue in new born and adults.

Materials and methods

The current study was an interventional experimental research. 40 Albino white rabbits (32 female, 8 male) of 5 months old and weighing 1.8-2 kg were obtained from animal facility of Pasteur institute of Iran. All animal experiments were supportedby the guidelines of the Iranian Council for Use and Care of Animals and were accepted by the Animal Research Ethical Committee of Tehran University of Medical Sciences. The rabbits werehoused individually in temperature controlled rooms (25C°) under 12-h light (07.00- 19.00)/12-h dark (19.00-07.00) cycle with free access to drinking water and standard commercial rabbit chow for one month prior to the experiment.

Experimental design

After one month of acclimation, the animals were randomly divided into 4 groups (8 females and 2 males in each group). In all groups, males separated from females after mating. In group 1 (C: Control group) animals received no injection. In the experimental groups 1.7mg/kg gentamicin sulfate[7] (Vial: 20mg/ml, Batch No: 115, Alborzdaru, Iran) was injected in gluteus muscles bilaterally every 8 hours as follow: In Group 2 (P: Pre differentiation stage) from days 1to 8 after mating. In Group 3 (E: Embryonic) from days 9 to 16 of pregnancy and finally in Group 4 (F: Fetal) from days 20 to 27 of pregnancy. It was used the guide for the Care and Use of Laboratory Animals published by the NIH (National Institute of Health) for the experimental procedures[19].

Tissue collection and processing

Samples were collected at two times: A) in 3-day-old rabbit newborns; and B) in adult Rabbits (8 months-old). In both instances, animals were anesthetized with sodium thiopental (0.5g, Batch No: 7083, SPECIA Company, France). The peritoneal cavity was opened by a transverse abdominal incision and the testes were quickly removed. At the end of the surgical procedure, the animals were sacrificed by an over dose injectingof sodium thiopental. The samples were fixed in 10% formalin[20], dehydrated through graded ethanol, and cleared in xylene. Tissues were infiltrated and embedded in paraffin. Finally five-micron thick sections were prepared and stained with hematoxylin and eosin (H&E) for light microscopic examination (Olympus, Model: CX31, Japan)[21].

For the quantitative study, we randomly selected 30 transverse sections of seminiferous tubules per animal from the four groups. Seminiferous tubules diameter and thickness of germinal epithelium measured using Image tools III Microsoft software.

Statistical analysis

Statistical analysis was performed using SPSS-16 software and one-way ANOVA followed by Tukey's post hoc test to evaluate the statistical significance among different groups. The data were expressed as mean ± SD. A value of $p<0.05$ was considered statistically significant.

Results

Morphologically, by light microscopic observations, the testicular tissue and seminiferous tubules of 3-day-old newborn rabbits in the group C appeared normal. Testicular cords were still solid without lumen (Fig. 1-C). As the photomicrographs in Figure 1 show, the lumen of the seminiferous tubules of 3-day-old newborn in groups P and E was completely defined and the diameter of the tubules have significantly increased (P<0.05) (Fig.1-P, Fig. 1-E, Fig. 2). Whereas, there was no morphological difference between groups F and group C, except expanded interstitial spaces (Fig. 1-F, Fig. 2).

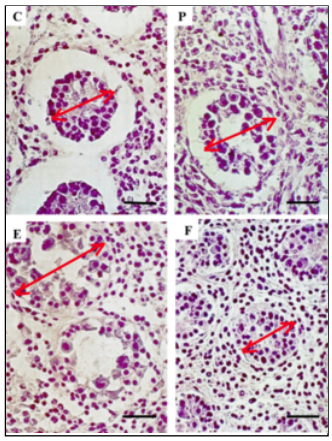

Figure 1: Comparative photomicrographs which show seminiferous cords and tubules of 3-day-old newborn rabbits in different groups: C: shows seminiferous tubules in control group without lumination. A distinct lumen is observed in P and E (Pre-differentiation and Embryonic groups). In photomicrograph F (Fetal group) there are no changes compared with control group. Arrows show the diameter of seminiferous tubules. In comparison with the control group, in group P and E seminiferous tubule diameter was significantly increased, whereas no changes were seen in group F. (H & E, 400X). Scale bar: 10µm.

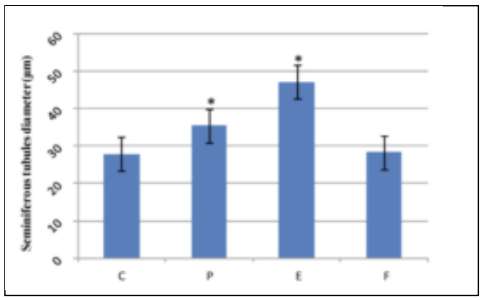

Figure 2: The diameter of seminiferous cords and tubules of 3-day- old newborn rabbitsin different groups: (C) Control, (P, E, F) animals which received gentamicin during the Pre-differentiation, Embryonic and Fetal stages of pregnancy respectively. It was increased significantly in groups P and E (*P<0.05).

Histological study in adult rabbits showed that the cycle of spermatogenesis was regular in the control group. The data demonstrated seminiferous tubules with normal size, function and spermatogenesis in group C. Sperm bundles were seen inside the lumen of seminiferous tubules (Fig. 3-C).

In group P the shape and diameter of seminiferous tubules were different from those in control group. Results showed that the number of undeveloped seminiferous tubules in group P was increased (Figs 3-P, 5-P).

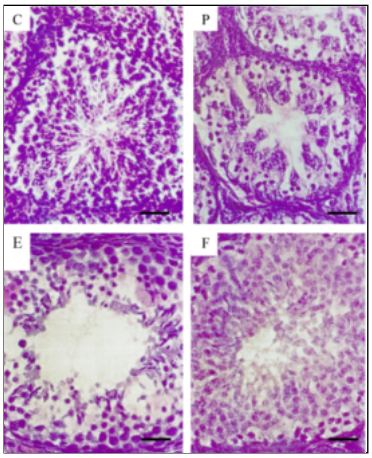

Figure 3: Comparative photomicrographs which show seminiferous tubules of adult rabbits among different groups: C: shows seminiferous tubules in control group with normal size, function and spermatogenesis. P: shows seminiferous tubules in Pre-differentiation group. E: shows significant increase in diameter of seminiferous tubules in group E compared to control group. F: Fetal group with no changes in the seminiferous tubules in comparison with control group. (H & E staining, 400X). Scale bar: 20µm.

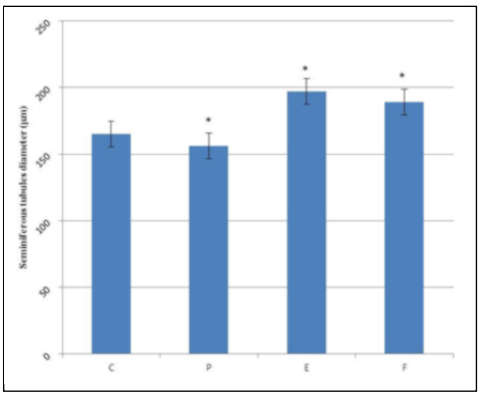

Figure 4: Seminiferous tubules diameter of adult rabbits in different groups:(C) Control, (P, E, F) animals which received gentamicin during Pre-differentiation, Embryonic and Fetal stages of pregnancy respectively. It was significantly increased in groups E& F and decreased in group P (*P<0.05).

In quantitative examinations, the diameter of the seminiferous tubules in group E was significantly increased and their growth was more complete compared to control group(P<0.05) (Fig. 3-E, Fig

4). The shape of their lumen was also changed from the normal polyhedral to oval shape (Fig. 5E).

No changes were indicated between groups F and C regarding the seminiferous tubules profiles and they remained in the form of normal polyhedral shape (Fig. 5-F, 5-C).

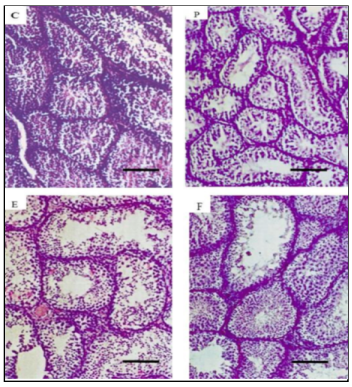

Figure 5: A: photomicrograph of sections of the testis of adult rabbits in different groups. C: control. Groups P, E, F: gentamicin administered during Pre-differentiation, Embryonic and Fetal stages respectively. As photomicrographs show seminiferous tubules in groups C and F have polyhedral shape but in two other groups it changed from normal polyhedral to oval shape. (H & E, 100X). Scale bar: 100µm.

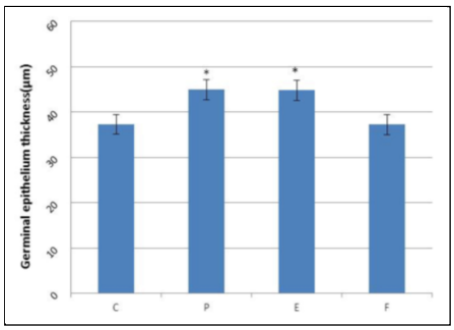

Figure 6: Germinal epithelium thickness of seminiferous tubules in 3-day old rabbits in different groups: (C) Control, (P, E, F) animals which received gentamicin during Pre-differentiation, Embryonic and Fetal stages of pregnancy respectively. Whereas there was no significant changes in group F, it was increased significantly in groups P & E compared to control group (*P<0.05).

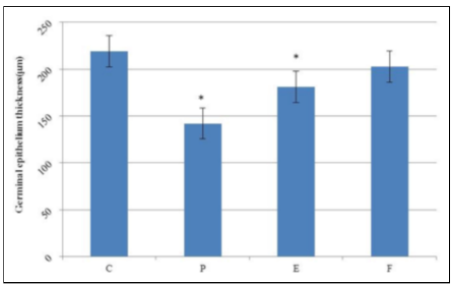

Figure 7: Germinal epithelium thickness of seminiferous tubules in adult rabbits in different groups :(C) Control, (P, E, F) animals which received gentamicin during Pre-differentiation, Embryonic and Fetal stages of pregnancy respectively. Whereas there was no significant change in group F, it was decreased significantly in groups P&E compared to control group (*P<0.05).

Moreover, the quantitative data demonstrated a significant increase in germinal epithelium thickness in groups P and E in 3-day-old rabbits (P<0.05) (Fig. 6).

However in adult rabbits, germinal epithelium thickness was reduced significantly compared with-that in the control group (P<0.05) (Fig.7).

Discussion

Gentamicin as an aminoglycoside antibiotic is widely used in clinic for treatment of pyelonephritis, urinary tract and gram negative bacterial infections. It has also effect against some gram positive organisms, e.g. Staphylococcus. Administration of this antibiotic in pregnancy especially during organogenesis may result in structural anomalies in embryo[4, 22].

In this study, teratogenic effect of gentamicin on testicular cords of 3-day- old newborn rabbits and adult rabbits during different stages of prenatal development were assessed. According to our results, administration of this antibiotic in the pre differentiation and embryonic periods resulted in early lumination of testicular cords and early maturity in rabbit newborns. Also, the other results of studies on newborn rabbits showed increase in diameter of seminiferous tubules and germinal epithelium thickness in groups P and E. Seminiferous tubules appeared with irregular and sloughing of germinal epithelium into the lumen. It may be due to the defect of cellular junctions. It should be noted that because of organogenesis, these two mentioned stages are the most sensitive times to teratogenic effects.

On the other hand in adult groups, diameter of seminiferous tubules in groups E and F significantly increased but a significant reduction was observed in group P. Moreover in comparison to control group, germinal epithelium thickness in groups P & E reduced significantly whereas in group F this reduction was not significant.

Fetouh et al. reported reduction in diameters of seminiferous tubules and height of the germinal epithelium in gentamicin treated guinea pigs[18]. Their observations were consistent with our data in adult groups.

In some previous studies the effects of gentamicin, neomycin, streptomycin, ofloxacin and ciprofloxacin were compared. Those studies showed that these aminoglycosides had adverse effect on sperm parameters and testicular tissue in rats and

caused apoptosis in testicular germ cells[13, 20].

In another research, the effects of different dose of gentamicin in adult rats were studied. They found dose dependent side effects of gentamicin on sperm count, concentration and motility. Also structural changes such as epithelial sloughing and gaps, germ cell degeneration, tubular atrophy and reduction of seminiferous epithelium thickness were reported which were consistent with our findings[14].

Although there are several studies which show the influence of gentamicin on testis structure, Literatures on the structural and histomorphometric changes in newborns reproductive system in gentamicin-treated animals are infrequent.

In a recent study, researchers assessed testicular structures during different stages of postnatal development in rabbits. Testicular parenchyma comprised of solid straight cords with no lumen and abundant interstitial tissue till 8 weeks after birth. They showed that seminiferous cord lumination started after 12 weeks. According their report, animals reach sexual maturity after 16-24 weeks. At this time, increase in lumination of the seminiferous tubules was observed and spermatogenesis was completely active[23].

Whereas according to our findings, gentamicin administration during pre-differentiation stage induced early lumination in 3 day-old newborn rabbits and resulted in less growth in seminiferous tubules. Moreover, conformation changes from polyhedral to oval shape,increase in diameter of tubules and early spermatogenesis were observed in seminiferous tubules in adult rabbits who obtained gentamicin during their embryonic period (Fig 4&5). But newborns and adult rabbits in group F whose mothers received gentamicin during fetal period had no difference with control group.

In addition to the important immediate effects of antibiotics on reproductive system of mammals, it may have delayed effects too. A previous study showed that tetracycline treatment induced decrease in sperm counts and increase in oxidative stress in rat males[24]. Also recent investigations reported reduction in the thickness of seminiferous epithelium, increase the number of apoptotic germ cells in the testis of adult rats who were exposed to tetracycline during their puberty[25].These reports are consistent with our data. According to our results, there was an increase in seminiferous tubules diameter and germinal epithelium thickness in newborns whereas it decreased during puberty. This reduction in germinal epithelium may be due to delayed effects of gentamicin, which result in epithelial sloughing, cell degeneration and tubular atrophy[14].

According to the recent studies, theses toxic effects of gentamicin maybe related to induction of oxidative stress damage in the testis through increasing formation of ROS(reactive oxygen species)[26] which can result in DNA destruction, lipid peroxidation, and other cell components[27-29].

Conclusion

It could be concluded that gentamicin administration during pre- differentiation and embryonic stages of pregnancy even at therapeutic dose can have adverse effects on development of testis. Whereas, it seems safe to be used during fetal period of pregnancy. Also histomorphometric changes in testis of newborns and adult rabbits are completely different and vice versa.

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