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Original article

Effect of gentamicin on proximal convoluted tubules of kidney in developing chicks

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KEYWORDS

Gentamicin, Aminoglycoside, Teratogenic, Proximal convoluted tubules, Cystic dilatation.

ABSTRACT

Aim: To study any teratogenic effect of gentamicin as a result of single-dose injection given therapeutically or accidentally. *Materials and methods*: The study was conducted on 60 fertilized eggs of white leghorn species in the Department of Anatomy, Pt BD Sharma PGIMS, Rohtak, Haryana. The eggs were incubated for 20 days and divided into treated (n = 30) and control (n = 30) groups. Eggs of treated and control groups were injected with 0.2 mg of gentamicin sulfate and 'sterilized water for injection', respectively, on the 4th day of incubation. On the 20th day, the chick embryos were extracted and then dissected to remove the kidneys. *Results*: No effect of gentamicin was found on either the mortality of chick embryos or the gross appearance of the newborn chicks and kidney as compared to the control group. The mean weight of both the right and left kidneys was found less in the treated group, though not statistically significant. On light microscopy, various changes in proximal convoluted tubules were noticed in both the groups which included cystic dilatation and cloudy swelling. Statistical analyses showed that these changes were significantly higher in both the kidneys of treated group in comparison to the control group. *Conclusion*: Gentamicin has a teratogenic effect on proximal convoluted tubules of kidney even if administered in a single dose during the development of an embryo.

1. Introduction

Gentamicin is a well-known aminoglycoside antibiotic that was obtained from Micromonospora purpurea in 1964 as reported by Finland.[1] Gyselynck et al reported that it is not metabolized to a measurable extent and is eliminated from the body principally by renal excretion.[2]

Various side effects are reported by Regec et al such as nephrotoxicity, ototoxicity, and neurotoxicity.[3] It is estimated that up to 53% of patients treated with gentamicin suffer some form of renal damage.

Use of gentamicin is increasing during pregnancy for the following purposes:

(a) Infections by *Pseudomonas aeruginosa*, most enteric bacteria and *Staphylococcus aureus* reported by Yoshioka.[4]
(b) Maternal infections such as pyelonephritis and pneumonia reported by Weinstein et al.[5]
(c) Chorioamnionitis reported by Gilstrap et al.[6]

Because gentamicin rapidly crosses the placenta into the fetal circulation and amniotic fluid, knowledge about its teratogenic potential is essential.[4–8]

Mallié et al reported degenerative changes such as swollen cells, cloudy cytoplasm, PAS-positive inclusions, brush border atrophy, and luminal enlargement in proximal convoluted tubules in rat neonates born to pregnant Wistar rats after subcutaneous gentamicin administration at doses of

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75 mg/kg/day, 50 mg/kg/day, 25 mg/kg/day, or 7.5 mg/kg/day during the 2nd and 3rd weeks of gestation.[9]

Czeizel et al observed the teratogenicity of aminoglycoside antibiotics, including parenteral gentamicin during pregnancy, and reported congenital malformations in 0.08% among 22,865 women who received gentamicin during pregnancy.[10]

2. Material and methods

The present study was conducted in the Department of Anatomy, Pt BD Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, on 60 successfully fertilized eggs of white leghorn species. The eggs were brought in seven series. In each series, the fertilized eggs were divided into two groups:

Group I: The control group (total no. = 30).
Group II: The treated group (total no. = 30).

The prior permission was taken from the Institutional Animal Ethics Committee.

2.1 *Incubation of eggs*

The eggs were incubated in a Digital incubator manufactured by "Universal LABAID equipment" maintaining the temperature ranging between 38 ºC and 39 ºC and humidity was maintained around 85%. The eggs were kept with their broad ends up and were rotated twice daily along their longitudinal and vertical axes as advised by Olsen and Byerly.[11] After 48 hours of incubation, candling of eggs was done. Then, the unfertilized eggs were removed out of incubation. In each series, the unfertilized eggs ranged from 10% to 20% (Table 1).

The fertilized eggs were then divided into control and treated groups. Both the groups were incubated further and on the 4th day of incubation, the eggs were injected. The fertilized eggs of both the groups were then labeled using a black marker pen and were marked G for gentamicin (treated) group and C for control group. After the injection, eggs were placed back in the incubator and were allowed to remain there till the 20th day of incubation. A variable degree of mortality was noted both in control and treated groups of all the seven series as shown in Table 1.

Gentamicin was taken in the form of gentamicin sulfate, 80 mg in 2 ml ampule. The fertilized eggs of study group were injected single dose of 0.2 mg of gentamicin sulfate by a tuberculin syringe on the 4th day of incubation. The dose was calculated by recommended human dose 3–5 mg/kg bodyweight/day by Tripathi and taking the weight of a newborn chick as 40 g (normal weight 38–42 g).[12]

For the injection purpose, the following things were used:

(a) Rectified spirit
(b) Cotton
(c) Hypodermic needle for making hole
(d) Tuberculin syringe (100 divisions in 1 ml)
(e) Hypodermic needle no. 24 for injection
(f) Molten paraffin wax.

The eggs were cleaned with the cotton soaked in the rectified spirit, and then with the help of a needle (item 3), two holes were made in the egg shell. One was made at the broad end of the egg for the displacement of air, while the second hole was made at the pointed end of the egg for the purpose of injection. The injection was given by a sterilized hypodermic needle no. 24 fitted to a tuberculin syringe having 100 divisions in 1 ml. The eggs of control group were injected with a corresponding volume of 'sterilized water for injection' on the same day. The holes were then sealed with molten paraffin wax.

The eggs of both groups were again kept for incubation after injection till the 20th day of incubation. Sufficient measures were taken to maintain continuous electricity supply. On the 20th day of incubation, the eggs were taken out and their shell was broken so as to take out the chick.

Any gross malformation was recorded. Each chick was dissected after anesthetizing with chloroform. Photographs of dissected chick were taken with the help of a digital camera (Fig. 1). The kidneys were located and any gross malformation in kidneys was noted. The kidneys of both right and left sides were dissected out intact carefully. But some pairs of kidneys were damaged during dissection (Table 2). The weight of right and left kidneys of both control (n = 30) and

Table 1 – The number and distribution of eggs at various stages of experiment.

	Series I	Series II	Series III	Series IV	Series V	Series VI	Series VII	Total
Eggs kept for incubation	10	10	10	20	20	35	15	120
Number of unfertilized eggs	2	2	1	3	4	7	1	20
Number of eggs for experimental use	8	8	9	17	16	28	14	100
Grouping of eggs								
Treated	4	4	5	10	8	20	0	51
Control	4	4	4	7	8	8	14	49
Number of dead embryos								
Treated	1	2	1	3	6	1	0	14
Control	4	2	1	2	2	3	1	15
Number of living embryos								
Treated	3	2	4	7	2	19	0	37
Control	0	2	3	5	6	5	13	34

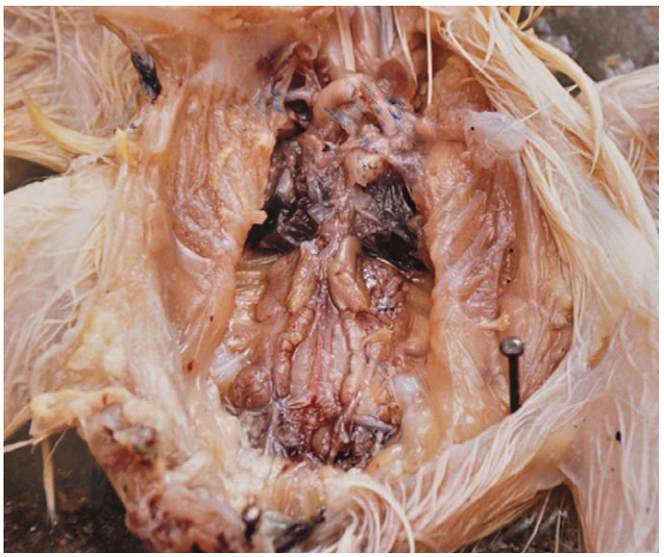

Fig. 1 – Photograph of dissected chick (magnified view).

Fig. 2 – Photograph of the right and left kidneys of control group. The kidney is an elongated organ consisting of three successive lobes (cranial, middle, and caudal) connected with each other by parenchymatous.

treated (gentamicin) groups (n = 30) was recorded separately by the Balance Analytical manufactured by MeOpta Praha, Czechoslovakia. The photographs of kidneys of both the groups were taken with the help of a digital camera (Figs. 2 and 3).

Fig. 3 – Photograph of the right and left kidneys of gentamicin group. No gross malformation was observed.

Both the right and left kidneys of each chick were kept in the 10% formalin solution in separate-labeled containers for 48 hours. The kidney was taken out of fixative and washed thoroughly in running tap water. The kidney was dehydrated in ascending series of alcohol (50%, 70%, 80%, 90%, 95%, absolute), cleared in benzene for 3 hours, and embedded in paraffin. The blocks of two groups were labeled as follows:

sGnRK or sGnLK and sCnRK or sCnLK

where s is the series in roman numerals to which the kidney belongs, G is the gentamicin (treated) group, C is the control group, n is the number of newly hatched chick in respective series to which the kidney belongs, RK is the right kidney, and LK is the left kidney.

Sections of each tissue were cut using a Rotary Microtome at 6 μm thickness. Sections from each block were then stained by hematoxylin and eosin (H&E).

Slides of both control and treated groups were then observed under a light microscope and changes due to gentamicin were noted down in proximal convoluted tubules and microphotographs were taken.

3. Results

The present study was based on observations made in 60 newborn chicks of two groups comprising 30 chicks of the control group and 30 chicks of the treated group.

Table 2 – The number of damaged pairs of kidney in each series during dissection.

	Series I	Series II	Series III	Series IV	Series V	Series VI	Series VII	Total
Number of living embryos								
Treated	3	2	4	7	2	19	0	37
Control	0	2	3	5	6	5	13	34
Number of pairs of kidneys damaged during dissection								
Treated	3	1	2	1	0	0	0	7
Control	0	2	1	1	0	0	0	4
Number of pairs of kidneys left for processing								
Treated	0	1	2	6	2	19	0	30
Control	0	0	2	4	6	5	13	30

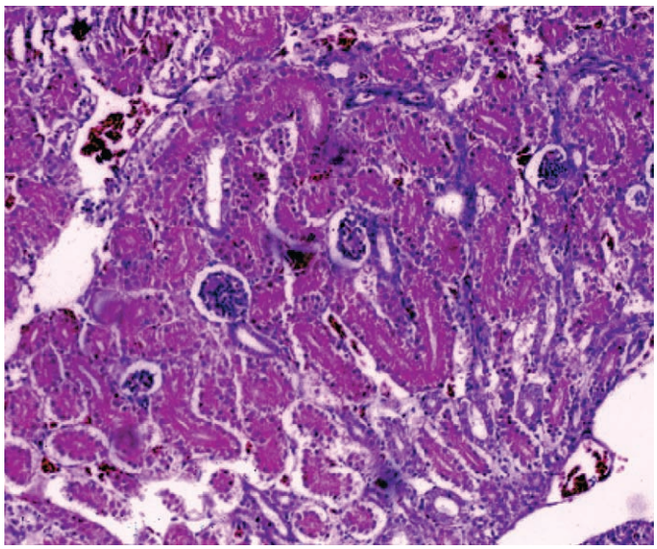

Fig. 4 – Microphotograph of kidney of control group (H&E, 40×) showing a magnified view of glomeruli, proximal convoluted tubules, and distal convoluted tubules.

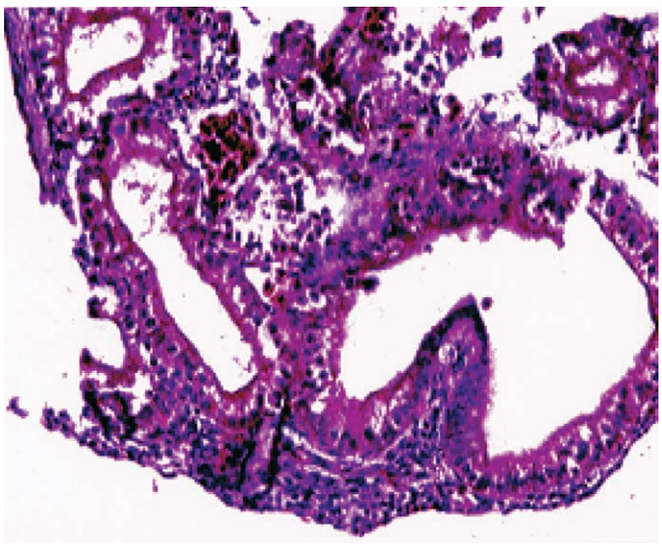

Fig. 5 – Microphotograph of kidney of treated (gentamicin) group (H&E, 10×) showing cystic dilatation of proximal convoluted tubules.

In the control group, 15 chick embryos were found dead out of total 49 eggs i.e., 30.6%, whereas in the treated (gentamicin) group, 14 chick embryos were found dead out of total 51 eggs i.e., 27.4% (Table 1).

No gross malformations were observed in chicks or kidneys of both control and treated (gentamicin) groups (Figs. 1–3).

3.1 *Effect of gentamicin on the weight of kidney of chick embryo*

The mean weight of both right and left kidneys of the control group is more than that of the treated (gentamicin) group which means that there is reduction in weight although this difference is found statistically nonsignificant on applying the unpaired t-test ($p > 0.05$). In addition to that, no significant difference was found between the weights of right and left kidneys of both control and treated (gentamicin) groups on applying the paired t-test ($p > 0.05$) (Table 3).

Table 3 – The mean and range of weight (in mg) of right and left kidneys of control and treated (gentamicin) groups.

	Range (mg)	Mean ± SD (mg)
Right kidney of control group	132–198	164.43 ± 17.97
Left kidney of control group	130–197	163.37 ± 18.61
Right kidney of treated (gentamicin) group	127–192	155.2 ± 18.44
Left kidney of treated (gentamicin) group	130–190	155.87 ± 17.3

3.2 *Effect of gentamicin on histology of kidney (proximal convoluted tubules) of chick embryo (Tables 4 and 5)*

1. Cystic dilatation: In total, this change was noticed in kidneys of 8 out of 30 chick embryos of control group (26.67%), out of which it was bilaterally found in 2 and unilaterally in right kidney in 3 and left kidney in 3 chick embryos (Table 4). On the other hand, this change was noticed in kidneys of 21 out of 30 chick embryos of treated group (70%), out of which it was bilaterally found in 8 and unilaterally in right kidney in 7 and left kidney in 6 chick embryos (Table 5 and Figs. 4 & 5).
2. Cloudy swelling or hydropic transformation: In total, out of 30 chick embryos of control group, this change was observed in kidneys of 7 chick embryos (23.33%), out of which it was bilaterally found in 1 and unilaterally in right kidney in 1 and left kidney in 5 chick embryos (Table 4). On the other hand, out of 30 chick embryos of treated group, this change was observed in kidneys of 22 chick embryos (73.33%), out of which it was bilaterally found in 12 and

Table 4 – The various changes in proximal convoluted tubules in the control group as seen by a light microscope.

		Changes in proximal convoluted tubules	
		Cystic dilatation	Cloudy swelling
Number of chick embryos showing a positive change	Bilateral	2	1
	Unilateral in right kidney	3	1
	Unilateral in left kidney	3	5
	Total	8 (26.67%)	7 (23.33%)

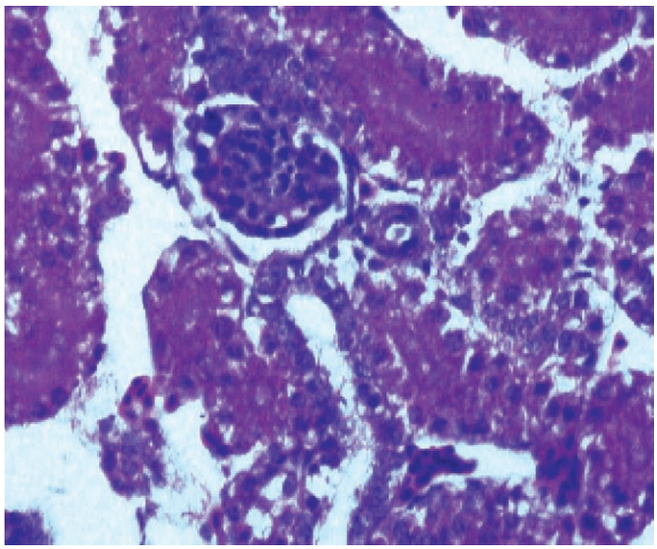

Fig. 6 – Microphotograph of kidney of treated (gentamicin) group (H&E, 40×) showing cloudy swelling of proximal convoluted tubules.

unilaterally in the right kidney in 6 and the left kidney in 4 chick embryos (Table 5 and Figs. 4 & 6).

3.3 *Statistical analysis (chi-square test) (Table 6)*

Histological changes in proximal convoluted tubules were found to be significantly higher in both right and left kidneys of treated group in comparison to those of control group.

Table 5 – The various changes in proximal convoluted tubules in the treated (gentamicin) group as seen by a light microscope.

		Cystic dilatation	Cloudy swelling
Number of chick embryos showing a positive change	Bilateral	8	12
	Unilateral in right kidney	7	6
	Unilateral in left kidney	6	4
	Total	21 (70%)	22 (73.33%)

Table 6 – The p-values related to the comparison of histological changes in the right and left kidneys of control and treated (gentamicin) groups.

		Right kidney of control vs treated (gentamicin) group	Left kidney of control vs treated (gentamicin) group
Changes in proximal convoluted tubules	Cystic dilatation	0.006*	0.012*
	Cloudy swelling	0.000*	0.007*

*Statistically significant ($p < 0.05$).

4. Discussion

The study was planned to know the teratogenic potential of gentamicin, if administered even in a single dose, to pregnant females unaware of their pregnancies especially in the first few months of pregnancy. The study was performed in chick embryos since the embryogenesis in chick is similar to human beings. Particular attention was paid to the effect of gentamicin on the mortality of chick embryos, gross appearance of newborn chick, gross appearance of kidney and its weight, and histological structure of the proximal convoluted tubules of the kidney.

4.1 *Teratogenic effect of gentamicin on the mortality of chick embryo*

No significant effect of gentamicin on the mortality of chick embryos was found when compared to the control group in the present study in a single therapeutic dose.

Lelievre-Pegorier et al found a similar mean number of dead pups per litter in both saline and gentamicin treated groups after daily injection of 4 mg/kg gentamicin to pregnant guinea pigs from days 48 to 54 of gestation.[13]

Mallié et al also found a similar mean number of dead neonates per litter in both control and gentamicin groups after subcutaneous gentamicin administration to pregnant Wistar rats at doses of 7.5 mg/kg/day, 25 mg/kg/day, 50 mg/kg/day, or 75 mg/kg/day during the 2nd and 3rd weeks of gestation.[9]

Similarly, no effect of gentamicin on the mortality of neonates was observed by Smaoui et al[14] after intraperitoneal administration of 75 mg/kg/day gentamicin to pregnant Wistar rats on days 7–11 and 14–18 of gestation.

Hence, all findings in the available literature regarding the teratogenic effect of gentamicin in multiple doses on the mortality of neonates matched with our findings in a single therapeutic dose.

4.2 *Teratogenic effect of gentamicin on the weight of kidney of newborn chicks*

In the present study, it was found that though the mean weight of both right and left kidneys of gentamicin-treated chicks was less than that of the control group, but this difference was not statistically significant (Table 3).

Lelievre-Pegorier et al found that the mean weight of kidney of pups born to gentamicin-treated guinea pigs with multiple doses was less than that of pups born to saline-treated guinea pigs though this difference was not statistically significant.[13] Hence, their findings matched with our findings.

On the other hand, Smaoui et al found significantly decreased kidney weights in prenatally gentamicin-exposed neonates born to pregnant Wistar rats who were administered 75 mg/kg gentamicin on days 7–11 and 14–18 of gestation.[14]

4.3 *Teratogenic effect of gentamicin on the histological structure of kidney of the newborn chick*

In the present study, out of 30 chicks of treated group, 21 showed cystic dilatation of proximal convoluted tubules (70%) and 22 showed cloudy swelling of proximal convoluted tubules (73.33%).

Mallié et al[9] and Smaoui et al[14] reported degenerative changes such as swollen cells, cloudy cytoplasm, PAS-positive inclusions, brush border atrophy, and luminal enlargement in proximal convoluted tubules in rat neonates born to pregnant Wistar rats after subcutaneous gentamicin administration.

The changes in the proximal convoluted tubule noted in our study are similar to those reported by Mallie et al[9] and Smaoui et al[14] but they have not mentioned any statistically significant difference in comparison to the control group, whereas in our study, these changes are found to be statistically significant when compared with the control group.

In the present study, various histological changes in the structure of the proximal convoluted tubules of the kidney were noted even with a single dose of gentamicin. The effect with multiple dose administration during pregnancy in human beings will obviously be much more.

5. Conclusions

With the analysis of the results, it can be definitely concluded that gentamicin has a teratogenic effect on kidney even if administered in a single dose during the development of an embryo and further studies need to be conducted to prove its teratogenic potential in human embryo.

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