Original Article

The Effect of Prenatal Administration of Sodium Phenytoin on the Survival and Hatching of Chick Embryos

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ABSTRACT

Objective: To determine the effects of prenatal exposure to sodium phenytoin on survival and hatching of chick embryos.

Study Design: Experimental study.

Place and Duration of Study: This study was carried out in the Anatomy Department, Regional Centre of College of Physicians and Surgeons, Islamabad from January 2012 to January 2013.

Materials and Methods: The study was carried out on three experimental (B1,B2,B3) and three control (A1,A2,A3) groups. The chick embryos of the experimental groups were injected with 3.5 mg of sodium phenytoin per egg whereas the controls were administered same volume of normal saline just before incubation. The experimental group was dissected on day 4, day 9 and day 22 or hatching whichever was earlier. The survivability was compared with age-matched controls.

Results: Survival was less in the experimental groups as compared to the controls. The percentage of mortality was 3.84% in group B1, 14.28% in group B2 and 21.42% in group B3. This difference between control and experimental groups was found to be statistically significant ($p<0.05$). In group B3, 90% of the live chicks were able to crack open the shell on their own. Rest of the chicks had to be assisted after waiting till 22nd day of the incubation. All of the chicks belonging to the control group A3 cracked open the shell on their own on the 21st day of incubation but this difference between groups A3 and B3 regarding mode of hatching was found to be statistically insignificant (p 0.1812).

Conclusion: In this study, prenatal sodium phenytoin exposure resulted in decreased chick embryo survival with increasing embryonic age and increased duration of exposure but there was no significant effect on the hatching of the chicks.

Key Words: Chick Embryo, Phenytoin, Survival, Hatching

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INTRODUCTION

Women with a history of seizure-related illnesses require antiepileptic medication throughout pregnancy. Phenytoin is a widely used non-sedative antiepileptic drug included in pregnancy category 'D' of teratogenic potential according to the FDA (United States Food and Drug Administration) which justifies the use of this drug if the potential therapeutic benefits outweigh the potential risks.[1]

Intrauterine exposure to phenytoin leads to a broad spectrum of fetal anomalies collectively known as the 'fetal hydantoin syndrome'. It comprises a number of birth defects including facial dysmorphism, mental retardation, neurobehavioural disorders, heart defects, abdominal wall defects and limb abnormalities.[2,3]

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The chicken (Gallus domesticus) embryo develops and hatches in 20 to 21 days and has been extensively used in embryological studies due to completion of developmental processes over a short period of time. A large numbers of eggs can be incubated at one time to obtain embryos at the precise stages of development.[4]

This research was conducted to expose chick embryos to sodium phenytoin to determine the number of dead and alive embryos and compare it with age-matched controls. Poor development of the nervous system can lead to troublesome hatching, therefore the mode of hatching was also observed and noted to be natural or assisted.

MATERIALS AND METHODS

This study was carried out on two main groups A and B, each having 90 eggs. These groups were further subdivided into three experimental (B1,B2,B3) and three control (A1,A2,A3) subgroups. There were 30 eggs in each subgroup. The freshly laid chicken eggs of 'Egyptian fayoumi' breed were collected from Poultry Research Institute (PRI) Rawalpindi. Eggs stored for more than 3 days and cracked eggs were excluded. The eggs were randomly selected according to the random

selection table. All the eggs were first wiped clean with swabs soaked in 70% alcohol and then placed in racks with the blunt end facing upward for ten minutes. This gave the eggs time to dry and allowed the blastoderm to float upward and settle at the blunt end just beneath the air sac. This prevented damage to the embryo from drug injection at the lower pointed end. Two holes were drilled into each egg with the help of a thumbpin, one at the upper blunt end and the other a fingerbreadth above the pointed lower end. The hole at the upper end allowed air to escape from the egg creating a space for the entrance of the drug or normal saline at the lower end. A sterile insulin syringe (needle length 8 mm, 30 gauge) was used to inject 3.5 mg sodium phenytoin per egg in the experimental group and an equivalent amount of normal saline in the control group directly into the egg albumen.[5] The holes were immediately sealed with melted wax and eggs were placed in the incubator. The day the eggs were placed in the incubator was taken as day 0. The temperature inside the incubator was kept at 38±0.5ºC. The relative humidity was kept between 60 and 70%. Adequate ventilation was also maintained. The eggs were rotated ½ turn twice daily.

On day 4 of development the eggs belonging to subgroups A1 and B1 were taken out of the incubator and placed horizontally on a table for ten minutes. This allowed the blastoderm to float upward and take a position over the yolk sac just beneath the shell. The eggs were then broken open in a bowl of warm normal saline. This was a delicate procedure.[6] Starting from the broader end, the shell cap was removed exposing the underlying embryo. Survivability was noted and easily determined by observing the pulsatile beating of the heart, cleanly dissected out and transferred to a petri dish avoiding unnecessary traction and hence, trauma.

On day 9 of development the eggs belonging to subgroups A2 and B2 were taken out of the incubator and the embryos were extracted by the same method as mentioned before. The protective membranes were cleanly dissected away from the embryos. Survivability was noted and the embryos were carefully observed for any gross anomalies.

Chicks belonging to subgroups A3 and B3 were allowed to hatch by themselves till the 22nd day after incubation, after which shells of the chicks, which failed to hatch from the shells on their own were cracked open. Once again the number of dead and alive chicks was noted and recorded. The data was analysed statistically with Statistical Package for Social Sciences (SPSS) computer software program, version 16. Chi-square test was applied to detect any significant difference in survivability between the control and experimental groups. To detect any significant difference between mode of hatching, Fisher's Exact test was applied. A p-value of ≤0.05 was considered to be statistically significant.

RESULTS

The percentage of mortality in each subgroup was calculated from the total number of fertilized eggs.

Percentage of mortality = (no. of dead embryos/total no. of fertilized eggs) x 100

All the chicks belonging to the control subgroups A1, A2 and A3 were alive and well. From the 26 fertilized eggs in subgroup B1, 25 survived and 1 was found dead indicated by a coagulated mass of blood on opening the egg shell. The percentage of mortality calculated for subgroup B1 was 3.84%. In subgroup B2, from 28 fertilized eggs, 24 were alive and 4 died. Of the four dead embryos in the experimental subgroup B2, three showed drastically reduced size and restricted development while one was only macerated blastoderm. The percentage of mortality was found to be 14.28% in subgroup B2. In subgroup B3, from 28 fertilized eggs, 22 survived and 6 were found dead. All the 6 dead chicks exhibited gross reduction in size, whereas 4 of them had abdominal wall defects and one had limb deformities. The percentage of mortality in this subgroup B3 was calculated to be 21.42%. The difference of survival between chick embryos belonging to control and experimental subgroups was found to be statistically significant ($p<0.05$). Also, the rate of survival of the chick embryos decreased with increasing age and duration of exposure. (Table-1)

Table No.1: Comparison of control and experimental subgroups regarding mortality in chick embryos.

Subgroup	Total	Fertilized	Alive	Dead	p-value
A1	30	27	27	0	0.491
B1	30	26	25	1	
A2	30	26	26	0	0.112
B2	30	28	24	4	
A3	30	29	29	0	0.010*
B3	30	28	22	6	

*= statistically significant

Table No.2: Comparison between subgroups A3 and B3 regarding mode of hatching.

Subgroups	Mode of Hatching		
	Natural	Assisted	Total
A3	29	0	29
B3	20	2	22

p=0.181

In subgroup B3, 90% of the live chicks were able to crack open the shell on their own. Rest of the chicks had to be assisted after waiting till 22nd day of the incubation. All the chicks in control subgroup A3 opened the shell on their own on the 21st day of incubation. This difference between subgroups A3 and B3 regarding mode of hatching was found to be statistically insignificant (p 0.1812). (Table-2)

DISCUSSION

The teratogenicity of phenytoin has already been well-documented. Exposure to this anticonvulsant drug during pregnancy leads to increased rate of mortality, growth retardation, dysmorphogenesis and neurobehavioural problems in the newborn.[7,8]

In the present study, the experimental chick embryos exposed to sodium phenytoin showed decreased survival which was statistically significant in comparison to the controls. This is in accordance with a previous study conducted by Singh and Shah[9] in which they directly injected a single dose of 3 mg sodium phenytoin in each egg. This resulted in death of embryos whereas the surviving embryos showed several birth defects including craniofacial, limb and abdominal wall abnormalities.

Several mechanisms have been proposed to explain the cause behind the lethal effects of this drug on the developing embryo. Phenytoin may act as a folic acid antagonist since it produces folic acid deficiency anemia in many patients. The folic acid deficiency can cause neural tube and limb defects with increased mortality rate. The incidence of malformations has been seen to decrease with folic acid supplementation in the diet.[10]

Many developmental processes are directly linked to the redox status of the embryo. Disturbance in the oxidative metabolism by sodium phenytoin increases the production of free radicals and reduces glutathione levels. This oxidative stress can lead to increased mortality in the embryo.[11,12]

Sodium phenytoin exposure can also lead to decreased embryo survival by causing fetal hypoxia, edema and vascular disruption as shown by previous studies.[13,14]

Another proposed mechanism of the phenytoin teratogenicity is the disturbed retinoid metabolism. Retinoic acid is a key player in numerous developmental processes.[15] Previous studies have shown that phenytoin causes an altered expression of genes involved in key morphogenetic events of embryological development including the retinoic acid receptor (RAR) isoforms.[16] Also the plasma levels of retinoic acids were found to be markedly decreased in patients treated with phenytoin.[17]

All of these previous studies throw light on the fact that prenatal exposure to sodium phenytoin leads to teratogenicity and increased mortality of embryo which could occur by a number of different mechanisms. Therefore in our study there was decreased survival of the chick embryos exposed to this drug. Also, the normal gestational period in chicks is 21 days. On day 20 of incubation, the process of pipping begins in which the chick breaks through the eggshell with the help of its beak, the allantois ceases to function and dries up. The process of hatching is completed on day 21 of incubation. In our study, regarding hatching, most of the chicks in experimental group were able to hatch on their own. In the remainder, the delayed hatching was either due to mortality or weakness and diminished mobility in the surviving chicks.

CONCLUSION

Prenatal exposure to sodium phenytoin decreased survivability in chick embryos with increasing age and increased duration of exposure but there was no significant effect on hatching of chicks.

Conflict of Interest: The study has no conflict of interest to declare by any author.

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