

STUDY OF AN EPILEPTIC DRUG ON GROWING CHICK EMBRYO OF WHITE LEGHORN CHICKEN TO OBSERVE GROSS MALFORMATIONS.

Nilam R. Madhale¹, Uday Kudalkar²

1. Associate Professor,
2. Associate Professor, Department of Anatomy, Goa Medical College, Goa.

ABSTRACT

The following study was conducted using Phenytoin Sodium on growing chick embryo. Fertilised eggs were injected with the drug, and fourth day effects observed on day 20. Gross malformation like Beak deformity, Ramplessness, limb deformity, ecl,opiacardia, ectopia viscera, omphalo, colour change, stunted growth & dead at hatching were observed.

Key words: Epileptic drug, Malformations, Teratogenic risk, Check Embryo

Introduction :

The statements that the Uterus is 'the only ivory tower that man ever known' was being quoted for years. Thalidomide penetrated that Ivory tower with such devastating results that the effect is being felt in medical practice and pharmacological investigations for generations.

Before the thalidomide disaster, teratogenic risk of drugs had not been taken in to Account. After the demonstration of noxious effects of the drug, a drastic change in opinion on the potential danger of drugs in pregnancy has been observed.

Epilepsy accounts for 1% of the world's burden of diseases. The same as for breast cancer in women or lung cancer in man (Reynolds 1990). The issue of epileptic woman particularly in her child bearing age is complicated because of potential

implication on her offspring. Epileptic women account for one in every 200 pregnancies (Tamer, Mishra & Jaiswar 1996).

The Epileptic pregnant women taking phenytoin either alone or in combination with other anticonvulsant has a two or three times greater risk for delivering a child with congenital defects over the general population (Rataboli, Bhandare, Diniz D'Souza & Dhume 1998). It is not known if the increased risk is due to antiepileptic drugs, the diseases itself genetic factors or a combination of these, although some evidence indicator that drugs are the causative factor (Hanson & Buchler 1982).

Many studies have been done by using phenytoin Sodium on rats (Tachibana, Terada, Fukunishi & Tanimura 1996) mice (Mino, Muzusaw & Shiotak

1994) cats (Khera 1979) & sea urchin (Eshus, Blumer 1989) in the past. Kucera & Bernand (1988) used chick embryo culture to do a comparative study of teratogenesis using dexamethasone & diphenylhydantion. Singh & Shah (1889) studied teratogenic effects of phenytoin on chick embryo by injecting single dose of 3 mg in yolk sac of leghorn chick embryo.

The present study was designed to study the effects of phenytoin Sodium absorbed across the Chorio Allantoic membrane following its injection in air sac.

Materials & Method

Materials Used were

- Fertilized eggs of white leghorn chicken
- Phenytoin sodium in injectable form marketed by Parke Davis as Dilantin.
- Distilled water for Dilution
- Tuberculin Syringe
- Black tube & bulb
- Incubator manufactured by MSCO Pvt. Ltd.

Methods

- Zero Day fertilized eggs of white leghorn chicken were incubated at 37°C-38°C
- On the fourth day of incubation, the growth of the embryo was checked by transillumination test.
- An instrument consisting of a black tube open at one end and closed at the other was used for the test. The open end was placed over a light source. The egg was placed over the other end of the tube over a small aperture.
- Eggs showing satisfactory embryonic growth were taken for the experiment.

- About 100 fertilized eggs were injected with single dose of 6mg of freshly prepared solution of phenytoin sodium diluted in distilled water.
- 2cc of above mentioned solution was injected into the Air Sac at the broad end of the Egg using Tuberculin syringe.
- This was compared with hundred eggs, which were kept as control by injecting same ml of distilled water under similar conditions.
- Eggs were turned manually twice a day throughout the period of incubation.
- Care was taken to provide adequate moisture in the incubator.
- Eggs were sacrificed on 20th day of incubation
- These were then evaluated for gross malformations.

Results

On the treated group of embryos, maximum incidence of lethality and abnormalities was seen. 64% of the embryos were found dead at hatching. The surviving embryos showed marked reduction in weight as compared with those of the control group. 60% of embryos showed stunted growth. Omphalocele with protrusion of abdominal contents was seen in 20% of the embryos, whereas ectopia cordia due to defective development of the thoracic wall was seen in 2% of the embryos. Ectopia Viscera was seen in 22% of the embryos. Beak deformities like short beak and crossed beak were seen in 40% of the embryos. Limb deformities like crossed limbs, crossed toes, hyper flexed toes and hyper extended toes were seen in 50% of the embryos.

Deformity	Control Group	Experimental Group
Beak Deformity	NIL	40%
Rumplesness	NIL	30%
Limb Deformity	NIL	50%
Ectopia Cordis	NIL	02%
Ectopia Viscera	NIL	22%
Omphalocele	NIL	20%
Feathers (Colour Change)	NIL	08%
Stunted growth	NIL	60%
Dead at Hatching	NIL	64%
Average Weight	17GMS	6 GMS

Fig 1: Shows experimental embryo on the right side treated with phenytoin sodium. On the left is seen chick belonging to the control group. The experimental chick shows ectopia viscera with protrusion of the lobes of the liver along with the coils of intestine.

Fig 2: shows experimental embryo on the right side and the chick belonging to control group on the left. The experimental embryo shows hyperflexion of the digits on the right side. The experimental chick also shows growth retardation.

Discussion:

The commonly used anti-convulsants are phenytoin, phenobarbitone, ethosuximide and sodium valproate. Reports stated that epileptic mothers receiving anticonvulsant therapy have borne children with deformities involving wide range of organ system including facial deformities, skeletal deformities, heart defects, and coagulation abnormalities etc.

Most epileptic patients are put on multiple drug therapy. Therefore the drug or combination of drugs responsible for the birth defects cannot be deducted with certainty.

In the present study, chick embryos were used as convenient experimental tool.

They were preferred to other laboratory animals like rats and mice because of the difficulty for seen in obtaining the desirable individual observation of parents and litter. The chick embryo test for detecting teratogenic effect by injecting the chemical into the air sac is a very easy, rapid and comparatively inexpensive procedure. Moreover they can be maintained under controlled environmental conditions. Chick embryos are accepted as an ideal experimental tool by a number of other investigators also.

The present study was designed to ensure that phenytoin was absorbed across the chorio-Allantoic membrane, thus stimulating the passage of drug across the placental membrane.

It was decided to administer phenytoin in the air sac on fourth day of incubation of eggs because it offered certain advantages.

- Infertile eggs could be eliminated by trans-illumination test.
- Embryos of uniform size could be segregated.
- Survival rate of such pre-incubated eggs was higher.
- The chorioallantoic membrane was well developed and vascularized thus allowing absorption of drug across the membrane.

The exact mechanism of phenytoin teratogenicity is unknown, however multiple factors are suspected to be involved in the causation of birth defects due to phenytoin:

- Phenytoin may induce folic acid deficiency either by impairing gastrointestinal absorption or by increasing hepatic metabolism of the vitamin. Folic acid is required for synthesis of DNA and is essential in normal development of human foetus.
- It may cause early hemorrhagic disease of the newborn.

The tests performed on animals are of limited value for predicting such effects on human embryo. The extrapolation to man to date obtained in animals remains a challenging problem. Although chick is a non-placental species its reaction to the teratogenic effect of phenytoin sodium has been similar to that of human foetus.

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