THE TERATOGENIC EFFECT OF RIFAMPICIN ON THE DEVELOPING CHICK EMBRYO

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ABSTRACT

Rifampicin is one of the antibiotics against bacteria with the key action of anti-tuberculosis drug. The duration of treatment is about 3-6 months and the dosage is of 10 mg/kg. Thus, it is interesting to study about the teratogenic effect of rifampicin when it is used during pregnancy as it might cause abnormality to the newborn. This study is conducted to elucidate the teratogenic effect of rifampicin which different concentrations by using chick embryo as an animal model to compare the results with normal. The 120 fertilized white leghorn eggs was randomly divided into 4 groups, one control group and 3 experimental groups with in different concentrations of rifampicin 2 mg/ml, 4 mg/ml and 8 mg/ml with normal saline (NSS). After 21 hours of incubation the drugs and NSS were injected into yolk sac and re-incubated. All the eggs from each group were collected in day 3 of incubation and studied total mount and serial section. The results in experimental groups showed increase in mortality rate and growth retardation as increasing concentration of rifampicin. There were retardation of brain vesicle, heart constricted to U-shaped loop, neutral tube lines were non-parallel, opened of posterior neuropore. Thus, rifampicin produced teratogenic and embryotoxic effects to developing chick embryo.

Keywords: Rifampicin, teratogen, chick embryo

1. Introduction

Drugs is necessary for human, when taken into the body for relieving and treating the symptoms. In pregnant women, drug is one of the teratogenic agent, the drug intake can harm her own health, can effect the fetus when drug molecules cross placental membrane (Kamuhabwa *et al.*, 2011). In general, pregnant women should not consume drugs during pregnancy. Pregnant women with underlying diseases or infectious diseases can not avoid using a certain drug, they need to advice by doctor or pharmacist for properly dose.

Rifampicin is one of the antibiotic drug used to treatment of tuberculosis and other infections, only bacterial infections. The duration of treatment about 3-6 months with combine of medication. The dosage of rifampicin 10 mg/kg was introduced in 1971 based on pharmacokinetic and toxicity, not to exceed 600 mg/kg (Boeree *et al.*, 2015). The U.S. Food and Drug Administration has classified rifampicin in pregnancy risk category

C. This category, the fetal risk revealed in studies in animals but not established or not studied in humans. Animal reproduction studies have shown an adverse effect on the fetus, there are no adequate studies in humans (Thakur *et al.*, 2011). Thus, it is interesting to study about the teratogenic effect of rifampicin to the benefits when use of the drug in pregnant women may be acceptable despite its potential risks.

However, few previous researches were found in consider to the teratogenic and toxicity of rifampicin in the development of chick embryo. Thus, this research is to study about the teratogenic effect of rifampicin using animal models by using chick embryo because of short gestation period, inexpensive and development is rapidly increasing in size, similar to the situation in the human embryo (Nusrat *et al.*, 2011). The main objective of this research to study about the teratogenic effect of rifampicin on the developing chick embryo at a different developmental stages to compare the result with human and to adjust on using drugs in pregnancy.

2. Objectives of the study

This study is conducted to elucidate the teratogenic effect of different concentrations of rifampicin by using chick embryo as an animal model, the gross structure malformations and morphological changes on the developing chick embryo will be compare with normal and apply the knowledge to human.

3. Materials and methods

The 120 fertilized white leghorn eggs were cleaned with 70% ethyl alcohol and randomly divided into following 4 groups, one control group and 3 experimental groups with different concentrations of rifampicin 2 mg/ml, 4 mg/ml and 8 mg/ml with NSS. All eggs were incubated at 36.5-37.5 ℃ and 70-80% humidity. After 21 hours of incubation (“0” embryonic day), the eggs were taken out from egg incubator carefully and maintained their position as they were incubated. Then cleaned at the blunt ends of egg shell with 70% ethyl alcohol and drilled through to get a hole by dental driller. Then injected into yolk sac with different doses concentrations of rifampicin solution. The hole in the egg shell were sealed with transparent adhesive tape carefully for protecting pathogens. After that the eggs re-incubated all eggs were collected in day 3 of incubation.

The day 3 were removed from each egg and washed with NSS, then each embryo were trimmed at diameter about 1-1.5 cm and fixed with Dietrich’ s FAA solution as fixative for 4 hours. After that transferred to washing with 70% ethyl alcohol several times until the embryo changed to transparent or white color, and stained the embryo with Mayer’ s carmalum staining for 8-10 minutes to studied morphological malformations with total mount. And studied histological changes with serial section by paraffin embedding and hematoxylin-eosin staining. Then the embryos were transferred to washing with 70% ethyl alcohol several times until the embryo changed to transparent or white color and trimmed tissue around the embryo. All the embryo were dehydrated with series of ethyl alcohol and cleared with xylene. After finished all of process, the embryonic specimen were embedded in the mold with the paraffin wax by correct position for sectioning in the next step. Before section, the embedded

embryos were trimmed paraffin like T-shaped and then serial section with rotary microtome for the thickness 6 micron. Then that tissue were attached to the microscope slide by using egg albumin solution, expand on hot plate and stained with hematoxylin-eosin.

4. Results and Discussion

The percentage of survival and mortality rate were examined by observing heartbeat and blood circulation showed in Table 1

Table 1 The survival and mortality rate of day 3 chick embryos in each group

Group	n (%)	Survival n (%)	Mortality n (%)
Control group	10 (100)	10 (100)	0 (0)
2 mg/ml	16 (100)	14 (87.5)	2 (12.5)
4 mg/ml	18 (100)	15 (83.33)	3 (16.67)
6 mg/ml	16 (100)	12 (75)	4 (25)
Total	60 (100)	51 (85)	9 (15)

The result showed fertilized eggs were exposed to rifampicin which different concentrations, a decrease in number of survival chick embryos was observed as increasing concentration. The percentage of survival chick embryo in 3 experimental groups included 2 mg/ml, 4 mg/ml and 8 mg/ml showed 87.5%, 83.33% and 75% survival rate respectively and compared with control group resulted in 100% of survivor.

The total mount of day 3 chick embryo

Figure 1 The micrograph of day 3 chick embryos by total mount preparation in each group including A. control group, B. 2 mg/ml, C. 4 mg/ml and D. 8 mg/ml of rifampicin treated groups. (TC = Telencephalon, DC = Diencephalon, MS = Mesencephalon, MT = Metencephalon, MC = Myelencephalon, OT = Otocyst, OC = Optic cups, LV = Lens vesicle, CeF = Cephalic flexure, CvF = Cervical flexure, H = Heart loop, ALB = Anterior limb bud, PLB = Posterior limb bud, So = Somite, Vv = Vitelline vessel, T = Tail fold)

The control group (**Figure 1** A.) showed normal development of the chick embryo on day 3 of incubation showed about approximately 36 somites or HH stage 18 as characterized by Hamburger Hamilton stage (Hamburger *et al.*, 1951). The general appearances were head and tail fold, the long axis of 2/3 part of head twisted about 90 degrees to the right side and bend down the head. Following the torsion appeared the 2 flexures including cervical flexure and cephalic flexure. At the cervical flexure, the axis of the medulla forms approximately a right angle to the axis of the posterior trunk, the rotation extends to the posterior part of the body, the tail bud is turned to right. In this stage, the development of the brain vesicles were prominently observed including telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon. Otocyst was closed vesicle at the myelencephalon. Optic cup was large size like horse-shoes and lens located on middle of optic cup. The heart loop appeared S-shaped. The lateral side found that vitelline vessels which supplied the wall of yolk. In cervical regions there were 4 pharyngeal arches. And caudal part of chick embryo were appeared anterior, posterior limb buds and tail fold. Somites were arranged parallel to the neural tube and extend to caudal end.

The effects of rifampicin on day 3 of chick embryo treated with 2 mg/ml and 4 mg/ml (**Figure 1** B., C.) showed that the embryo were smaller than the control group and growth retardation of head and heart. Heart loop was like U-shaped and small size of eye and ear. Somites was incomplete formation, it does not extends to the caudal end.

The effects of rifampicin on day 3 of chick embryo, treated with 8 mg/ml (**Figure 1** D.) showed the embryo was to severely smaller than the control group and composed of severe congenital abnormities including open anterior and posterior neuropores, heart loop swang to cephalic position than that head, no eye and ear primordia, absent of limb bud and incompleted of somites formation.

The serial section of day 3 chick embryo

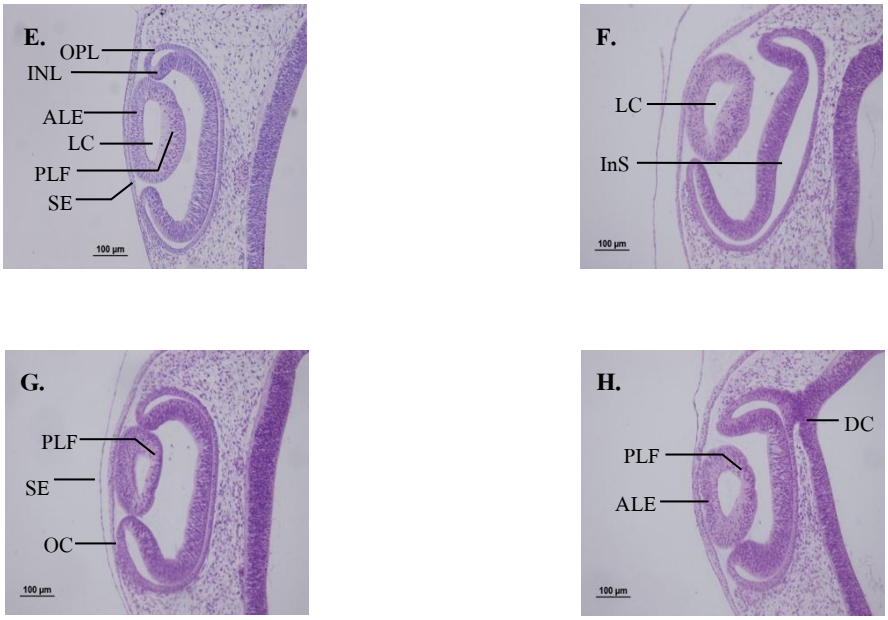

Figure 2 The micrograph of day 3 chick embryos at the level of eye development by serial section preparation in each group including E. control group, F. 2 mg/ml, G. 4 mg/ml and H. 8 mg/ml of rifampicin treated groups. (LC = Lens cavity, ALE = Anterior lens epithelium, PLF = Posterior lens fiber, OC = Optic cup, SE = Surface ectoderm, OPL = Outer pigment layer, INL = Inner nervous layer, InS = Intraretinal space, DC = Diencephalon)

From serial section, the control group (**Figure 2** E.) showed normal development of the eye, which developed from invaginated of diencephalic wall and extended laterally to form the optic vesicle. When optic vesicle was interacted with surface ectoderm induces that area of the ectoderm to form lens placode, then distal end of optic vesicle were distended and invaginated to form double-walled like cup-shape appearance called optic cup. The optic cup divided into 2 layers including the outer layers was thinner which was characterized by small pigment granule and the inner nervous layer was thicker. The inner and outer layers were separated by the intraretinal space. The anterior lens epithelium was cuboidal-shape cell and showed cell division. The posterior lens fiber was slightly elongated.

The experimental groups (**Figure 2** F., G. and H.) showed development and growth retardation of the eyes formation and smaller than control group. Lens vesicle was not closed, the posterior lens fibers were not-elongated and the space between inner and outer layers called intraretinal space persisted.

5. Conclusion

The result was consistent with the experimental of Asmatullah, Bashir F, Ara C. in 2012. It was concluded that the higher the concentration of rifampicin cause increasing the mortality rate. In this study, rifampicin produced and embryotoxic effects to developing chick embryo. Thus, the pregnant women should avoid consuming this during pregnancy.

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