Effects of Cafergot in Development of Chick Embryos

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ABSTRACT

Cafergot, one of the most well-known medical regimen for migraines is composed of ergotamine tartrate and caffeine. It has the porperties of analgesic, vasoconstriction, and increase the uterine contraction. However, it may cause fetal harm in pregnant woman. Nowadays Food and Drug Administration (FDA) has classified the cafergot as contraindicated in woman who becomes pregnancy. Nevertheless, Teratogen Information System (TERIS) is still undetermined the cafergot due to lack of empirical data support. The purpose of this study is to evaluate the affected fetal development period and to specify the effector organs that cafergot acts as the teratogenic agent in chick embryo as an animal model by using whole mounting technique. The fertilized white leghorn eggs were divided four treated groups that were injected with 6mg, 5mg, 2.5mg and 1.25mg cafergot into the yolk sac of fertilized eggs of group1, group 2, group 3, and group 4, respectively. The mortality rates were compared among the doses and developmental periods. Gross morphological changes of control and treated groups were studied on day 3. The results showed that the mortality rate increased as the concentration of the drug increased. The morphology showed retardations of the brain development, head region flexion, and somite formation. There was few branchial arches and absent the limb buds. The heart showed U-shape loop whereas the eyes were small (micropthalmia).

Keywords: Cafergot, Teratogen, Chick Embryo

1. Introduction

Globally, approximately 15 percent of people are affected by migraines (4) is more occurance among women than men. The important one of all trigger factors is influence of female sex hormones which appeared in 53% of all factors which lesser than 81% of sleep problem and 64% of emotional stress trigger (4). Migraine varies in female reproductive events involving menstrual period, menopause including pregnancy, even if pregnant women who has never experienced with migraine before. There were affect about 25% of pregnant women (2). Probably related to the rapid falling of estrogen levels during pregnancy (13). The treatment for the pregnancy should be deliberated because the vulnerable fetus inside will be affected.

The following drugs should be preferred as the first choice of acute migraine treatment in pregnancy are

paracetamol, NSAIDs and sumatriptan. However, over 60 years, ergotamine has been used in clinical practice for migraine treatment with vasoconstrictor, analgesic properties and increase the uterine contracting activity (2). So, Food and Drug Administration (FDA) considered as a contraindicated in pregnancy (catergory X), while the Teratogen Information System (TERIS) still undetermined because the lack of empirical data support (3). Estimate 3-5% of newborns are complicated by a congenital defect each year totaling around 3.2 million cases. In addition, a worldwide record in 2013 estimate presented of nearly 276,000 newborns die before one month of life each year, as a consequence of congenital anomalies (14). So, drug using is considered as one cause of birth defects which can increase the likelihood of developing a birth defect. Current evidence found that about 65%-94% of women take at least one prescription drug during pregnancy, with nearly 70% of women are taking a medication in the first trimester gestation (12).

Nevertheless, the percentage of migrainous pregnant women were not high, but the severe morbidity and mortality rate of newborns were shown in high rate, especially in case of maternal exposure to ergotamine during pregnancy. The previous studies showed the variety of anomalies such as microcephaly, neural tube defects, intestinal atresia, renal agenesis, urethral atresia, limb reduction defects and paraplegia. The congenital anomalies may severe and are the main cause of infant mortality (8), almost twice as in who without defects (6). These also impact on their families or health care providers, health expenses with specialized care, qualifying teratogens as a public health problem. Maybe mentioned that use of ergotamine should be avoided during pregnancy for preventing the possibility of congenital abnormally in variety of organs. However, the potentiating effect of concomitant exposure to multiple vasoconstrictive agents could be suggested for the further research. Previous experiment tried to fulfill the gap of mechanism of action, which organs and how that ergotamine acts as teratogent.

The purpose of this study is to confirm that teratogenic effect of cafergot on the development by using chick embryos as a model. Chick embryos are suitable than other animals models as they are inexpensive and easily available all year round. Moreover, they have short gestation period and plenty of database available about their development which can be compared to human. Including, gross morphological changes were studied. Also can be applied the database to the further studies.

2. Objectives of the study

1. To investigate the teratogenic effects of Cafergot which really induce abnormalities in chick embryos.
2. To confirm the morphology from total mount technique of chick embryos on 3rd day incubation by comparing the development between experimental and control groups.

3. Materials and methods

1. Prepare the teratogenic substance which is Cafergot tablets in oral form could be purchase at pharmacy in Thailand.

2. Prepare working solution. First step were divided the groups into four groups. There were control and 4 treated group. Control group was injected by 0.9%NSS amount 1 ml. For treated groups were injected by Carfergot solution which diluted with 0.9% NSS in different dosage based on LD50 at room temperature. There were 6, 5, 2.5, 1.25 mg. respectively (Figure 1).

Figure 1 Solution preparing

3. Animal model in this study were used the fertilized white leghorn (Gallus gallus Domesticus) eggs which obtained from Department of Animal Sciences, Faculty of Agriculture, Kasetsart University (Kamphaengsaen Campus). Used dried gauze for cleaned all eggs with carefully before placed in an incubator for reducing external contamination factor. The standard incubators have to maintain the temperature setting at 37ºC, the relative humidity at about 68-70%. Then, left the eggs for 24 hours of incubation. When reached 24 hours brought them out and made a tiny hole at the blunt end of eggs through air cell by a dental driller (Figure 2).

Figure 2 Dental driller

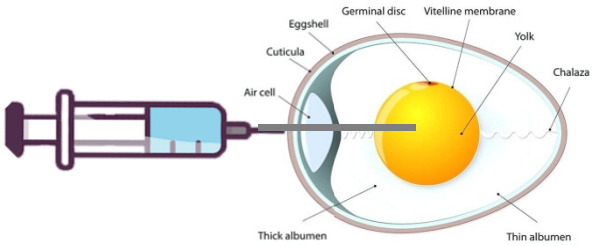


Figure 3 Injection of the chick embryo method

5. Followed by injected control group with 0.9% NSS 0.1ml to the yolk sac passed the same technique with experimental group (Figure 3). Others groups were Injected with Cafergot solution in each concentrations in equal volume as 0.1ml. Implied that all groups, except control group were injected with Cafergot solution in 6, 5, 2.5, and 1.25mg. consecutively. Next step, sealed the tiny hole with adhesive tape after injection. Placed them back to be continue incubated under careful. All groups were opened on 72 hours of incubation.

6.When the time reached 72 hours, brought them out for recorded the mortality and survival rate of each dosage. Only survive chick embryos were passed to total mount technique (Figure 4).

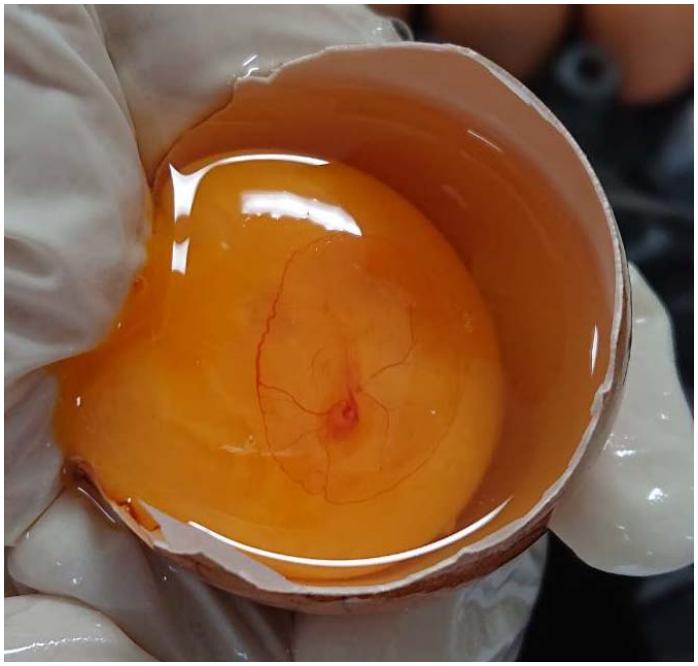


Figure4 The survive chick embryos

7. Washed the survival embryos by warm 0.9% NSS and preserved in Formaldehyde-acetic acid-alcohol (FAA)(5).

8. They were trimmed to get the diameter about 0.3 mm. before staining by Mayer's carmalum) (7).

9. After staining was mounting slide. Dropped Canada Balsam permount 2-3 drops on glass slide by avoiding air bubbles. Put the embryonic specimen on glass slide. Dropped permount one more time on it. Put some tiny glasses around the specimens adjusted to use the appropriate size of glasses. Finally, covered the specimens with circle coverslips and left it drying at room temperature.

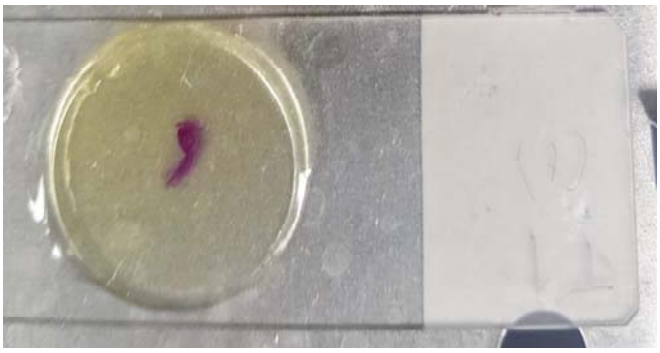


Figure 5 The total mount slide

10. Studied morphological abnormalities of embryos under light microscope and compared with the control groups.

4. Results

The purpose of this study was fulfill the gap that really rare of data in journals were not available at the present. The other one, to confirmed that Cafergot was a teratogenic agent. From these repeated 3 times laboratory confirmed that Cafergot correctly to be considered in catergory X for pregnant and acted as teratognic substance.

Method of separated mortal or survive embryos by opened the eggs carefully and evaluated the red vasculature extended around them. One was founded the red small heartbeat still beating. The survive embryos were placed in warm 0.9%NSS before fixation and recorded the mortality and survival rate as below

The survival and mortality rates of injected- Cafergot on the 3rd day incubator of all of 3 times experimental repeated excluded non-fertilized eggs. These repeated 3 times laboratory were following;

Table 1 : The first round of laboratory on the 3rd day incubator

Group	Average mean of survival (%)	Average mean of mortality (%)
Control	100	0
Group1 (6mg)	60	40
Group2 (5mg)	56.62	43.38
Group3 (2.5mg)	46.39	53.61
Group4 (1.25mg)	65.74	34.25

Table 2 : The second round of laboratory on the 3rd day incubator

Group	Average mean of survival (%)	Average mean of mortality (%)
Control	85.71	14.29
Group1 (6mg)	10	90
Group2 (5mg)	42.86	57.14
Group3 (2.5mg)	25	75
Group4 (1.25mg)	100	0

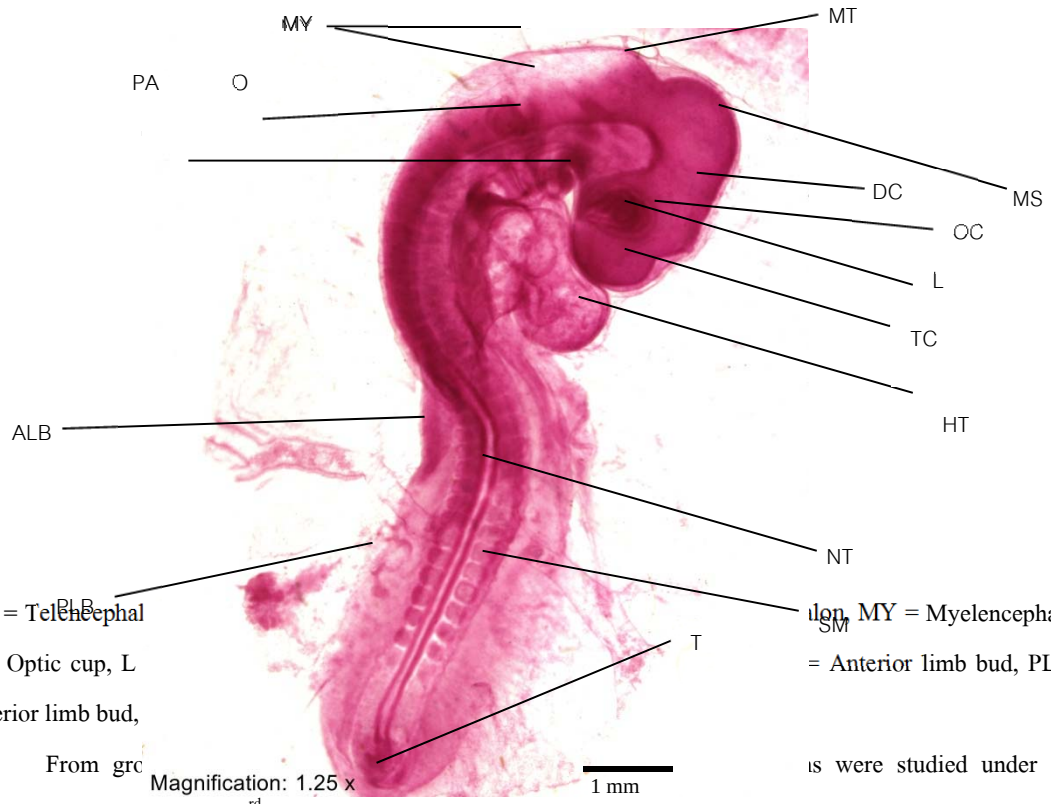
Table 3 : The third round of laboratory on the 3rd day incubator

Group	Average mean of survival (%)	Average mean of mortality (%)
Control	100	0
Group1 (6mg)	55.56	44.44
Group2 (5mg)	71.43	28.57
Group3 (2.5mg)	57.14	42.86
Group4 (1.25mg)	71.43	28.57

All 3 tables presented of the survival and mortality rate in percentage of each group were not corresponding to concentration of Cafergot solution. The control group showed the survival rate more than 85%.The first round of laboratory showed in group 4 > 1 > 2 > 3. The second round of laboratory showed in group 4 > 2 >3 > 1. And the last group showed in group 2 = 4 > 3 > 1. When compared the percentage of each dosage founded that concentration of Cafergot were not related among all groups.

In general, chick embryo on day3 should be founded prominent cervical flexure. Posterior and anterior neuropores were closed. Optic cups was identified and got a pigment. Also optic lens. Otocyst or auditory vesicle (otic) were growth at the myelencephalon area. The somites have 40-43 pairs extended to the caudal end in parallel line. Swelling pharyngeal arches that eventually developed. Maxillay process was the most protruded of the first arches. Heart loop was formed and became S-shape. Both wing buds were symmetry development. However, the leg buds were slightly asymmetry development. When compared to human, chick embryo 3 days equal to 2-5 weeks in human.

Total mount of 3rd day embryo in the control group



(TC = Telencephal lon, MY = Myelencephalon, OC= Optic cup, L = Anterior limb bud, PLB = Posterior limb bud,

From grc ıs were studied under light microscope in each group of 3rd incubation that we founded as below

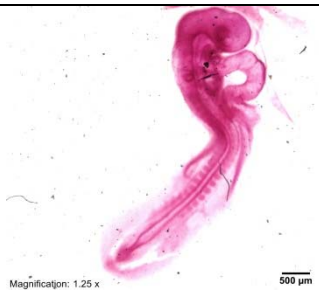
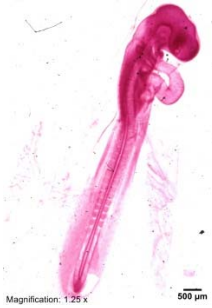
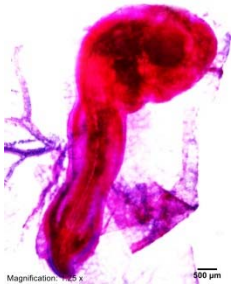
Group 1 : retardation of flexure of brain, microphthalmia, 4 complete formed of pharyngeal arches, small heart diameter of tube(figure7), somite not extended to caudal end, had limb buds

Group 2 : retardation of flexure of brain, had only cephalic flexure, microphthalmia, incomplete pharyngeal arches foming, swelling with thin wall of heart, somite not extended to caudal end. (figure8)

Group 3 : retardation of flexure of brain, had only cephalic flexure, microphthalmia, incomplete pharyngeal arches foming, swelling with thin wall of heart, somite not extended to caudal end. (figure9)

Group 4 : retardation of brain, neural tube widen parallel extened to caudal end, microphthalmia, incomplete pharyngeal arches foming (only 1 arch), heart was in normal size and suitable location, opened of posterior neuropore, No forming of limb bud. (figure 10)

Table 4 : Showed some chick embryos with abnormalities

Time	Group1	Group 2	Group 3	Group 4
3rd day	 Figure7 retardation of flexure of brain, microphthalmia, 4 complete formed of pharyngeal arches, small heart diameter of tube, somite not extended to caudal end, had limb buds	 Figure8 retardation of flexure of brain, had only cephalic flexure, microphthalmia, incomplete pharyngeal arches forming, swelling with thin wall of heart, somite not extended to caudal end.	 Figure9 retardation of flexure of brain, had only cephalic flexure, microphthalmia, incomplete pharyngeal arches forming, swelling with thin wall of heart, somite not extended to caudal end.	 Figure10 retardation of brain, neural tube widen parallel extened to caudal end, microphthalmia, incomplete pharyngeal arches forming (only 1 arch), heart was in normal size and suitable location, opened of posterior neuropore, No forming of limb bud.

5. Discussion

Cafergot is a registered trademark of the combination between ergotamine tartrate 1 mg and caffeine 100 mg. Its delivative was used for strengthening uterine contraction to prevent post-partum hemorrhage. During labor period is strictly prohibited because of harmful to the child. For example hypoxia, gastrointestinal atresia, congenital heart disease, low birth weight, preterm labor and even miscarriage (3). Therefore, Food and Drug Administration (FDA) considered it in catergory X as a contraindicated in pregnancy (2).

The reason why caffeine added is to facilitate drug absorption and more effective analgesic effect without the necessary of increasing ergotamine dosage. It has not only a central nervous system (CNS) stimulating properties and also impact on blood circulating system. Many studies have been suggested that excessive caffeine consumption during pregnancy could be induced congenital malformations, fetal growth retardation, preterm birth and spontaneous abortion. (9) Refer to 2 factors which may cause of these hazards. The first one is from the maternal side, the theory on the rising of caffeine's elimination half-life especially in late trimester of gestation then

can pass through the placental barrier freely (1). The other one is from the fetal side, in the same time, the fetus and infants until 3 months of age have poorly qualities or unable to metabolize caffeine(1). World Health Organization (WHO) recommended the pregnant should limit the caffeine intake less than 300 mg daily; corresponding to about 3 cups of instant coffee, to avoid harmful of both mother and child (15).

Thus, the result might not come from pure ergotamine. But, this study focus on the migraine in pregnant woman, chose cafergot seem to be reasonable. Moreover, every concentration and time founded not corresponding at all. Observed that the survive embryo, mostly malformation or retardation of body, brain, heart and no limb bud.

How much cafergot passed through the placenta, assumed that equal to caffeine. That was freely 100 percent. Because the caffeine help drug absorption and more effective. Besides, poorly quality of eliminate, drug might be accumulated and produced abnormalities.

In process of collected dead or live chick embryo, might miss interpreted from a very tiny heartbeat or small and less of vessels around them. Anyway, if got lived embryo, assumed that retardation.

In the past, pregnant mice, rats, and rabbits were used in the experiments. Significantly, ergotamine could increase the fetal mortality rate in rats and evidence of growth retardation in all three species. The researchers confirmed the hypothesis that ergotamine using involved in vasoconstrictive property and oxytocic effects may also be related. But, no evidence of specific teratogenic activity in any of those species (7). Rarely data in chick embryo. In human presented in small subject reports by limitation of harm. So that, further study is very necessary condition to explain the actual mechanism of teratogenicity of drug.

The survival and mortality rate of this study was non-corresponding. Maybe causing from sterile technique, injected force, non-fertilized eggs, and so on.

Although FDA considered it in catergory X, the reason why we had tested it because the babies from pregnants with migraine attack is the point. This study would like to confirm and caution the danger to the fetus and future research build on these observations by this database.

6. Conclusion

This study concluded that cafergot can cause developmental defects by gross morphology in various organ of chick embryo on day 3rd when compared to control group. It should be further studied in histological changes from serial section technique or bone and cartilage formation by dyeing alcian blue and alizarin red solution of other period and so on.

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