The teratogenic effects of celecoxib on developing chick embryo

Parinya Rachalotorn[1], Jantima Roongrungchai[2]

Yadaridee Viravud[3], and Vasana Plakornkul[4]

[1]Master of Science Program in Anatomy - Faculty of Medicine Siriraj Hospital, Mahidol University, f.teemah@hotmail.com

[2]Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, jantima.roo@mahidol.ac.th

[3]Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University, siyvv@mahidol.ac.th

[4]Department of Anatomy, Faculty of Medicine Siriraj Hospital, Mahidol University,

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ABSTRACT

The various benefits of celecoxib mean that it is frequently used clinically, including for woman of child bearing age. However celecoxib is still related to cardiovascular risk. Nowaday, there was no study about the use of cox-2 selective inhibitors in term of embryo-fetal effect. This study was to study the developmental toxicity of celecoxib on the development of chick embryo by injected with 2, 3 and 4 mg/0.1ml concentration of celecoxib in the treated groups and normal saline in control group. On day 3 of incubation chick embryos were evaluated the teratogenic effects by total mount technique. Results revealed that celecoxib induced more mortality and malformation of chick embyos which related to increase concentration. The abnormalities included brain, eye, heart and branchial arch development.

Keywords: Celecoxib, Teratogen, Chick Embryo

1. Introduction

Celebrex is a registered trademark of oral capsules containing 200 or 400 mg of celecoxib. It is a nonsteroidal anti-inflammatory drug, which selectively inhibits cyclooxygenase-2(COX-2) affect to inhibit prostagrandin synthesis that is important physiological and pathological mediators as inflammation. Celecoxib reduces the adverse effect as gastrointestinal complication, and is prescribed for the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis and acute pain. Furthermore, Epidemiological study reveal celecoxib can affect in prevention of cancer, and reduce the number adenomatous colorectal polyps, familial adenomatous polyposis. The study in human revealed that celecoxib were no significant difference from magnesium sulfate to prevent preterm labor (Najmich Saadati et al, 2014). Similar study showed celecoxib might be benefit for treatment of preterm labor similar efficacy as indomethacin and decrease amniotic fluid but no affect to close the ductus arteriosus (Sawdy RJ et al, 2003). The excellent therapeutic effects of celecoxib has been widely used in women of child-bearing age (Ostensen ME. Skomsvoll JF, 2004 and Olesen C et al, 1999). However, the celecoxib has side effects relative with cardiovascular risk (S.D Solomon et al, 2005). Celecoxib is categorized C prior to 30 weeks

gestation and category D beginning at 30 weeks gestation in the US FDA's pregnancy. Dao-ji Xu, M.D. et al (2011) found that Zebrafish embryo treated celecoxib induced markedly defects in heart development and induced mortality and various malformations as gut miscoiling, haemorrhage and oedema on Xenopus embryo development (Yeon-Hee Yoon et al ,2018). Moreover, it could induce retardation in the body as weight, size and length as congenital malformations associated with decrease in ossified lengths of axial and appendicular bone by missing of ossification centers in neonatal albino rat (Gamal M. et al, 2011). Nowaday, there was still no report about the effects of COX-2 selective inhibitors to the embryo and fetus. It is important to use animal to test many environmental agents before, during pregnancy, and newborn near term. Chick embryo screening plays important role to use as animal model because it has more advantages than other animals including easy to provide fertilization eggs, large size embryo, low cost, known stages of embryonic development and plenty of database comparing to human development. Thus this study is to elucidated the teratogenic effects of celecoxib in chicken embryo and used this knowledge to remind the pregnant women about the safety use of celecoxib

2. Objectives of the study

2.1. Investigate the effect of celecoxib on developing in chick embryo

2.2. Study the developmental toxicity of celecoxib in form of total mount of chick embryo on day 3

2.3. Make a fundamental knowledge of teratogenic effect of chick embryo, benefit for further study

3. Materials and methods

The pilot study in identifying the LD50 of celecoxib in chick embryo was 3 mg/0.1ml in the experiment add more than and less than LD50 concentration which were 4, and 2 mg/0.1ml respectively, celebrex 200 mg was purchased from drugstores in Thailand and then prepare of celecoxib solution as 2, 3, 4 mg/0.1ml dilute with 0.9 normal saline at room temperature.

Animal experiment in this study were used fertilized white leghorn eggs derived from Suwanvajok-kasikit Research station, Kasetsart University. The chick egg were devided into four groups, three groups were celecoxib treated groups and one was control group. After clean each egg with gently and marked the side for making a hole at the blunt end of egg. Place all eggs in sterile incubator which control the temperature at 37°C and humidity control at 68-70% including rotate each eggs manually in position 180° three times a day. After incubate 24 hours, use of dental driller for making a small hole at the blunt end of egg. Group treated eggs were Injected with 0.1ml of three concentration of celecoxib : 2, 3 and4 mg/ml and control group with 0.9 normal saline and sealed the eggs with tape, then incubated. On day 3 of incubation, all eggs were opened and record survival and mortality embryos. For survival chick embryos observed abnormality development by wash with warm normal saline before fixing in formaldehyde-acetic acid-alcohol (FFA) (Geoffrey Rolls et al., 2020). After that they were prepared for total mount preparation by Mayer's staining, dehydration, clearing, and mounting with Canadian Balsam .

4. Results

Fertilized eggs exposure with celecoxib to evaluating the teratogenic effects on chick embryo. The experiment groups were treated with celecoxib as 2, 3, 4 mg/0.1ml and the control with 0.9 normal saline after 27 hours of incubation and reincubated until day 3. All eggs were opened to record mortality and survival rate (Table 1) that were evaluated by observing heartbeat in the center of the body and blood vasculature around

Table 1 The mortality and survival rate of 3rd day chick embryo (all of 3 times experimental repeated).

Group	Mortality(%)(n)	Survival(%)(n)
Control	0	100
Group 1(2 mg/0.1 ml)	20	80
Group 2 (3 mg/0.1ml)	50	50
Group 3(4 mg/0.1 ml)	55.56	44.44

The table 1 showed that the percentage of mortality rate in control group is zero and the treated group as 2, 3, 4 mg/0.1 ml were 20, 50, 55.56 respectively. In 3 mg/0.1ml dose of celecoxib was equal between the mortality rate and survival rate. The mortality rate in 4 mg/0.1ml concentration was the highest when compared with other group

The Total mount

In control group, the result after treated with normal saline solution. For microscopic view showed normal development which presented two flexures as cephalic flexure and cervical flexor, brain area is 5 vesicle consist of telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon, chick embryo's eye were protudeed from the lateral side of diencephalon part, otocyst or auditory vesicle was located at the level of myelencephalon area, the spinal cord was continuously from myelencephalon, somites extended to the caudal end(approximately 36 somite). In cervical area, there were swelling of 3-4 pharyngeal arches that eventually developed to many organ in head and neck area, heart loop formed s-shape. Two bulg of anterior and posterior limb buds were at the lateral side of the body, tail fold located at the caudal end

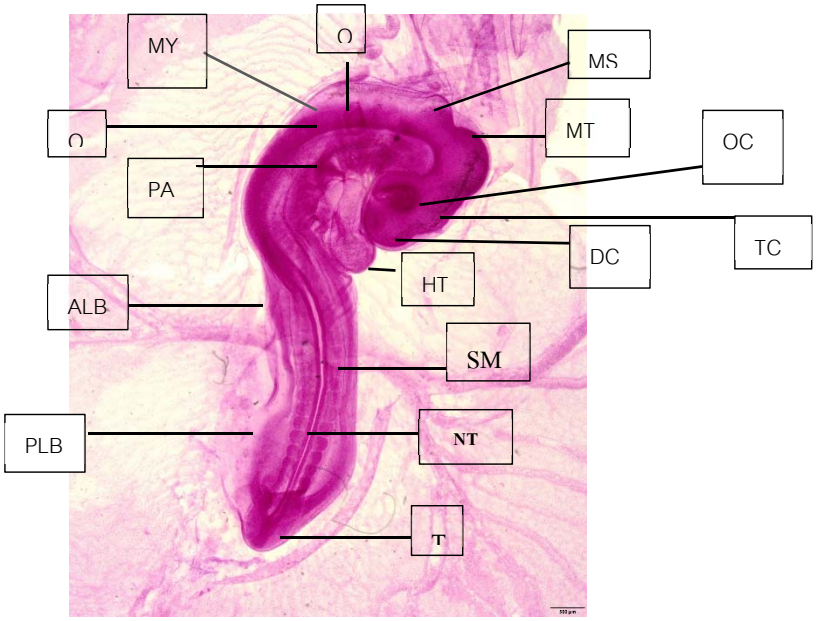


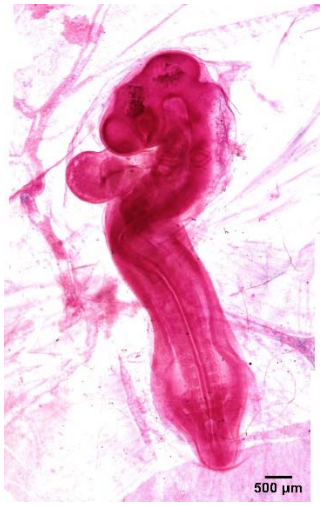
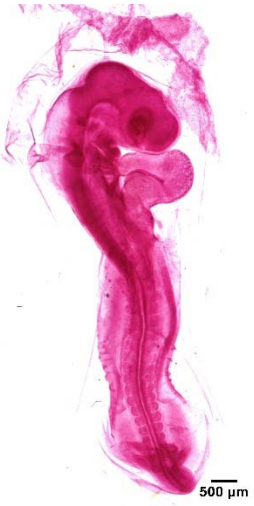
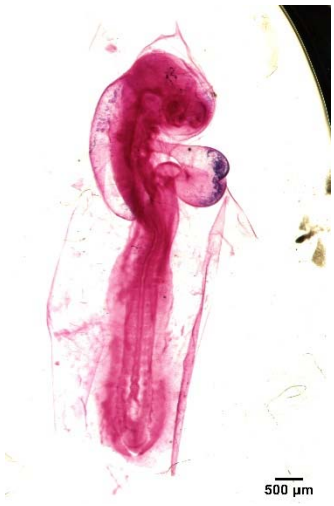
Figure 1. was the day 3 chick embryo in group control (DC = Diencephalon, TC = Telencephalon, MS = Mesencephalon, MT = Metencephalon, MY = Myelencephalon, OC = Optic cup, O = Otocyst, PA = Pharyngeal arch, HT = Heart, ALB = Anterior limb bud, PLB = Posterior limb bud, SM = Somite, NT = neural tube, T = Tail fold)

The result after treated with different concentrations of celecoxib were studied under light microscope as follow

Group 1(2mg/0.1ml) : showed brain vesicle was smaller than control, cervical flexure was absent, otocyst was smaller than control, pharyngeal arch were fewer as two pairs, abnormal flexure of the body. (Figure 2)

Group 2(3mg/0.1ml) : showed brain vesicle was smaller than control and could be identified about 3 parts, fore brain, mid brain and hind brain. Smaller flexure of cephalic and cervical flexure than control. The eye and otocyst were smaller than control. Heart loop was formed u-shape. Pharyngeal arch were fewer as two pairs. Body was twisted irregular pattern. Limb bud were absent. (Figure 3)

Group 3(4mg/0.1ml) : The brain was underdeveloped and presented only 3 parts and the flexure showed only cephalic flexure without cervical flexure. The telencephalon was irregular, pontine flexure was absent. Irregular shape of spinal cord. The eye and otocyst were small. Somites exteneded only ¾ upper of the body. The anterior limb bud were absent. The posterior neuropore still open, pharyngeal arch were fewer as one pairs. heart loop was formed as U-shaped. (Figure 4)

	Group 1 (2mg/0.1ml)	Group 2 (3mg/0.1ml)	Group 3 (4mg/0.1ml)
Day 3rd	 Figure 2	 Figure 3	 Figure 4

5. Discussion

The drug treatment during pregnancy must be evaluated about maternal disease and a successful pregnancy outcome for awareness the adverse effect of drug. Celecoxib is categorized C prior to 30 weeks gestation and category D beginning at 30 weeks gestation in the US FDA's pregnancy. The study was nescessary to establish the safety of administering this drug to pregnant women.

The mean LD 50 value for celecoxib was 3 mg/0.1ml these result that celecoxib was teratogenic in chick embryo. The mortality rate was increased as the concentration of the drug increase, indicated that the drug was affected to the development of chick embryo. There was no study about the mortality rate of embryo due to this drug so this was the first report.

The result in microscopic view all three concentration of celecoxib as 2, 3, 4 mg/0.1ml of the chick embryo show markly defect in heart development is formed u-shape of heart loop. The present finding are in agreement with the study (Dao-jie Xu,MD., et al., 2011) reported that celecoxib exposure cause abnormal heart looping in zebrafish embryo by measured the angle between the midline and atrioventricular canal

In this study showed retardation of development in several area. There were abnormal flexure of the body, pharyngeal arch were few, limb bud were absent, somite extened only ¾ upper of the body may be lead to morphologicalabnormality. Similar finding have been reported in newborn abino rat maternally treated with the celecoxib Gamel M. et al, 2011). The morphological abnormalities were kyphotic body, malformed fore and hind limb, reduced neck region. and the presence of missing ossified bones or retarded ossifications. The retardation development of chick embryo was brain vesicle, eye and otocyst were smaller than control, irregular of neural tube, and posterior neuropore were opened. There is no data report before. However, the study reported that high dose of

aspirin as NSAID reduced the total brain weight in antenatal rats. (Elkarmi, A.et al., 2007). The rate of mortality and malformation increase with increasing concentration of celecoxib.

The side effects of celecoxib relative with cardiovascular risk by imbalance of prostacyclin (PGI_2) and thromboxane A_2 (Kimmel et al., 2005). Including induction of apoptosis (Jendrossek V et al., 2003)

6. Conclusion

This study, it could be concluded that celecoxib can cause malformation and retardation formation of brain, eye, heart and branchial arch on the third day incubation of chick embryo. It should be further studied to evaluate the possible adverse histological by serial section technique.

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