The Teratogenic Effects of Ibuprofen on Developing in *Ovo* Chick Embryo

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ABSTRACT

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) which commonly used for the treatment of high fever, pain or inflammation. Ibuprofen is used in adults and children who are more than or at least 6 months old. The dosage that the patient should take is vary according to the symptoms and age of the patient. Side effects of ibuprofen include dizziness, headache, nausea and vomiting. Especially the usage of ibuprofen should be careful in patients with heart, liver or kidney diseases and asthma. It's not recommended during last trimester of pregnancy prior to 30 week gestaton because ibuprofen is categorized to category C which may induce a congenital malformation to the fetus. However, only a few studies investigated the teratogenic effect of ibuprofen. In this study, the objective is the teratogenic effects of ibuprofen to the development of chick embryos. For this experiment, fertilized eggs of white leghorn were randomly divided into 2 groups, control group and treated group. Control group was injected with normal saline and treated group was injected with the different concentrations of ibuprofen 10, 5 and 1 mg/ml in 0.9% normal saline solution (NSS) into yolk sac. After that, they were incubated until 3rd day of development. The chick embryos were removed for studying gross morphological malformation. By using total mount. The results of this study, demonstrated that the effects of ibuprofen in early chick embryos showed the morphological abnormalities such as retardation of brain development, abnormal looping of heart, the branchial arches were absent, eye and otocyst were very small, and no limb bud and tail fold. The number and percentage of the mortality rate in the treated group which increased in higher concentration. The percentage of the mortality rate were 16.67%, 50% and 60% at 1 mg/ml, 5 mg/ml and 10 mg/ml. It was concluded that the higher the concentration of ibuprofen cause increasing the mortality rate. This results may apply to study the teratogenic effects of this drug to the human embryo.

Keywords : Ibuprofen, Teratogen, Chick Embryo

1. Introduction

Nonsteroidal anti-inflammatoryndrugs (NSAIDs) are commonly used to the treatment of pain, inflammation and fever (Kristensen DM *et al*.,2016). Ibuprofen is one of NSAIDs and used in adults and children who are at least 6 months old. In 2013, 28.3% of pregnant women was reported for ibuprofen intake as tablets with a potency of 200 to 800 mg for three times a day as the second most used NSAIDs at some stages during the pregnancy (Van Marter LJ *et al.,*2013)(Thorpe PG *et al*.,2013). It is a non-selective inhibitor of cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2), main enzymes involved in the first rate-limiting step in the conversion of arachidonic acid into prostaglandins (PG) (Rainsford KD, 2009). In 2015, the Food and Drug Administration (FDA) pregnancy risk letter categories on prescription and biological drug categorized Ibuprofen to category C for animal reproduction. The studies have shown an adverse effect on the fetus and warrant use in pregnant women. The adverse effects of Ibuprofen may include upset stomach, mild heartburn, nausea, vomiting, bloating, gas, diarrhea, constipation, dizziness, headache, nervousness, decreased appetite, mild itching, rash or ringing in your ears. Ibuprofen is clearly indicated from 24 weeks of gestation onwards because the well-known risks of ibuprofen is one of teratogenic agent.

Teratogen is any agent that can induce or increase a congenital malformation. The teratogenic agent is a chemical, infectious agent, physical condition on fetal exposure can alter fetal morphology or subsequent function. Teratogenicity depends upon the ability of the agent to cross the placenta and usually kill the embryo rather than cause congenital malformations. The congenital malformation is an anatomical or structural abnormality present at birth and may be caused by genetic factors or environmental insults or a combination of the two that occur during prenatal development (Barness GB, 2010).

However, the teratogenicity of ibuprofen has not been reported in the development of chick embryo. Thus, this research is to study about the teratogenic effect of ibuprofen using chick embryo for animal model because of short gestation period, inexpensive and rapidly increasing in size, similar to the situation in the human embryo (Nusrat *et al*., 2011). This study has main propose to study the teratogenic effect of Ibuprofen on developing in chick embryos at day 3 stages of development compare to the normal.

2. Objectives of the study

The aim of the present study was therefore to evaluate the teratogenic effects of ibuprofen on chick embryo development. We evaluated the mortality and morphological malformations of the embryos after exposure to ibuprofen on the 3rd embryonic day in chick embryos. This study will be apply to study the teratogenic effects of this drug to the human embryo.

3. Materials and methods

Chemical : Ibuprofen was purchased from pharmacy in Thailand. The stock solution of ibuprofen was prepared in 0.9% normal saline solution (NSS)

Animal model : The fertilized white leghorn eggs (*Gallus gallus domesticus*) from Department of Animal Science, Faculty of Agriculture, Kasetsart University Kamphaeng Saen. Nakorn patom, Thailand.

The 120 fertilized white leghorn eggs were randomly divided into 2 groups: the control group and treated group. All eggs were incubated for 24 hours at 36.5-37 ºC and 70-80% humidity. At the 24 hours of incubation, the eggs were removed from egg incubator and cleaned with 70% alcohol at the blunt ends of eggshell. The eggshell was drilled at the blunt end through the air cell by dental driller to the yolk sac in each group. In the control group were injected 0.1 ml with 0.9% normal saline solution and treated group was subdivided into 3 subgroups and injected 0.1 ml of different concentration of ibuprofen at 10, 5 and 1 mg/ml in 0.9% normal saline solution (NSS) were randomly determined by preliminary study for LD_{50} of chick embryos. After injection, the hole in the egg were sealed by scotch tape and placed back to incubation until 3rd day of development. All eggs of control group and treated group were opened for observing and recording the results of the survival, mortality rates and morphological malformation. Next, the sample of survived chick embryos in each group were collected and fixed with Dietrich's FAA solution for 4 hours.

The total mount technique: The sample of survived chick embryos have been fixed with Dietrich's FAA solution. The embryonic specimens are placed into 70% ethyl alcohol, cut tissue around embryos to round shape and far from embryo about 1-1.5 cm and put in the cassettes. And then, the embryonic specimens were processed including distilled water, stained with Mayer's carmalum, dehydration, clearing and mounting, respectively. the embryonic specimens to studied morphological malformations.

4. Results

The effects of ibuprofen in different concentrations on chick embryo day 3 were recorded on percentage of survival and mortality rate which compared with control group. Survival chick embryos were examined by observing heartbeat and blood circulation that compared to stage 18 of Hamburger and Hamilton in normal development chick embryo (Hamburger et al.,1992) showed in Table 1

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

Group	N (%)	Survival n (%)	Mortality n (%)
Control group	30 (100)	30 (100)	0 (0)
Ibuprofen at 1 mg/ml	30 (100)	25 (83.33)	5 (16.67)
Ibuprofen at 5 mg/ml	30 (100)	15 (50)	15 (50)
Ibuprofen at 10 mg/ml	30 (100)	12 (40)	18 (60)
Total	120 (100)	82 (68.33)	38 (31.67)

Table 1 showed the number and percentage of the mortality rate in the treated group which increased in higher concentration. The percentage of the mortality rate in the treated group included at 1 mg/ml, 5 mg/ml and 10 mg/ml were 16.67%, 50% and 60% mortality rate respectively and compared with control group.

The total mount of day 3 chick embryo

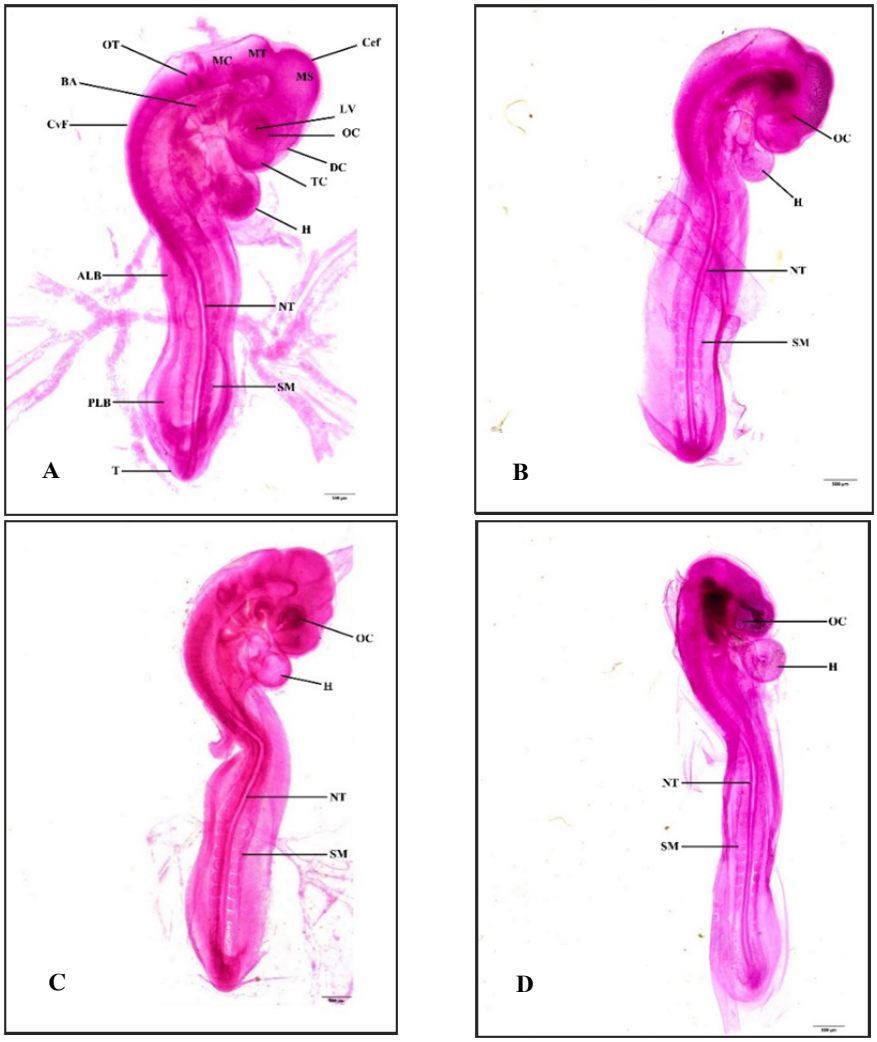

Figure 1 The micrograph of the development of day 3 chick embryos by total mount technique. A. control group, B. 1 mg/ml, C. 5 mg/ml and D. 10 mg/ml of ibuprofen treated groups. (TC : Telencephalon, DC : Diencephalon, MS : Mesencephalon, MT : Metencephalon, MC : Myelencephalon, OT : Otocyst, OC : Optic cups, LV : Lens vesicle, CeF : Cephalic flexure, CvF : Cervical flexure, H : Heart loop, BA : Branchial arch, ALB : Anterior limb bud, PLB : Posterior limb bud, NT : Neural tube, SM : Somite, T : Tail fold)

The control group of the development of day 3 chick embryos in **Figure 1 A** showed normal development. Normal characteristic typically as described by Hamberger and Hamilton as stage 18 or 36 somites

(Hamburger et al., 1951). In total mount showed head and body lifting. Head twisted 90 degree in longitudinal axis and flexed, producing 2 flexures : a cephalic flexure at mesencephalon and cervical flexure between myelencephalon and spinal cord. At upper part, brain developed into 3 parts of forebrain, midbrain and hindbrain. Forebrain was divided into telencephalon and diencephalon. Midbrain gives rise to mesencephalon. Hindbrain was divided into metencephalon and myelencephalon. Eye located at level of diencephalon which showed large optic cup with lens locating at the center. At the level of myelencephalon, the closed vesicle of otocyst was identified. At cervical flexure, level of stomodeum showed 4 branchial arches. Right side of the body showed heart tube. Normal heart of this period showed S-shaped loop. Heart was divided into 4 parts : sinus venosus, atrium, ventricle, and bulbus cordis, respectively. It composes of sinus venosus, atrium, ventricle and bulbus cordis. Each structures was varied in development while ventricle was the largest area and well development. At the middle of body showed two parallel lines of neural tube with somites which adjacent to it and extended to the caudal end. In addition the body showed anterior limb bud and posterior limb bud. The caudal end also showed a tail fold.

In treated group of ibuprofen showed the abnormality of several structures compared with the control group. Ibuprofen at 1 mg/ml in **Figure 1 B** showed unclearly development of brain into 3 parts. Chick embryo had small eye and otocyst although they were normal in shape. The branchial arches were absent. The heart loop was not formed s-shape (abnormal looping of heart). Ibuprofen at 5 mg/ml in **Figure 1 C** showed retardation of brain development only 3 parts which consisted of prosencephalon,mesencephalon and rhombencephalon. The branchial arches were absent. Heart loop was like U-shaped. The eye and otocyst were very small when compared with the control group. Somites were incomplete formation. The limb bud and tail fold were absent. Ibuprofen at 10 mg/ml in **Figure 1 D** showed the embryo was too severely smaller than the control group. Retardation of brain development by 3 parts presented without telencephalon development. there are only 3 parts. Both eyes were abnormal smaller in sige designed as microphthalmia. The observation revealed abnormal loop of heart, absent branchial arches, retardation somite (appear only ¾ of body), no limb buds and tail fold.

5. Discussion

The present study the development of day 3 chick embryos by total mount technique in all experimental groups showed growth retardation of several areas. Ibuprofen treated at the concentration of 1 mg/ml, 5 mg/ml and 10 mg/ml showed retardation with poorly development of cephalic and cervical flexures brain were not clearly developed, eye and otocyst were very small, The branchial arches were absent. Ibuprofen treated at the highest concentration of 10 mg/ml showed the most severity of retardation, abnormal brain development and microphthalmia. All of concentration in treated group, Development of heart showed U-shape looping, the branchial arches were absent. Somites extended to ¾ of body with the absent of limb bud and tail fold. These effects of ibuprofen are attributed to the inhibition of prostaglandin (PG) synthesis mediated by either of COX

isoenzymes, cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2).(Prescott and Yost., 2002)(Grosser et al., 2002)

The result corresponding with early studies regarding NSAIDs in animal models have demonstrated the effects of acetaminophen (paracetamol) in the embryonic development of zebrafish, *Danio rerio* (John Wiley & Sons, Ltd, 2009) reported that the higher concentration of ibuprofen cause increasing the mortality rate, and severely impaired in embryos which exposed. In 2009, there was a study developmental anomalies induced by a non-selective COX inhibitor (ibuprofen) in zebrafish (*Danio rerio*), the results indicate that exposure to higher doses of ibuprofen caused retarded development, induces mortality and the compound is potentially teratogenic in zebrafish embryos, thus posing threats to zebrafish development. (David and Pancharatna, 2009)

In conclusion, the teratogenic effect of high concentration of ibuprofen was reported that it affected to the 3rd day chick embryo induced growth retardation ana abnormality of organs more severe that low concentration of ibuprofen. It was concluded that the higher the concentration of ibuprofen cause increasing the mortality rate. However, the congenital retardation and malformation exhibit in present study showed ibuprofen is teratogenic agent. Thus, the pregnant women should avoid consuming this during pregnancy. So, the further molecular mechanisms which lead to toxicity and teratogenicity of ibuprofen are to be explored. The evaluation of ibuprofen teratogenicity in other models is also necessary.

Acknowledgements

This thesis was supported from my major advisor and co-advisors for all suggestions. I would like to thanks all advisors and technical assistances from Department of Anatomy, Faculty of Medicine Siriraj hospital, Mahidol University for support services, animal model and microtechnique laboratory.

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