THE TERATOGENIC EFFECT OF METFORMIN ON THE DEVELOPMENT OF CHICK EMBRYO

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ABSTRACT

Diabetes mellitus is a common complication of pregnancy, which affects mother and fetus, and lead to serious complications. Metformin is primarily used for type 2 diabetes and has been used in a woman with gestational diabetes. There is still no information to confirm the embryotoxic or teratogenic of this drug. This study aimed to evaluate the teratogenic effect of metformin on the development of chick embryos by comparing between experimental groups and control group. The fertilized eggs were divided into two main groups; group A was the control group (normal saline injected) and group B was the experimental group (metformin injected). The experimental group was subdivided into four subgroups with different concentration of metformin injection. Eggs from group A and group B were injected at 21-hour incubation and were opened on day 3 of incubation. The experimental group with the highest concentration of metformin showed the delay in neural tube closure and growth retardation of several organs. The mortality rate was correlated with the increase in the concentration of metformin. Metformin with high concentration affected to morphology and growth rate in developing chick embryos.

Keywords: Metformin, teratogenic, developing chick embryo

1. Introduction

Diabetes mellitus is a common complication of pregnancy, it can be divided into 2 types are pre-gestational diabetes mellitus (type 1 and type 2 diabetes mellitus) and gestational diabetes mellitus (GDM) [1]. Pregnant women may have diabetes before or during pregnancy. Diabetes on pregnancy has been associated with increase rate of fetal loss in early and late pregnancy, as well as congenital defects, accelerated fetal growth and increased risk of shoulder dystocia and birth trauma.

Metformin is primarily used for type 2 diabetes, but it is increasingly being used in polycystic ovary syndrome (PCOS), because of these two conditions are associated with insulin resistance. Metformin has been used in woman with gestational diabetes and it is currently classified as a category B drug during pregnancy. The previous study, some animal studies showed metformin not cause changes in embryonic growth and no major malformations. One study examined the embryonicity of phenformin and metformin in the mouse embryo, the results indicated that phenformin has greater toxicity in mouse whole embryo culture, suggesting that metformin

might be safer to use during pregnancy [2]. Other studies regarding the use of metformin-treated in women with polycystic ovary syndrome (PCOS) throughout their pregnancies, the women who received metformin have had no adverse maternal side effects, and no birth defects have occurred. Therefore, metformin does not appear to be teratogenic [3].

In summary, no evidence to shows that metformin increased the risk of malformations in pregnancy. Accordingly, present need more information to confirm metformin is an embryotoxicity or teratogenicity to animal and human development. Thus, this study was to evaluate the teratogenic effect of metformin on the development of chick embryos. The gross morphological and histological change were studied from total mount and serial section.

2. Objectives of the study

This study was to evaluate the teratogenic effect of metformin on the development of chick embryos and can be applied the database to the future studies.

3. Materials and methods

The fertilized eggs *(Gallus gallus domesticus)* were obtained from Suwanvajok- kasikit Research Station, Department of Animal Sciences, Faculty of Agriculture, Kasetsart University. The study was divided into two main groups; Group A was the control group (normal saline injected, n=10) and group B was the experimental group (metformin injected, n=30). The experimental group was subdivided into three subgroups; B1, B2, and B3, each comprising 10 eggs. All eggs were cleaned with 70% ethanol before incubation to decrease external contamination. Incubation was under careful and standard monitoring. The temperature was maintained at 37℃, the relative humidity kept between 68-70%, and eggs were rotated every one hour. After 21 hours of incubation, eggs were brought out from the incubator. A hole was drilled into its upper blunt pole where the air sac was located. The control group was injected with normal saline 0.1 ml to the yolk sac. The experimental group was injected with 0.1 ml of metformin (B1: 1 mg/ml, B2: 1.5 mg/ml and B3: 2 mg/ml) into the yolk sac. After injection sealed the hole with adhesive tape and bring it back to continue incubating. Eggs from group A and group B were opened on day 3 of incubation.

Experimental procedure

After the eggs were opened, the embryonic specimens of day 3 were washed in normal saline and then preserved in Dietrich's FAA solution and fixed for 4 hours. The specimens were selected for total mount and serial section to study morphology and histology. The total mount slide was stained by Mayer's staining and the serial section was processed for tissue processing routine, embedding in paraffin and stained with hematoxylin and eosin. The slides were mounted and observed the results under a light microscope.

Data analysis

The data on survival and mortality rates were showed in percentage and the slide of chick embryos with 3 different concentrations of metformin were analyzed compare with control (normal saline solution).

4. Results

The survival and mortality rate

The result showed that the survival rate of the experimental groups, the concentration of metformin injection from 1, 1.5 to 2 mg/ml., were decreased from 100, 85.71 and 60% respectively, while the survival rate of control group was 100% . The mortality rate the experimental groups were increased from 0, 14.29 and 40% respectively (Table 1.). The survival chick embryos were continue studied developmental malformations by total mount and serial section.

Table 1. The percentage of survival and mortality of embryos on day 3 of chick embryos which were injected by 3 different concentrations of metformin compared with control group.

Group (mg/ml)	Survival (%)	Mortality (%)
Control (n=10)	100 (10)	0 (0)
1 (n=10)	100 (10)	0 (0)
1.5 (n=7)	85.71(6)	14.29(1)
2 (n=10)	60 (6)	40 (4)
Total (n=37)	86.49 (32)	13.51 (5)

Total mount

The day 3 of chick embryos or 72 hours after incubation can be referred to stage 18 of the Hamburger characterization, this stage was indicated by having 36 somites. In this stage, it appeared head and tail fold and several organs such as optic vesicle, lens vesicle, optic cup and olfactory pit. The heart loop showed S-shaped. Anterior and posterior limb buds were presented. The control group showed normal development of chick embryos. In experimental groups, the concentration of 1, 1.5 and 2 mg/ml of metformin showed a growth retardation and abnormalities of brain vesicle, the cervical flexure in the head region and heart loop when compared with the control group (Fig.1).

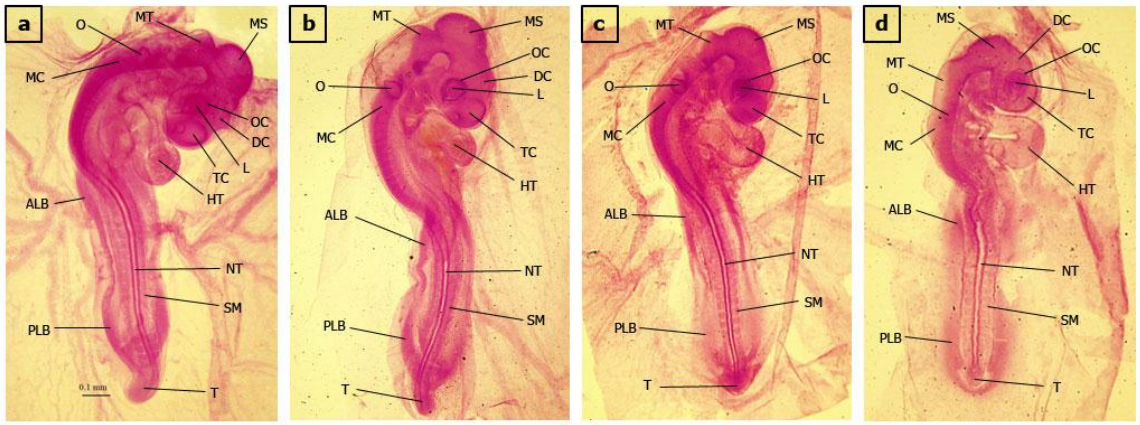

Figure 1. The micrograph of total mount preparation of the 3rd day of chick embryo in the control group (a), 1 mg/ml (b), 1.5 mg/ml (c) and 2 mg/ml of metformin injected (d). (DC: Diencephalon, TC: Telencephalon, MS: Mesencephalon, MT: Metencephalon, MC: Myelencephalon, OC: Optic cup, L: Lens, O: Otocyst, HT: Heart loop, ALB: Anterior limb bud, PLB: Posterior limb bud, SM: Somite, NT: Neural tube, T: Tail fold)

Serial section

The serial section was processed to study histological of day 3 chick embryos and to evaluated malformation of several organs such as ear, eye, heart, pharyngeal arches, liver and neural tube. The control group showed normal development. In the experimental groups of 1 and 1.5 mg/ml of metformin showed growth retardation when compared with the control group. From stage 16 of the Hamburger characterization the neural tube has become closed dorsally along its whole length. In the concentration of 1 and 1.5 mg/ml of metformin showed the neural tube closed but the concentration of 2 mg/ml of metformin showed the delay in neural tube closure (fig. 2).

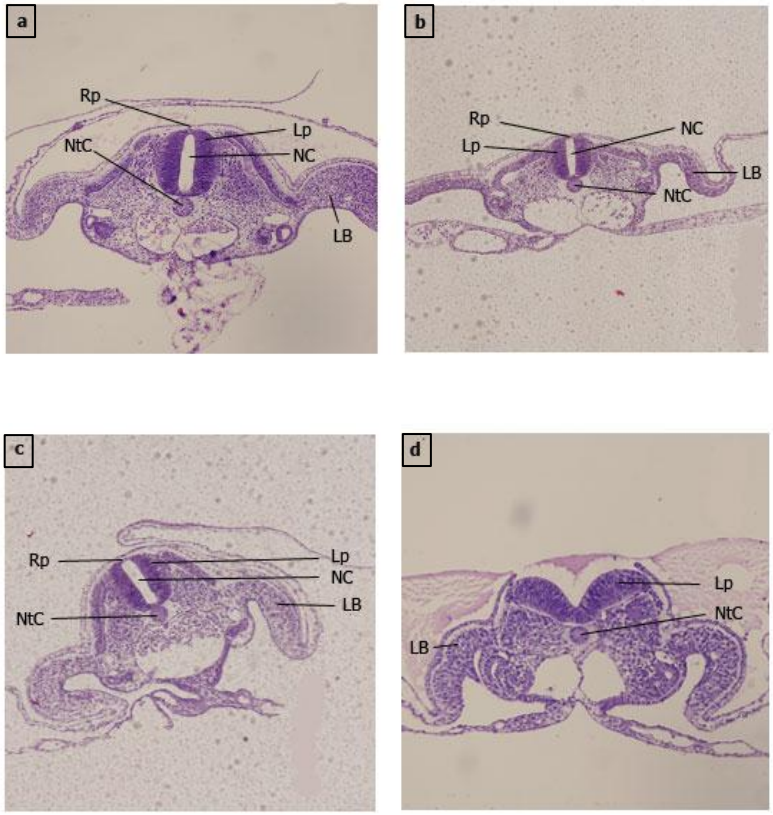

Figure 2. The micrograph showed serial section of the 3rd embryonic day at the level of spinal cord in the control group (a), 1 mg/ml (b), 1.5 mg/ml (c) and 2 mg/ml of metformin injected (d). (Rp: Roof plate, Lp: Lateral plate, NtC: Notochord, NC: Neural canal, LB: Limb bud).

5. Discussion

Metformin is an antihyperglycemic agent which improves glucose tolerance in patients with type 2 diabetes, lowering both basal and postprandial plasma glucose. The most common adverse effect of metformin is gastrointestinal irritation, including diarrhea, cramps, nausea, vomiting, and increased flatulence. The most serious potential side effect of metformin use is lactic acidosis. In women with PCOS are frequently insulin resistant, and hyperinsulinaemia is thought to be a major contributing factor to its pathogenesis by increasing ovarian androgen secretion and decreasing the synthesis of sex hormone binding globulin by the liver. Metformin used at a dosage up to 1500 mg/day can decrease body weight, insulin, total and free testosterone and luteinizing hormone (LH) levels. The previous studies showed the rate of early pregnancy loss as high as 30–50% in women with PCOS, the continuation of metformin therapy throughout gestation has been associated recently with a dramatic decline in the miscarriage rate. These data cause metformin is currently classified as a class B drug during pregnancy [4].

Only a few studies have described the use of metformin during the first trimester of pregnancy for the control of maternal diabetes mellitus. There are studies regarding the use of metformin treated in women with polycystic ovary syndrome (PCOS) throughout their pregnancies, the result is the women receiving metformin have

had no adverse maternal side effects, and no birth defects have occurred. Sonography showed normal fetal development without congenital defects in the ongoing pregnancies. Thus, metformin therapy throughout pregnancy in women with PCOS reduces the otherwise high rate of first-trimester spontaneous abortion seen among women not receiving metformin and does not appear to be teratogenic [3].

For this study to evaluate the teratogenic effects of metformin on day 3 of chick embryos, the result showed the concentration of exposure teratogen and effect to the development with a high factor to fetal resorption. The mortality rate increased from dose 1.5 mg/ml to 2 mg/ml. In the results of total mount showed a growth retardation and abnormalities such as the small and unclear of brain vesicle, the heart loop was loosened and the cervical flexure was not completely bending. In the results of serial section, a highest concentration of metformin showed the delay in neural tube closure. The neural tube formation might be interrupted by metformin. According to Denno and Sadler in 1994, studied the embryotoxic effects of metformin in mouse embryos when exposed metformin 500–2,550 mg per day and 50–400 mg per day for 24-48 hour. Metformin showed a delay in neural tube closure but did not result in gross morphological abnormalities. However, it showed no alterations in embryonic growth and no major malformations.

According to the result of teratogenic study of metformin were increasing the mortality rates, it depends on concentration. It showed abnormalities and retardation to structures and affected to several organs. So usage of metformin in pregnant woman that without knowledge can be harmfuls.

6. Conclusion

Metformin is primarily used for type 2 diabetes and used in polycystic ovary syndrome (PCOS), this study was about teratogenic effects of metformin on chick embryos. The percentage of mortality increased as using the high dose of metformin solution. The concentration of 2 mg/ml. showed the delay in neural tube closure.

The teratogenic effects of metformin were the induction of retardation and abnormality of several organs including eye, otocyst, limb, heart and spinal cord and induced to severity abnormal such as anencephaly, microphthalmia/anophtalmia, ectopia cordis, abnormal of otocyst and abnormal heart loop. Thus, the further study of the teratogenic effect of metformin should be developed in other animal models to gain more knowledge about diabetes drug in a pregnant woman.

Acknowledgements

I would like to offer my special thanks to my research advisors for their suggestions during the planning and development of this research work, and the technicians of the microtechnique laboratory for their support in the process of materials and methods.

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