Original Article

Preventive Role of Folic Acid in Artemether-Induced Teratogenicity on Fetal Heart in Albino Mice

Muhammad Shahid Akhtar, Fatima Inam and Muhammad Amin

Objective: To determine the role of folic acid in preventing the adverse effects of artemether, on fetal heart in Albino mice.

Methods: Eighteen pregnant Albino mice were randomly divided into three groups A, B and C each consisting of six mice. The control group A was given intramuscular injection of solvent Arachis oil 10.7mg/kg, group B was given intramuscular injection of artemether 10.7mg/kg and group C was given intramuscular injection of artemether, 10.7mg/kg and folic acid 4.93 mg/kg, dissolved in 0.1 ml. of distilled water orally, from 6th to 10th day of pregnancy. On 18th gestational day, the mice were sacrificed, dissected to deliver the fetuses; the heart of each fetus was isolated and fixed in 10% formalin. The macroscopic features and cardiac weight were noted. Then it is processed in a usual way for histological examination with the light microscope after H&E staining, using X4, X10 and X40 objectives. The sections were evaluated for any histological changes and septal defects. For statistical analysis, SPSS version 18 was used.

Results: Gross examination of the heart revealed no structural malformation. Post-Hoc Tukey's test indicated statistically significant difference in mean cardiac weight between groups A and B (p=<0.001), groups B and C (p=<0.001) and between groups A and C (p=<0.001). No atrial and ventricular septal defects observed. Histological examination showed myocardial development in current study was delayed in groups B and C, showing presence of mesenchymal cells.

Conclusions: Folic acid prevented the adverse effects of artemether on the development of heart.

Keywords: artemether, cardiac weight.

Introduction

Any agent that induces structural malformations and abnormal development or increase the incidence of an anomaly in the population is called a teratogen.[1] Malformations caused by drugs and other therapeutic agents are important, however, we should be careful in prescribing these drugs during pregnancy, which cause teratogenicity.[2]

For DNA and RNA synthesis, folic acid, a component of vitamin B complex is required. It promotes erythropoiesis and is used commercially in food supplements.[3] It is a dietary requirement, since it cannot be synthesized by the body and only 10 percent or less of folic acid in active form is present in the normal diet.[4] The risk of congenital anomalies, like cardiac anomalies induced by retinoic acid (Vitamin A), craniofacial deformities, neonatal mortality from neural tube defects, cleft lip and palate and imperforate anus are reduced by periconceptional use of folic acid.[5-8]

New antimalarial drugs like artemether and other derivatives of artemisinin, artesunate, dihydro artemisinin and arteether are now used commonly for the treatment of malaria due to emergence of malarial parasites, resistant to drugs, like chloroquine and quinine. The mechanism of action of artemether and other artemisinins against malaria is not well understood, despite the extensive research. It is suggested that production of the free oxygen radicals are responsible for killing the parasite.[9]

As resistance to quinine is common in Asia and decrease of sensitivity to quinine has been reported in Africa, intramuscular artemether appears to be an excellent alternative to intravenous quinine.[10] Pregnant women are highly susceptible to malaria infestation. Malaria during pregnancy, causes severe maternal complications like abortion, premature labour and still-births which are higher in Plasmodium falciparum infestation.[11] Severe maternal anemia due to malaria is a leading cause of maternal mortality.[12,13]

A study was conducted in New Halfa Hospital eastern Sudan, from October 1997 to February 2001; twenty-eight pregnant women infested with Plasmodium falciparum, after failure of chloroquine and quinine therapy, were given artemether intramuscularly (six injections, a total of 480 mg) during three different periods of gestation. One patient was treated with injection of artemether in the tenth week of twelve patients received the drug during the second

trimester and fifteen patients during the third trimester. The patients were free of symptoms within three days as the parasite was eliminated from the blood and the drug was well tolerated. Only one patient (3.5%) delivered at 32 weeks and six hours after birth the baby died. There were no abortion, stillbirth and congenital anomalies in the newborn and there was no mortality reported among the pregnant women.[10] When artemether and other derivatives of artemisinin were given orally or by injection, during vulnerable period of organogenesis, caused death of the embryo, blood vessels anomalies, ventricular septal defects, malformed ribs, shortened or bent long bones, defects in scapulae and incompletely ossified pelvic bones in rodents. These embryotoxic effects were due to destruction of primitive erythroblasts, which are present early in the developing embryo, by artemether and other derivatives of artemisinin, resulting in transient deficiency of the primitive erythroblasts.[14] Artemether and other derivatives of artemisinin are not recommended in the first trimester of pregnancy due to limited safety data on its use in human, although these can be given during second and third trimesters. Various studies showed that artemether caused miscarriage and cardiac anomalies like ventricular septal defect when given during vulnerable period of gestation in animals, whereas preventive role of folic acid on cardiac anomalies induced by it has not been previously investigated. The present study was therefore designed to investigate the preventive role of folic acid on the adverse effects of artemether on development of heart in Albino mice.

Methods

It was a randomized controlled experimental study conducted at the Department of Anatomy, University of Health Sciences, Lahore. Twenty four adult BALB/c strain Albino mice (eighteen females and six males) 6 - 8 weeks old, weighing from 30 to 35 grams were kept under control conditions of temperature 23 ± 2 0C, humidity 50 ± 5 % with regular 12 hours light/dark cycles. Male and female mice were put together in a ratio of 1:3 in a single cage for mating. Females were examined early morning everyday for the presence of vaginal plug; its presence indicated that mating had occurred, the day was considered as day 0 of gestation. The pregnant mice were separated, housed in a separate cage and randomly divided into three groups; A, B and C having six female mice each. Commercially available preparations of artemether, folic acid 97% and Arachis oil, the solvent for preparation of artemether injection were used. Group A was treated with single intramuscular injection of solvent, Arachis oil 10.7mg/kg, Group B was given artemether, 10.7mg/kg once daily by intramuscular injection and Group C was treated with artemether, 10.7mg/kg by intramuscular injection and folic acid 4.93 mg/kg in 0.1 ml. of distilled water once daily, from 6th to 10th day of pregnancy.

The pregnant mice were sacrificed on the 18th gestational day to deliver the fetuses.14 All live male and female fetuses were included in the study. The live fetuses were then euthanized with chloroform, examined for any gross malformations under dissecting stereo microscope and fixed in 10% formalin solution for 72 hours after decapitation. The thoracic cavity of the fetuses was opened by midline thoracoabdominal incision; heart was identified and observed under dissecting microscope for its position and that of the great vessels and any visible gross anomalies. The heart was carefully dissected and removed with the root of great vessels for histological examination. The heart was isolated immediately after the animal was sacrificed and rapidly washed with distilled water to clear its blood contaminants, weighed and fixed in 10 % formalin for 48 hours. The fixed complete fetal hearts were processed in automatic tissue processor. The tissue blocks were made; sections were cut at 5µm thickness and mounted on the albumenized glass slides, which were allowed to dry on a slide warmer. These sections were stained with Hematoxylin and Eosin (H&E) for histological study.15 The stained sections were studied under light microscope using X4, X10 and X40 objectives. These sections were evaluated for presence of the atrial and ventricular septal defects. The collected information of the study groups was analyzed using Statistical Package for Social Sciences (SPSS) version 18. The difference in the quantitative measurement was tested by one way ANOVA. Post-Hoc Tukey's test was applied to identify which group mean differed. Relevant descriptive statistics was mean and Standard deviation. The p-value of ≤ 0.05 was considered statistically significant.

Results

1. Macroscopic features of the heart:

The heart of each animal in control and experimental groups were light brown in colour. The position of heart, lungs and diaphragm were normal in fetuses of

trimester and fifteen patients during the third trimester. The patients were free of symptoms within three days as the parasite was eliminated from the blood and the drug was well tolerated. Only one patient (3.5%) delivered at 32 weeks and six hours after birth the baby died. There were no abortion, stillbirth and congenital anomalies in the newborn and there was no mortality reported among the pregnant women.[10] When artemether and other derivatives of artemisinin were given orally or by injection, during vulnerable period of organogenesis, caused death of the embryo, blood vessels anomalies, ventricular septal defects, malformed ribs, shortened or bent long bones, defects in scapulae and incompletely ossified pelvic bones in rodents. These embryotoxic effects were due to destruction of primitive erythroblasts, which are present early in the developing embryo, by artemether and other derivatives of artemisinin, resulting in transient deficiency of the primitive erythroblasts.[14] Artemether and other derivatives of artemisinin are not recommended in the first trimester of pregnancy due to limited safety data on its use in human, although these can be given during second and third trimesters. Various studies showed that artemether caused miscarriage and cardiac anomalies like ventricular septal defect when given during vulnerable period of gestation in animals, whereas preventive role of folic acid on cardiac anomalies induced by it has not been previously investigated. The present study was therefore designed to investigate the preventive role of folic acid on the adverse effects of artemether on development of heart in Albino mice.

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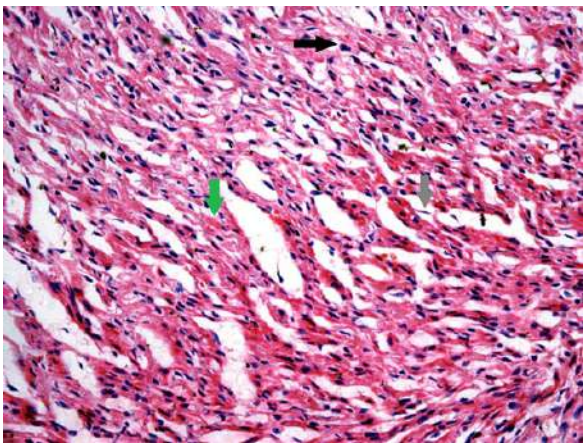

Fig-3: Photomicrograph of myocardium of ventricular wall of group C; myocardial cells are partially mature having branching pattern, with oval to elongated nuclei (black arrow), unstriated cytoplasm (green arrow) and having mesenchymal cells (gray arrow) (Stain H&E. X400).

groups. Gross examination of the heart revealed no structural malformation. Histological examination showed myocardial development in current study was delayed in groups B and C, showing presence of mesenchymal cells (Fig. 2 and 3). This observation had not been reported earlier as an effect of artemether and other derivatives of artemisinin on myocardium. Cardiac weight of animals were reduced in groups B and C and were reduced statistically significant when compared to group A. There were statistically significant decrease in cardiac weight, in group B when compared with group C **(Table 1 and 2).**

The first system which develops in embryo is cardiovascular system, which provides nutrients to the developing embryo. In mouse it begins on 7th day of gestation.[16, 17] Ventricular septal defects and various defects of skeleton, like malformed ribs, cleft sternebrae, shortened or bent long bones were seen in orally administered artesunate, a derivative of artemisinin, in pregnant rats, at doses of 6, 10 and 16.7 mg/kg, once daily, starting from Day 6 of gestation for 12 days throughout, the period of organogenesis. In rabbits, however doses of 5, 7 and 12 mg/kg once daily, starting from Day 6 of gestation for 13 days produced comparable results.[18, 19] There was an increased incidence of anomalies, when a single oral dose of 17 mg/kg of artesunate was given on day 10 of gestation to rats; in rabbits the embryolethal effect was observed as abortions and total loss of litter. These developmental effects were seen without any evidence of maternal toxicity.[18,19] It has been observed that, abnormalities of heart appeared in the rat embryo, after a single oral administration of 17 mg/kg of artesunate on day 10 of gestation; these changes are manifested in the form of reduced thickness of ventricular and atrial walls and cardiac cavity due to thin trabeculae carneae which became more evident over the next few days. The heart showed signs of recovery in the rat embryo that survived to day 14 of gestation, but its development got retarded.[20]

As evident from various studies, supporting the conclusion that periconceptional multivitamin supplementation containing folic acid may reduce the risk of congenital heart defects; the Hungarian randomized clinical trials in 1984-1991 have demonstrated that the risk for congenital anomalies of cardiovascular system and urinary system was reduced significantly after the periconceptional multivitamin supplementation containing folic acid.[21]

Conclusion

This study investigated the preventive role of folic acid on the adverse effects of artemether on the developing heart of the mouse. Cardiac weight was significantly decreased in artemether treated group. The statistically significant improvement in cardiac weight was observed in folic acid treated group. The values of the parameters were nearly comparable to those in the control group and maturation of myocardial cells on histological examination is evident indication of preventive effect of folic acid on heart.

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