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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (24): 2665-2669, 2008
ISSN 1028-8880

Histopathological Effect of Enalapril Maleate on Fetal Heart in Rat

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Abstract: Enalapril Maleate is an antihypertensive drug that reduces blood pressure by dilatation of blood vessels as well as diminishing systolic and diastoles blood pressure. We wanted to assess its histopathology effects on fetal heart in rat. In this survey twenty male and female Wistar rats were selected and matched together, after getting pregnant, they divided into two groups: control group (n = 10) and test group (n = 10). The test group was administered 0.4 mg kg^{-1}, Enalapril maleate daily during pregnancy. Heart tissue of the neonates was provided for investigation throughout light microscopic observation (LM) in the first day of birth. Microscopic studies of the heart tissue slices in the test group revealed histopathological change in myocardial cells in comparison with the control group. Body weight of neonates in test group was reduced in comparison with control group ($p<0.05$). Since, in present study Enalapril maleate presented side effect on fetal heart development in rat, consequently it is suggested not to use this drug for human being during pregnancy.

Key words: Enalapril maleate, fetal heart, rat, pathologic

INTRODUCTION

The most likely users of antihypertensive drugs during gestation including Angiotensin Converting Enzyme (ACE) inhibitors, are women with hypertensive pregnancy complications who have already been reported with low background levels of Angiotensin II(ANGII), (With regard to the pregnancy complication per se) and also elevated background risk of associated adverse effects on fetus. ACE inhibitors add further decrease in lower ANGII women, which is physiologically necessary for normal fetal development. Enalapril maleate is an antihypertensive drug of the class of ACE inhibitors that used in pregnancy for treatment of pre-existing or pregnancy-induced hypertension (Amy and Karchy, 2001) and they have been used for treatment the women in reproductive age, in comparison with conventional antihypertensive agents (Joseph *et al*., 1999) and enhancement the distribution of blood flow to the kidneys, heart and brain with no alteration in cardiac output (Johnston, 1984). Enalapril maleate can increase Plasma-Rennin Activity (PRA) factor, obeying negative feedback of releasing rennin from kidney or increasing the stimulation of bar receptors (Ayan *et al*., 2001). In first step, ANGII causes decrease in secretion aldosterone from cortex of adrenal gland (De Mello, 2003). During pregnancy, It is seen a physiologic increase of rennin, ANGII and prostaglandin E_2 (PGE_2) in the plasma and uterus in both women and female animals (Rabbit, Dog), (Rcoustan and Nochizuki, 1998; Sonia and Carole, 2001) When ANGII synthesis is blocked, there is a fall in uterine PGE_2 synthesis, a slump in uterine blood flow, and a rise in fetal mortality (Ferris and Weir, 1983). The identification and quantification of ANGII receptors in developing rat heart (Hunt *et al*., 1995) shows an active role for Rennin-Angiotensin-Aldosterone System (RAAS) during early cardiac development. The aim of this study is demonstration the side effect of Enalapril maleate on the heart tissue of fetal rat.

MATERIALS AND METHODS

For this survey, 20 male and 20 female Wistar rats were selected, these animals were chosen for the study by the effort of Department of Reproductive Endocrinology and Embryology of Avicenna Research Institute (ACECR), Tehran, Iran. Their ages were approximately 17 weeks and their body weights were 300±20 g. They were maintained in a 12-12 h light-dark cycle (lights on at 9:00 am and off at 9:00 pm) room, with constant temperature (23.9-25.3°C) and appropriate humidity (55-60%). All animals were treated in accordance with the Principles of Laboratory Animal Care (National Institute of Health, 1985). After adjustment the first day of

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gestational age we divided 20 female rat into two groups, first group as the control group (n = 10) and second group as the experimental group (n = 10). We fed control and experimental groups, by plate, each rat took 20 g food daily. Enalapril maleate (Sigma Chemical Co., St. Louis, USA); 0.4 mg kg^{-1} b.wt. was used during pregnancy for 21 day, in experiment group and the method of drug administering was oral gavages. After delivery, the body weight of the neonates and heart weight of neonates in both groups (control and experimental) were assessed. At the end of the experiment, the animals were anesthetized with diethyl ether and killed by decapitation between 9:00 and 11:00 am and the neonates were killed by CO_2 inhalation.

Determination of the reproductive performance: Individual males were placed in a cage with a healthy adult female (body weight, 200±20 g). The animals were kept together overnight and were separated the following morning. Immediately after each separation, a vaginal scrape was performed to determine the presence of spermatozoa. Procedure for nights repeated continuously when the vaginal scrape was negative. A vaginal scrape was considered positive while the presence of a vaginal tap, adhered spermatozoa, or both was seen. When the vaginal scrape was positive the night-day routine was stopped and consequently the female was housed individually (one rat per cage) during the estimated period from conception till delivery.

Histological techniques for optic microscopic Observation: Myocardial Tissues of neonates (control and experimental) were immediately fixed in 3% formaldehyde in a buffered solution in both groups, containing 54 mM NaH_2PO_4 and 28 mM Na_2HPO_4 (pH 7.4) at 4°C (buffered formaldehyde), Additionally, Formaldehyde-fixed samples of myocardial tissue of both groups (control and experimental) were embedded in paraffin and then sliced (slice thickness, 5 mM) on silane-precoated slides. Later, they were deparaffined with xylol, then histological observations were performed after staining by the hematoxylin-eosin method (Stevens, 1982). Slides were examined under Microscopic observation by Olympus/3H microscope.

Statistical analysis: We used t-test to compare fetal body weight and heart weight in control group with the experiment group; the results expressed as Mean±SEM (standard error of means). A p-value that was less than 0.05 were considered as significant.

RESULTS

Neonates body weight average in control group was 5.98±0.378 and this criteria in experimental group was 4.58±0.55. As a conclusion, that body weight in test group was less than control group. The difference rate of birth weight in two groups was $p<0.05$ (Fig. 1). Neonates heart weight average in control group was 59±7.41 mg and this criteria in experimental group was 43±4.80 mg. As a conclusion, that heart weight in test group was less than control group. The difference rate of birth weight in two groups was $p<0.001$ (Fig. 2). The ratio of neonates heart

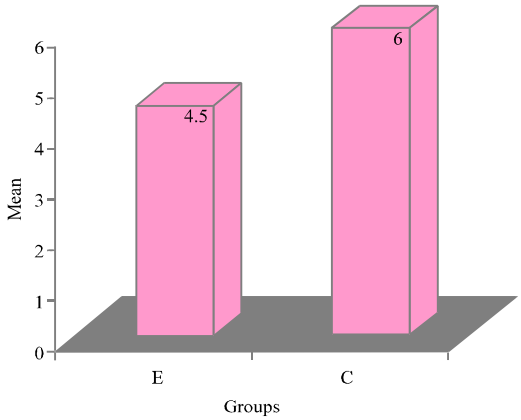

Fig. 1: Comparison of neonates weights in experimental group that received Enalapril maleate (E) and control group (C). Weight of neonates in experimental group was significantly different to control group ($p<0.05$, t-test)

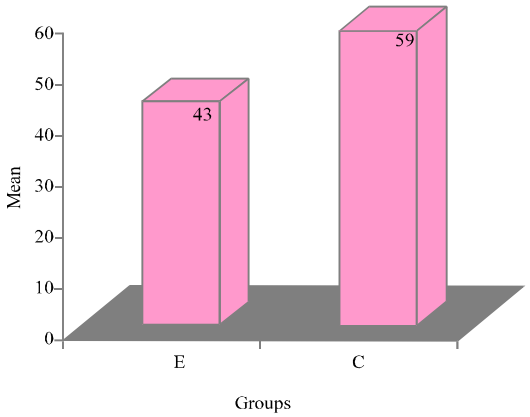

Fig. 2: Comparison of neonates heart weights in experimental group that received Enalapril maleate (E) and control group (C) Weight of heart in experimental group was significantly different to control group ($p<0.001$, t-test)

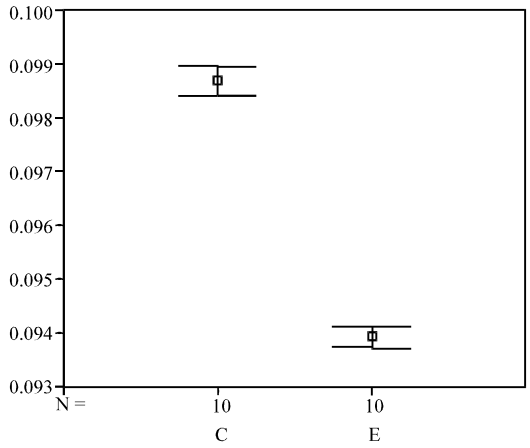

Fig. 3: Comparison of neonate heart weights ratio in experimental group that received Enalapril maleate (E) to control group (C). Weight of heart in experimental group was significantly different to control group ($p<0.05$, t-test)

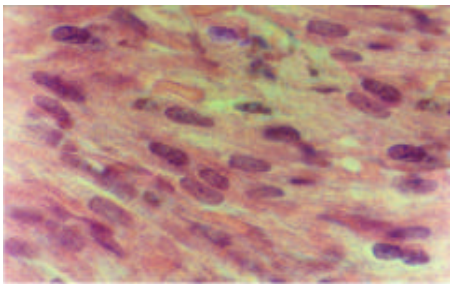

Fig. 4: Micrograph of rat neonate heart muscle slide in control group, H and E (x640)

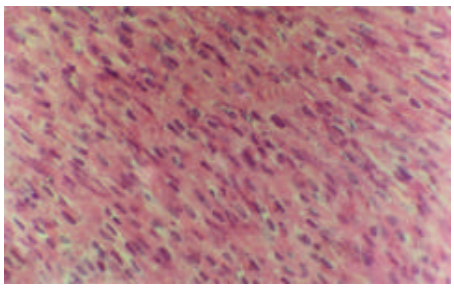

Fig. 5: Micrograph of rat neonate heart muscle slide in control group, H and E (x640)

weight to neonates body weight in control group was 0.0098±0.0003 and the ratio of neonates heart weight to neonates body weight in test group was 0.0093±0.0002. The difference rate of birth weight in two groups was $p<0.05$ (Fig. 3). Micrographic assessment of heart muscle in neonates rat of the control group demonstrated cardiac muscle contains normal structure, furthermore the nucleus

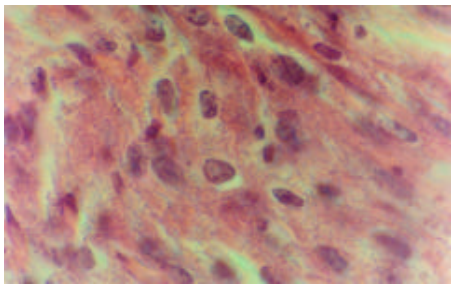

Fig. 6: Micrograph of rat neonate heart muscle in experimental group showed polymorph nuclei with perinuclear clearing area and loses of striation pattern, H and E (x640)

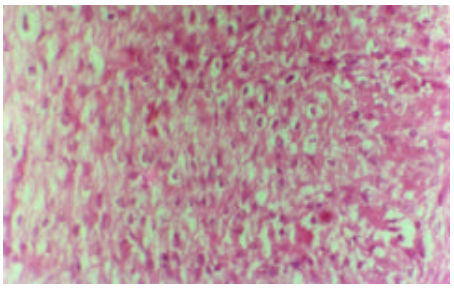

Fig. 7: Micrograph of rat neonate heart muscle slide in experimental group showed, hydropic degeneration in cytoplasm, H and E (400x)

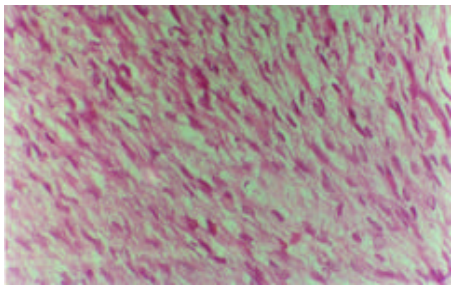

Fig. 8: Micrograph of rat neonate heart muscle in experimental group showed, myocardial cell was smaller and coagulative necrosis, H and E (x640)

size was normal (Fig. 4, 5). The same analysis in experimental group revealed that nucleus were enlarged with polymorphic appearance (arrow) and per nuclear clearing (triangle), area were developed (Fig. 6). Other micrographic analysis obtained from myocardial cell in experimental group presented cytoplasmic change such as hydropic degeneration (arrow) and coagulate necrosis (arrow) in myocardial muscle (Fig. 7, 8).

DISCUSSION

In nowadays the prevalence of cardiac hypertension disease is increasingly and called the new epidemic of cardiovascular disease (Braunwald, 1997). Enalapril maleate is an antihypertensive drug of the class of ACE inhibitors (Amy and Karchy, 2001). Important reasons for the higher vulnerability of the human fetus are its accessibility by Enalapril maleate and the earlier (relative to animal species) intrauterine development of organ systems (Al-Harbi *et al*., 1992). In the earlier study, researchers demonstrated maternal treatment process with Enalapril, one of the ACEIs exerts an inhibitory action on the spontaneous closure in heart ducts arteries of neonatal rat (Takizawa *et al*., 1994). When ANGII synthesis is blocked by ACEI drugs, there is a fall in uterine PGE_2 synthesis, a slump in uterine blood flow and a rise in fetal mortality (Ferris and Weir, 1983) and may result in neonatal renal failure in pregnancy (Hulton *et al*., 1990). Placental transferring of Enalapril has been demonstrated in the human (Reisenberger *et al*., 1996), nonhuman primate (Ducsay *et al*., 1996), sheep (Broughton and Wallace, 1986) and hamster. There is no documented report with regard to Enalapril (Barr and Teratogen, 1994) and there is little passage in the term of rat placenta (below 0.5 and 0.07% of the maternal plasma levels) has been found for other similarly structured ACE inhibitors, Imidapril and Quinapril, respectively (Dostal *et al*., 1991). Rennin-angiotensin system is active during fetal life and in comparison with adults, presenting the same procedure. The use of ACE inhibitors during the second and third trimester of pregnancy in humans is directly associated with specific laceration and injury on fetuses and neonates such as fetal death, fetal and neonatal morbidity including fetal cephalic hypoplasia, lungs hypoplasia and anuria fetal calvarias (Geraled, 1998; Pryde *et al*., 1993; Hall *et al*., 1977). Studying and research in rat revealed 200 mg kg^{-1} Enalapril maleate, with no teratogenity and toxicity for embryo however, in the fetal rats that received 1200 mg kg^{-1} Enalapril maleate, weight loss was seen. (Martinez-Castelao, 1998). On this basis and with consideration to above mentioned data, there is no animal model that would be an ideal predictor of Enalapril (and ACEI) developmental toxicity in humans. Among animal species, the most concordance of fetal pharmacodynamics with human being in the rhesus monkey is seen, but further studies are necessary to determine if similar developmental pathology is induced in this animal model with repeated administration of the drug during the relevant period of intrauterine development. In this study, administration of (0.4 mg kg^{-1}) Enalapril maleate, in gestational period may lead to weight loss in neonates rats and neonates heart weight that were born in experimental group -in comparison with control group. In other hand, photomicrographs of fetal heart rat muscle in experimental group showed Enalapril maleate, inducement cause to increasing perinuclear clearing area. in normal cell glycogen surrounds nuclei and in histological tissue proceeding it has been solved in solutions and created perinuclear clearing, but in our myocardial cell that were obtained from experimental group this perinuclear clearing area were increased, this finding may explain increasing of glycogen accumulation around the nuclei. Nuclei change showed highly denser nuclei with polymorphic appearance and pyknosis, karyorehhexis; karyolysis were seen and coagulate necrosis were observed in comparison with control group. Cytoplasm changes presented hydropic change and intracellular edema in myocardial cell in experimental group. It is concluded that above-mentioned result, this drug could be transferred through placenta and cause abnormal development of cardiac cells. This change could have detriments effect on the function of myocardial cells of this organ after birth.

ACKNOWLEDGMENTS

The authors wish to express their appreciations from the Departments of Pathology, Histology and Pharmacology, Tabriz University of Medical Sciences, Tabriz, Iran.

REFERENCES

Al-Harbi, M.M., O.A. Al-Shabanah, N.M. Al-Gharably and M.W. Islam, 1992. The effect of maternal administration of Enalapril on fetal development in the rat. J. Res. Commun. Chem. Pathol. Pharmacol., 77: 347-358.

Amy, M. and R.N. Karchy, 2001. Nursing Drugs guide. Lippincott and Wilkins Philadelphia, pp: 440-441.

Ayan, S., J.A. Roth, M.R. Freeman, S.H. Bride and C.A. Peters, 2001. Partial urethral obstruction dysregulates the renal renin-angiotensin system in fetal sheep kidney. J. Urol., 58: 301-306.

Barr, M. and J.R. Teratogen, 1994. Angiotensin-converting enzyme inhibitors. J. Teratol., 50: 399-409.

Braunwald, E., 1997. Shattuck lecture-cardiovascular medicine at the turn of the millennium: Triumphs, concerns and opportunities. N. Engl. J. Med., 337: 1360-1369.

Broughton P.F. and C.P. Wallace, 1986. The effect of Enalapril (MK-421), an angiotensin converting enzyme inhibitor, on the conscious pregnant ewe and her fetus. Br. J. Pharmacol., 87: 533-542.

De Mello, W.C., 2003. Further studies on the effect of intracellular angiotensins on heart cell communication: on the role of endogenous angiotensin II. J. Regul. Pept., 115: 31-31.

Dostal, L.A., S.N. Kim, J.L. Schardein and J.A. Anderson, 1991. Fertility and perinatal/postnatal studies in rats with the angiotensin-converting enzyme inhibitor, quinapril. J. Life Sci. Toxicol. Sci., 17: 684-695.

Ducsay, C.A., H. Umezaki, K. Kaushal, A. Barrish, Gilbert and J. Manson, 1996. Pharmacokinetic and fetal cardiovascular effects of enalapril at administration to maternal rhesus macaque. Am. J. Obstet. Gynecol., 75: 50-55.

Ferris, T.F. and E.K. Weir, 1983. Effect of captopril on uterine blood flow and prostaglandin E synthesis in the pregnant rabbit. J. Clinical Investig., 71: 809-815.

Geraled, K.M., 1998. Drug Information. American Society of Health-System Pharmacists Inc., Bethesda, USA., pp: 1328-1335.

Hall, J.E., A.C. Guyton, N.C. Trippodo, T.E. Lohmeier, R.E. McCaa and A.W. Cowley, 1977. Intrarenal control of electrolyte excretion by angiotensin II. Am. J. Physiol., 232: 538-544.

Hulton, S.A., P.D. Thomson, P.A. Cooper and A.D. Rothberg, 1990. Angiotensin-converting enzyme inhibitor in pregnancy may result in neonatal renal failure. Med. J., 78: 673-676.

Hunt, R.A., G.M. Ciuffo, J.M. Saavedra and D.C. Tucker, 1995. Quantification and localisation of angiotensin II receptors and angiotensin converting enzyme in the developing rat heart. J. Cardiovasc. Res., 29: 834-840.

Johnston, C.L., 1984. Clinical Pharmacology of Hypertensive Drugs Doyle. Elsevier, Amsterdam, pp: 272-311.

Joseph, R., G. Dipalma and J. Digregorrio, 1999. Basic Pharmacology in Medicine. 3rd Edn., McGraw Hill, New York, pp: 421-446.

Martinez-Castelao, A., M. Hueso, V. Sanz, J. Rejas and J. Alsina, 1998. Treatment of hypertension after renal transplantation: Long-term efficacy of verapamil, enalapril and Doxazosin. Kidney Int., 54: 130-134.

National Institutes of Health, 1985. The principles of laboratory animal care. National Institutes of Health publication, No. 86-23.

Pryde, P.G., A.B. Sedman, C.E. Nugent and M.J.R. Barr, 1993. Angiotensin-converting enzyme inhibitor fetopathy. J. Am. Soc. Nephrol., 3: 1575-1582.

Rcoustan, D. and T. Nochizuki, 1998. Hand Book for Prescribing Medication During Pregnancy. 3rd Edn., Lippincott Williams Wilkins, USA., pp: 1-192.

Reisenberger, K., C. Egarter, B. Sternberger, P. Eckenberger, E. Eberle and E. Weissenbacher, 1996. Placental passage of angiotensin-converting enzyme inhibitors. Am. J. Obstet. Gynecol., 174: 1450-1455.

Sonia, A.T. and A. K. Carole, 2001. Enalapril: pharmacokinetic/dynamic inferences for comparative developmental toxicity. J. Reproduct. Toxicol., 15: 467-478.

Stevens, A., 1982. The Hematoxylins. In: Theory and Practice of Histological Techniques, Bancorft, J.D. and A. Stevens (Eds.). Longman Group, London, pp: 109-122.

Takizawa, T., T. Oda, K. Arishima, M. Yamamoto, H. Somiya, 1994. Inhibitory effect of Enalapril on the constriction of the ductus arteriosus in newborn rats. J. Vet. Med. Sci., 56: 605-606.