Pakistan J. Zool., vol. 44(4), pp. 1045-1050, 2012.

Effects of Vitamin A on Fetal Kidneys in Albino Mice: A Histological Study

Uzma Naseer and Mohammad Tahir*
Department of Anatomy, University of Health Sciences, Khiaban-e-Jamia Punjab, Lahore-54600, Pakistan

Abstract.- This study describes the histological teratogenic effects of retinoic acid (RA), the active metabolite of vitamin A, on the fetal kidneys of the albino mouse. Twelve pregnant albino mice were divided into two groups, control and treated each of 6 mice. Mice of treated group were given high dose of retinoic acid (60 mg/kg/day) on 7th, 8thand 9thdays of gestation. The mice were sacrificed on 18th day of gestation; the fetal kidneys were removed for histological study. The fetuses in treated group showed high rate of fetal resorption. Kidneys were enlarged and showed extensive tubular necrosis with marked increase in the thickness of renal medulla. In view of this abnormality, it is suggested that vitamin A supplements and its metabolites like retinoic acid may be sparingly used for various diseases like dermatological disorders and malignancies during pregnancy.

Key words: Retinoic acid, tubulogenesis, kidney, histological changes.

INTRODUCTION

Retinoic acid (*RA*), an active metabolite of vitamin A is a requirement for vision, development and reproduction. During embryonic period it affects the normal development of different organs and tissues (Quadro *et al*., 2004). RA mediates these activities by binding to two families of nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs), each of these families comprises three isotypes (a, b and g)which affect transcription of a number of genes during vertebrate development (Snyder *et al*., 2005). In kidney both RARs and RXRs are cell specific in expression; these are therefore, practically responsible for activation of both receptors by their respective agonists (Wagner, 2001).

In addition to the nuclear receptors, there exist two cytosolic RA binding proteins, the cellular retinoic acid binding protein I and II (CRABP I & II). It has been proposed that the CRABPs could transport RA from cytoplasm to the nucleus where it would be transferred to the nuclear receptors (Sacchi *et al*., 1997), these safeguard the cells exposed to retinoic acid and buffer free retinoid concentrations in the cytoplasm by controlled synthesis and catabolism (McCaffery *et al*., 2003).

It is widely accepted that excessive *RA* intake during pregnancy results in a variety of craniofacial malformations, depending upon the dosage and gestational stage at the time of drug administration (Robens, 1970). In mammals, retinoids both in deficiency and excess are reported to be well known potent teratogens (Lammer *et al*., 1985). Development of the kidney is reported to be the result of interactions between the metanephric and ureteric bud mesenchyme in the presence of retinoids (Vilar *et al*., 1996). In early 1950s, Wilson *et al*. (1953) observed that maternal vitamin A deficiency resulted in renal hypoplasia in rats which was reported to have been prevented by vitamin A administration to pregnant animals. This was suggestive of direct involvement of vitamin A in kidney development. In another study, double knockout models *i.e.* mice in which both RAR and RXR have been blocked, produced several malformations, including kidney agenesis, hypoplasia or aplasia of the ureteral bud (Wagner *et al*., 2000). The World Health Organization (1998) recommends that a daily vitamin A supplement taken during any part of the reproductive period be limited to 10,000 IU (3000 Retinol Equivalents). In United States, about 25% of adults consume supplements containing vitamin A and about 5% take supplements of vitamin A alone (Braesco and Pascal, 2000; Hathcock *et al*., 1990). The occurrence of malignancies during pregnancy has increased over the last decade complicating 0.1% of pregnancies (Siegel *et al*., 2004). An extensive use

* Corresponding author: babari1@gmail.com
0030-9923/2012/0004-1045 $ 8.00/0

of retinoids in dermatology, particularly in psoriasis, acne, and malignancies, have been associated with additional side effects such as lipid and bone toxicity, visual and teratogenic effects (Simoni *et al*., 2001; Klvanova and Brtko, 2002)**;** it is used sparingly during the reproductive age of women on account of its toxicity (Siegel *et al*., 2004).

A lot of work has been done on teratogenic effects of retinoic acid on nervous system, heart and other organs of the body (Rothmann *et al*., 1995). Since there is hardly any work on the histological changes in fetal kidneys induced by retinoic acid administered to pregnant albino mice, the present work was consequently designedto investigate the effect of retinoic acid on the developing kidney.

MATERIALS ANS METHODS

Total of sixteen mice (twelve female and four male), 6-8 weeks old, weighing 25-30g, were obtained from NIH Islamabad. They were acclimatized for a period of one week and maintained under constant environment of temperature 22ºC±0.5, humidity 50±10% and dark and light cycle of 12 h each for the experimental period in the laboratory of University of Health Sciences, Lahore. They were fed on standard mouse diet and given water *ad libitum*.

The experiment was carried out using two groups, control and experimental, randomly created; each group containing eight animals, six females and two males. Four mice were placed together (1 male: 3 female) in a single cage overnight for mating, the presence of vaginal plug next morning was considered to have confirmed conception and was taken as gestational day 0 (Firat *et al*., 2005).

Control mice were given 0.1ml of olive oil orally on 7^{th}, 8^{th} and 9^{th} days of pregnancy, whereas the experimentals mice received RA, 60mg/kg/day dissolved in 0.1ml of olive oil orally on comparable days. The mice were anaesthetized under chloroform on 18^{th} day of gestation and a midline longitudinal incision was given from xiphi-sternum to pubic symphysis to expose the uterus; implantation sites in the uterine horns, number of intact fetuses and resorbed embryonic masses were counted. Fetuses were dissected using dissecting stereo microscope and their kidneys were removed and fixed in 10% formalin for 48 h for histological preparation. The specimens were dehydrated through a graded series of alcohol, cleared in xylene and infiltrated with molten paraffin before preparing the paraffin blocks; 5µm thick sections were obtained, using Leica rotatory microtome (RM 2125). The sections were stained with standard haematoxylin and eosin method before examining them under the light microscope (Leica DM 1000).

Micrometery

The ocular reticule was calibrated with the linear stage micro meter using 40X and 100X objectives. Serialized length wise sagittal sections of the kidney were used to determine its size. The length of the organ was measured using ocular and stage linear micrometerat 40X objective. The thickness of cortex and medulla was also obtained in similar way at 100X objective (Dziarmaga *et al*., 2003).

Statistical analysis

The statistical analysis was carried out using computer software Statistical Package for Social Sciences (SPSS) version 16. The arithmetic mean, standard deviation and the significance between two groups was calculated by Mann Whitney test. The difference was regarded statistically significant if the 'p' value was ≤ 0.05.

RESULTS

Litter size

The total available litter size was 91, of which 50 belonged to the control and 41 were from experimental group. In the latter group eight fetuses were resorbed and the difference between the alive fetuses of the two groups was statistically significant when compared to each other using Pearson chi square test.

Histological features

The kidneys were bilaterally present and normal in shape in all fetuses of both groups, whereas the size of fetal kidney in control group appeared to be enlarged.

On histological examination, the sagittal section of kidneys from the control group showed well developed cortical renal corpuscles; these

contained loops of glomerular capillaries, surrounded by Bowman's capsule, having visceral and parietal layers, with capsular space in between. The cortical region also showed cross section of proximal and distal convoluted tubules lined by simple cuboidal epithelium (Fig. 2). Renal medulla showed closely packed cross sections of collecting ducts, thin descending limb and thick ascending limbs of loop of Henle. All these structures were embedded in the interstitium. In experimental, the size of the kidney was increased and the difference in size when compared to those from control was statistically significant ($p<0.05$, Table I). In experimental group, the medulla was observed to be much thicker than that in control group, the difference was statistically significant ($p<0.05$, Table I). In experimental group, thickness of cortex was apparently thicker than in control and the difference was also statistically significant ($p<0.05$, Table I).

Table I.- Comparison of parameters from fetal kidneys of control and experimental groups.

Parameter	Control Mean± S.D	Experimental Mean± S.D	p-value
Kidney size (mm)	3.09±0.13	4.01±0.19	0.000*
Cortex thickness (μm)	293.8±26.56	357.56±31.68	0.000*
Medulla thickness (μm)	853.0±41.21	1341.21±80.53	0.000*

*p value<0.05 is statistically significant.

The architecture of renal tissue in fetal kidneys was well preserved in experimental mice, showing outer cortex and inner medulla; latter was larger than the cortex (Fig. 1) which was presumably responsible for renal enlargement in the group. Epithelial cells lining both the proximal and distal convoluted tubules showed small cytoplasmic vacuoles, lightly stained basophilic nuclei which were absent at some places, indicating different stages of necrosis (Fig. 2).

In renal corpuscles, glomeruli showed hypercellularity, obliterating the capsular space which was due to increased proliferation of endothelium (Fig. 2).

DISCUSSION

Retinoic acid plays important role during morphogenesis of different organs in mammals; this is on account of impaired vitamin A signaling or maternal vitamin A deficiency inducing syndromic urogenital-tract malformations similar to those in humans (Dame and Knutson, 2011). It also affects mesenchymal/epithelial interactions in the development of kidney, lung, central nervous system, and gut (Tulachan *et al*., 2003).

In the present study we focused on the effects of RA on histological structure of fetal kidney. The RA was given on 7^{th}, 8^{th} & 9^{th} of gestation, considered to be critical for developing kidney, since nephrogenic cords are reported to appear by 8^{th}day and pronephric tubules and duct are observed to be suspended in the coelom by 10^{th}day (Rugh, 1968). Moreover, Bhat and Manolesscu (2008) pointed out that cytoplasmic enzymes (retinaldehyde dehydrogenase), responsible for the synthesis of RA from retinol, are also expressed between gestational days 7-9 in mice embryo.

Examination of the sagittal section of kidneys from control group showed normal looking well developed organ with outer cortex and inner medulla, whereas those from experimental group, showed increase in the thickness of renal medulla with closely packed tubules due to increased tubulogenesis induced by RA (Fig.2). Similar findings were reported earlier in which development of the collecting duct system was greatly impaired in RAR $\alpha\beta2^{-}$ mutant mice embryo; fewer branches of ureteric bud were present, and their ends were positioned abnormally at a distance from the renal capsule (Mendelsohn *et al.*, 1999).

Retinoic acid is reported to control the pattern of developing kidney through expression of tyrosine kinase receptor, which modulates morphogenesis of branches of ureteric bud (Bhat and Manolesscu, 2008).These receptors are encoded by a proto-oncogene Ret; which is responsible for formation of the ureteric bud and its branching in the kidney (Dressler, 2002). Ret receptors are first expressed in the Wolffian duct at gestational day 8-11.5 and as ureteric arborization proceeds(gestational days 13.5-17.5) 'Ret' is expressed in ureteric bud tip cells (Pachnis *et al*., 1993). The branching and stromal

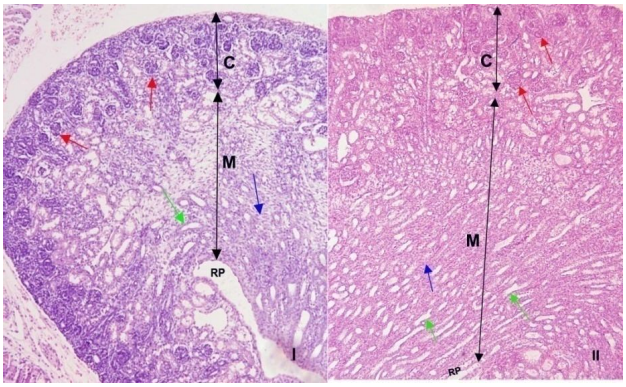

Fig. 1. (I); Histological structure of sagittal section of fetal kidney of (a) control showing cortex, C (↕) and medulla, M (↕); lower arrow head points to the renal pelvis.Renal corpuscles (red arrow), cross sections of collecting ducts (green arrow), interstitium (blue arrow) and (b) experimental renal pelvis (RP). showing outer cortex, C (↕), renal corpuscles (red arrow). Renal medulla, M(↕) shows significant thickening and contains cross section of collecting ducts(green arrow) which are closely packed and separated by small amount of interstitium(blue arrow) and renal pelvis (RP). H & E stain X 50.

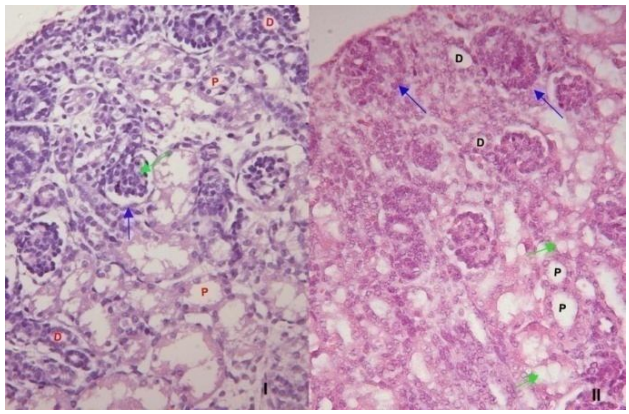

Fig. 2. (I); Histological structure of sagittal section of fetal kidney of (a) control showing cortical region having renal corpuscles; glomerulus (green arrow) and Bowman's capsule(blue arrow) lined by simple squamous epithelium. Cross sections of proximal convoluted tubules (P) and distal convoluted tubules (D) lined by simple cuboidal epithelium. (b) experimental showing renal corpuscles in the cortical region; gloemeruli show hypercellularity(blue arrow) resulting in obliteration of capsular space. There are cross sections of proximal convoluted tubules (P) and distal convoluted tubules (D); epithelial cells lining the tubules show hypochromatic nuclei and cytoplasmic vacuolization (green arrow) indicative of necrosis. H & E stain X 200.

cell abnormalities had been linked to impaired 'Ret' signaling in the ureteric bud (de Graaff *et al*., 2001*)*.

In the current study, histological examination of kidneys obtained from embryos of group B showed hypochromic nuclei and cytoplasmic vacuolization in the epithelial lining of the tubules, indicative of their necrosis. Our observations also indicate that there was an increase in number of the fetal resorption in experimental group. These findings are similar to those reported by Calaustro and Lichton (1967), implying cell damage and necrosis of junctional zone of placenta during pregnancy in retinol deficient rats receiving RA resulting in increased fetal absorption.

In the current study renal corpuscles showed hyper-cellularity in the glomeruli of treated group B, suggesting an increased proliferation of endothelial cells of the capillaries. Our findings are consistent with an earlier study by Saito *et al*. (2007), in which All-trans retinoic acid (ATRA) was reported to induce angiogenesis via RAR by stimulation of human umbilical vein endothelial cells (HUVEC) proliferation and by enhancement of signaling by endogenous vascular endothelial growth factor (VEGF). RA is reported to play a crucial role in the control of mammalian vascular development and in restoring cell cycle control. In another study, it was observed that Raldh2$^{-/-}$ mice embryos exposed to RA during gestational days 7.5-8.5, a period of endothelial tube formation, the yolk sacs exhibited formation of some large vessels, which were not formed in untreated mutants, and smaller vessels were significantly dilated (Lai *et al*., 2003), whereas Hoffmann *et al*. (2007) have demonstrated anti-angiogenic effects of *RA* in thyroid cancer. Since these results are controversial, effects and mechanism of ATRA on angiogenesis still remain unclear needing further investigations.

CONCLUSIONS

Vitamin A and its metabolites like retinoic acid have deleterious effect on developing kidney,

when given during the critical period of organogenesis, it is therefore suggested that these preparations may be avoided or sparingly used during pregnancy. Further investigations are required to see the effect of RA on vascular endothelium since both angiogenic and antiangiogenic effects had been reported by earlier investigations.

ACKNOWLEDGEMENTS

I am grateful to Professor Dr. Malik Hussain Mubbashar, Vice Chancellor of University of Health Sciences, Lahore, for providing facilities and funding support to complete my research project.

REFERENCES

BHAT, P.V. AND MANOLESSCU, D.C., 2008. E Role of vitamin A in determining nephron mass and possible relationship to hypertension. *J. Nutr*., **138**: 1407-1410.

BRAESCO, V.A. AND PASCAL, G., 2000. Vitamin A in pregnancy: requirements and safety limits. *Am. J. clin. Nutr*., **71**: 1325S-1333S.

CALAUSTRO, E.Q. AND LICHTON, I.J., 1967. Effects of retinoic acid and progesterone on reproductive performance in retnol deficient female rats. *J. Nutr*., **95**:517-525.

DAME, M.C. AND KNUTSON, D., 2011. Vitamin A in reproduction and development. *Nutrients*, **3**: 385-428.

DE GRAAFF, E., SRINIVAS, S., KILKENNY, C., D'AGATI, V., MANKOO, B.S., COSTANTINI, F. AND PACHNIS, V., 2001. Differential activities of the RET tyrosine kinase receptor isoforms during mammalian embryogenesis. *Genes Dev.*, **15**: 2433-2444.

DRESSLER, G.R., 2002. Tubulogenesis in the developing mammalian kidney. *Trends Cell Biol*., **12**: 390-395S.

DZIARMAGA, A., CLARK, P., STAYNER, C., JULIEN, JP., TORBAN, E., GOODYER, P. AND ECCLES, M., 2003. Ureteric Bud apoptosis and renal hypoplasia in transgenic *PAX2-Bax* fetal mice mimics the renal-coloboma syndrome. *J. Am. Soc. Nephrol.*, **14**:2767-2774.

FIRAT, D., KUNTSAL, L. AND SIRIN, Y., 2005. Protective effects of prenatal administration of folic acid induced cellular damages of Meckel's cartilage in rats. *Tohoku J. exp. Med*., **205**: 27-36.

HATHCOCK, J.N., HATTAN, D.G. AND JENKINS, M.Y., 1990. Evaluation Of vitamin A toxicity. *Am. J. clin. Nutr*., **52**:183-202.

HOFFMANN, S., ROCKENSTEIN, A., RAMASWAMY, A., CELIK, I., WUNDERLICH, A., LINGELBACH, S., HOFBAUER, L.C. AND ZIELKE, A., 2007. Retinoic acid inhibits angiogenesis and tumor growth of thyroid cancer cells. *Mol. Cell Endocrinol*., **264**:74-81.

KLVANOVA, J. AND BRTKO, J., 2002. Selected retinoids: determination by isocratic normal phase HPLC. *Endocr. Regul*., **36**: 133-137.

LAI, L., BOHNSACK, B.L., NIEDERREITHER, K. AND HIRSCHI, K.K., 2003. Retinoic acid regulates endothelial cell proliferation during vasculogenesis. *Development*, **130**: 6465-6474.

LAMER, E.J., CHEN, D.T., HOAR, R.M., AGNISH, N.D., BENKE, P.J. BRAUN, J.T., CURRY, C.J., FERNHOFF, P.M., GRIX, A.W. AND LOTT, JR. I.T., 1985. Retinoic acid embryopathy. *N. Engl. J. Med*., **313**: 837-84.

McCAFFERY, P.J., ADAMS, J., MADEN, M. AND ROSA-MOLINAR, E., 2003. Too much a good thing: retinoic acid as an endogenous regulator of neural differentiation and exogenous teratogen. *Europ. J. Neurosci*., **18**: 457-472.

MENDELSOHN, C., BATOURINA, E., FUNG, S., GILBERT, T. AND DODD, J., 1999. Stromal cells mediate retinoid-dependent functions essential for renal development. *Development*, **126**: 1139-1148.

PACHNIS, V., MANKOO, B. AND COSTANTINI, F., 1993. Expression of c-ret proto-oncogene during mouse embryogenesis. *Development*, **119**: 1005-1017.

QUADRO, L., HAMBERGER, L., GOTTESMAN, M.E., COLANTUONI, V., RAMAKRISHNAN, R. AND BLANER, W.S., 2004. Transplacental delivery of retinoid: the role of retinol binding protein and lipoprotein retinyl ester. *Am. J. Physiol. Endocrinol. Metab*., **286**: E844-E851.

ROBENS, J.F., 1970. Teratogenic effects of hypervitaminosis A in the hamster and the guinea pig. *Toxicol. appl. Pharmcol.*, **6**: 88-99.

ROTHMANN, K.J., MOORE, L.L. AND SINGER, M.R., 1995. Teratogenicity of high vitamin A intake. *N. Engl. J. Med*., **333**: 1369-1373.

RUGH, R., 1968. *The mouse; its reproduction and development*. Burgess Publishing Company, Minneapolis.

SACCHI, S., RUSSO, D., AVVISATI, G., DASTOLI, G., LAZZARINO, M., PELICCI, P.G., BONORA, M.R., VISANI, G., GRASSI, C., IACONA, I., LUZZI, L. AND VAZANELLI, P., 1997. All-trans retinoic acid in hematological malignancies. An update. *Haematologica*, **82**:106-121.

SAITO, A., SUGAWARA, A., URUNO, A., KUDO, M., KAGECHIKA, H., SATO, Y., OWADA, Y., KONDO, H., SATO, M., KURABAYASHI, M., IMAIZUMI, M., TSUCHIYA, S. AND ITO, S., 2007. All trans retinoic acid induces in vitro angiogenesis via retinoic acid receptor: possible involvement of paracrine effects of endogenous vascular endothelial growth factor

signaling. *Endocrinology*, **148**: 1412-1423.

SIEGEL, E.M., CRAFT, N.E., ROE, D.J., DUARTE-FRANCO, E., VILLA, L.L., FRANCO, E.L. AND GIULIANO, A.R., 2004. Temporal variation and identification of factors associated with endogenous retinoic acid isomers in serum from Brazillian women. *Cancer Epidemiol. Biomark. Prev*., **13**: 1693-1703.

SIMONI, D., RONDANIN, R., BARUCHELLO, R., ROBERTI, M., ROSSI, M., GRIMAUDO, S., D'ALESSANDRO, N., INVIDIATA, F.P. AND TOLOMEO, M., 2001. Retinoic acid and analogs as potent inducers of differentiation and apoptosis. New promising chemopreventive and chemotherapeutic agents in oncology. *Pure appl. Chem.*, **73**: 1437-1444.

SNYDER, J.M., MOORE, J.M., JACKSON, S.K., GOSS, K.I., DAL, H., BANGSUND, P. J., GIGUERE, V. AND MCGOWAN, S.E., 2005. Alveolarization in retinoic acid receptor β deficient mice. *Pediat. Res.*, **57**: 384-391.

TULACHAN, S.S., DOI, R. AND KAWAGUCHI, Y., 2003. All-trans retinoic acid induces differentiation of ducts and endocrine cells by mesenchymal/epithelial interactions in embryonic pancreas. *Diabetes*, **52**: 76-84.

VILAR, J., GILBERT, T., MOREAU, E. AND MERLET-BENICHOU, C., 1996. Metanephros organogenesis is highly stimulated by vitamin A derivatives in organ culture. *Kidney Int*., **49**: 1478-1487.

WAGNER, J., DECHOW, C., MORATH, C., LEHRKE, I., AMANN, K., WALDHERE, R., FLOEGE, J. AND RITZ, E., 2000. Retinoic acid reduces glomerular injury in a rat model of glomerular damage. *J. Am. Soc. Nephrol.*, **11**: 1479-1487.

WAGNER, J., 2001. Potential role of retinoids in the therapy of renal disease. *Nephrol. Dial. Transplant.*, **16**: 441-444.

WHO., 1998. *Safe vitamin A dosage during pregnancy and lactation. Recommendations and report from a consultation*. Micronutrient series. World Health Organization, Geneva.

WILSON, J.G., ROTH, C.B. AND WARKANY, J., 1953. An analysis of the syndrome of malformations induced by maternal vitamin A deficiency. Effects of restoration of vitamin A at various stages of gestation. *Am. J. Anat*., **92**:189-217.

(Received 19 December 2011, revised 21 July 2012)