IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 11, Issue 1 Ver. II (Jan. - Feb. 2016), PP 56-60
www.iosrjournals.org

Teratological Effects Of Retinoids On Liver Of Swiss Albino Mice

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Abstract: *The specific branch of developmental biology which deals with abnormalities caused as a result of failure of normal pattern of development is referred as teratogenesis.Retinoids are essential for spermatogenesis, oogenesis, placental development, foetal morphogenesis and growth. The role of vitamin A in vision is well known . Active derivative of vitamin A (Retinoids) play an important and multiple role in Mammalian development and homeostasis (Sapin, et.al. 1997, Dup et al 1997). These essential dietary compounds are needed in very small quantities, they are not synthesized by the animals and are obtained from external sources in the form of β carotene (C40H56). In cells of intestine β carotene is converted into vitamin A alcohol and is transported by blood to liver where it is esterfied and stored as vitamin A palmitate. RA is not stored in liver but is derived from retinal and it is biologically the most potent form of vitamin A. Vitamins are organic compounds which are devided into 2 categories*
I Fat soluble (A, D, E, K) and
II water soluble (B and C).
Vitamin A belongs to fat soluble category of vitamins. A fat soluble substance essential for life was first discovered in egg yolk in 1909 by Stepp (Pawson, 1981; Robert and Sporn, 1984). It was confirmed by Mc Collum Devis in 1913 when he found a compound with similar biological activity in butter fats, egg yolk and cod liver oiland was named fat soluble vitamin A. The development of an organism is a complex process of embryogenesis involving cell proliferation, differentiation, migration and organogenesis. Many agents interfering the development process can cause malformations in the embryo. The study of these congenital abnormalities is called teratology and agents which are responsible for causing these malformations are called teratogens. Susceptibility to teratogens depends on the genotype of the organism, including species as well as strain differences. Etienne Geoffrey Saint Hilaire and his son in 1820 started experiments on chick embryo by disturbing its environment in different ways at different embryonic stages. They found some anomalies like Trioncephally, atrophy of eyes and spina bifida in his experiments (Tuli, 1968). In 1877 Dareste reported found some anomalies like Trioncephally, atrophy of eyes and spina bifida in his Experiments (Tuli, 1968). 4mg RA severely damaged skin on later stage ie 11th and 14th day gestation period ,of development , shows disruption and folds of skin look like over verying skin flaps. In the present paper effects of different doses of vitamin A on Liver on different development stages of swiss albino mice are studied.

Key Words: *Retinoids, Teratology, Swiss albino mice, Liver.*

I. Introduction

The branch of development biology which deals with abnormalities of development or failure of normal development is referred as teratology.Retinoids are essential for spermatogenesis oogenesis, placental development, foetal morphogenesis and growth. The role of vitamin A in vision is well known . Active derivative of vitamin A (Retinoids) play an important and multiple role in Mammalian development and homeostasis (Sapin, et.al. 1997, Dup et al 1997). These essential dietary compounds are needed in very small quantities, they are not synthesized by the animals and are obtained from external sources in the form of β carotene (C40H56). In cells of intestine β carotene is converted into vitamin A alcohol and is transported by blood to liver where it is esterfied and stored as vitamin A palmitate. RA is not stored in liver but is derived from retinal and it is biologically the most potent form of vitamin A.Vitamins are organic compounds which are devided into 2 categories
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Sporn, 1984). It was confirmed by Mc Collum Devis in 1913 when he found a compound with similar biological activity in butter fats, egg yolk and cod liver oiland was named fat soluble vitamin A. The name of vitamin was given by Dummond In 1920. The natural and synthetically obtained vitamin A is found in several forms of alcohol (Retinol), aldehyde (Retinol palmitate), acetate and retinoic acid. They were collectively known as retinoids. Retinol or alcoholic form of vitamin A is a unstable organic compound. They are easily oxidized specially in the presence of heat. The ester form of vitamin A are fairly stable.When vitamin A is taken in the form of esters (Palmitate/acetate) , its converted into alcoholic form (Retinol) in the intestinal cells to be carried to liver, where it is converted to palmitate form for storage. Retinoic acid is not stored in the liver but it is derived from retinol and it is biologically most potent form.

The development of an organism is a complex process of embryogenesis involving cell proliferation, differentiation, migration and organogenesis. Many agents interfering the development process can cause malformations in the embryo. The study of these congenital abnormalities is called teratology and agents which are responsible for causing these malformations are called teratogens. Susceptibility to teratogens depends on the genotype of the organism, including species as well as strain differences. Teratological experiments was started from 1820s with the studies of Etienne Geoffrey Saint Hilaire and his son on chick embryo by disturbing its environment in different ways at different embryonic stages. They found some anomalies like Trioncephally, atrophy of eyes and spina bifida in his Experiments (Tuli, 1968). In 1877 Dareste reported some congenital malformation in the chick embryo by environmental disturbance.

II. Materials And Methods

The mice were obtained from mice breeding center , Department of Zoology M.D.S.U. Ajmer. Animals were fed synthetic diet mice feed pallets (Brook Bond Lipton India Ltd.)Supplemented with germinated grains, seasonal green vegetables, multivitamin drops and water ad libitum. Tetracycline mixed in water was regularly given.There are four virgin female mice 25+-1gm caged with fertile healthy male in the evening and these were examined for presence of vaginal plug, the next morning. The day appearance of vaginal plug was considered as day 0 of pregnancy.

The Veginal Plug

After copulation a secretion of seminal vesicles in the ejaculate of the male coagulate to form a plug in the vegina extending from the cervix to the valve , where it is ordinarily visible and is a convenient external sign that mating has occurred (Green , 1996).

Duration Of Pregnancy

The gestation period in this species is 19 days and occasionally 20 days. The youngs are most frequently born in the early hours of the morning between midnight and 4 AM.

III. Objectives]

Investigation of the effects of retinoic acid on different developmental stages (5^{th}, 8^{th}, 11^{th}, and 14^{th}) of mouse embryo.To study effects of RA on organogenesis of skin, liver, heart . Effect of RA on skeletal elements.

Experimental Design

The pregnant females were devided into following groups :(6 animals per group) Group A : untreatedGroup B : Treated

Doses

Suitable (non lethal or sublethal) doses of RA were screened on mice embryos on different stages of developments. The following doses were found to produce various teratological defects on developing mice embryos

1. 2mg RA /pregnant female.
2. 4 mg RA /pregnant female.

IV. Results And Discussions

Effect of RA on Liver

Development of liver takes place in the form of hepatic diverticulum which is the lobe of endoderm that extends out from the foregut into the surrounding mesenchyme . it is the mesenchyme induces the endoderm to proliferate , to branch and to form the granular epithelium of the liver. A portion of the hepatic diverticulum (region closest to the digestive tube) continues to function as drainage duct of the liver and a branch from this duct produces the gallbladder. In the adult body the liver occupies the upper right and central portions of abdominal cavity, just below the diaphragm it is reddish brown in color and highly vascularised.

Structure of Liver

The liver in mice is enclosed in a fibrous capsule and is divided by connective tissueinto two lobes.These two lobes are separated by falciform ligament, ultimately 4 lobes are visible Namely Right middle lobe, Right lateral lobe, left middle and left lateral lobe. A fold of visceral peritoneum hangs the liver with the anterior abdominal wall. On its superior surface liver is attached to the diaphragm with the help of Visceral peritoneum called coronary ligament . Each lobe is separated into numerous tiny Hepatic lobules, which are the functional unit of liver. Each lobule consist of numerous hepatic cells that radiate outward from a central vein. These plate like group of cells are separated from each other by vascular channels called hepatic sinusoids, blood supply to the liver carrying absorbed nutrients flow through the sinusoids and nourishes the hepatic cells. Often the blood in the portal veins contains some bacterial cells that have entered through the intestinal wall. However, large Kuffer cells which are present in the inner lining (endothelium) of the hepatic sinusoids, remove most of the bacteria from the blood by phagocytosis . Ultimately the blood passes into the central veins of the hepatic lobules and moves out of the liver.In liver lobule are many fine bile canals which receive secretions from the hepatic cells.These canals unite to form large hepatic duct. They merge to form common hepatic duct which carry bile for digestion. Treatment of 2mg, 4mg RA given to pregnant female at 5^{th},8^{th}, 11^{th}, 14^{th} day of gestation, to study the effect of dose of RA at various stages of development of liver.

2mg RA on 5^{th} Day Gestation

RA does not influence the development process of liver at early stage of development by treating low dose of RA (2mg/RA /pregnant female).

2mg RA On 8^{th} Day Gestation

RA treatment causes some distinct and some obvious teratological effects on the developing liver of on 8^{th} day gestation . One of the most significant observation , separation of liver lobes, particularly between the left middle and left lateral lobes.The right lateral lobe elongated anterio-posteriorly, hiding most of the portion of the right liver lobe. The major lobes are normally connected in the center of the liver. These morphological observations clearly indicates abnormal pattern formation of liver lobe under the influence of RA.RA also causes several changes in the internal organization of the liver lobes. CS (cross section) through lateral lobe of liver shows wide sinusoids. The nuclei are lightly stained in the hepatic cells.Very large number of kuffer cells are found with liver cells . The central vein and portal veins has also become enlarged.

2mg RA 11^{th} Day Gestation

Treat of 2mg RA on 11^{th} day gestation also cause separation of liver lobes but the extent of effect is lesser then that is found in the cases 8^{th} day treatment. Right lateral lobe is significantly increased and right middle lobe does not show pronounced effect as compared to the untreated controls. Left lateral lobe has not developed properly. Separation is also not very clear in between two left middle lobes and left lateral lobes . Both major lobes are connected by ligaments .Histological examination of lobules show central vein and portal vein are slightly enlarged. The sinusoid are also wider . Hepatic cells are in clusters with wide sinusoid space. Nuclei are lightly stained , Kuffer cells are present but there number is almost similar to the untreated control.

2 Mg RA 14th Day Gestation.

When pregnant female given 2mg RA 14TH day gestation parturiated neonet shows variety teratological defects significantly. The separation between right middle lobe and right lateral lobe is very distinct. Similarly left lateral and left middle lobe are also clearly separated, Both liver lobes are joined at the middle region. The CS shows scattered bundles of hepatocytes. The sinusoid spaces slightly enlarged but the central and portal are almost similar in dimension. Hepatocytes with poor stained nuclei. Kuffer cells are present with hepatocyte .

4mg RA On 8th Day Gestation

The liver shows enlarged right lobe . Formation of fringed or globular extension of lower margin. Liver lobes are joined on the middle line of the liver .
Internal organization shows lobules consist of clustures of hepatocytes distributed around the central veins. The radiating pattern of hepatic cells . plate is not observed. Kuffer cells present in the cluster of hepatocytes.

4mg RA On 11th Day Gestation

Treatment of 4mg RA on 11th day gestation shows smaller size of liver lobes. CS shows wide spaces sinusoids around the central vein. The peripheral veins and central veins are enlarged The number of kuffer cells are less than that found on 8th day gestation treated cases. The hepatic cells shows lightly stained nuclei in lobules.

4mg RA On 14th Day Gestation

There is extensive growth of right lateral lobe of liver. Separation of lobes is very clear. The size of left lateral lobe and left middle lobe is similar to untreated cases. Histological examination of right lateral lobe shows arrangement of central vein, portal vein, bile duct, sinusoids and hepatic cells in a definite pattern. The central vein are not enlarged in RA treated cases Hepatic cells are in cluster form sinusoids. Kuffer cells are present and number is almost similar to controls. These dark stained bodies(kuffer cells) present in the cluster of hepatocytes.

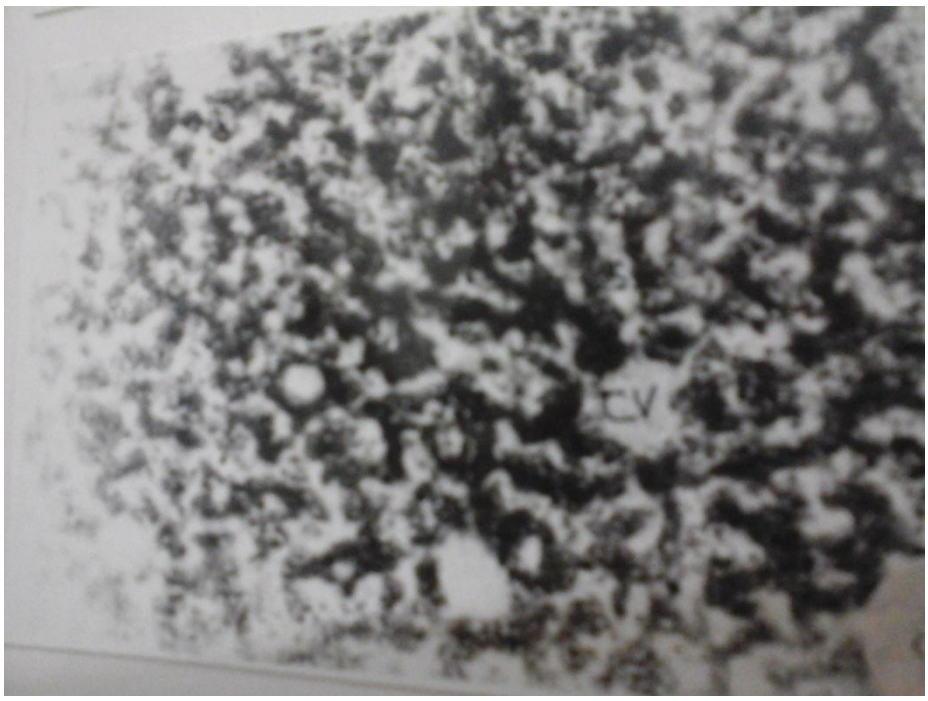

Image 1 Histological picture of liver of newly born mice treated with 4mgRA/pregnant female at 14 day gestation shoeing kuffer cells and hepatocytes arranged in ball like structure and central vein. HC: Hepatocytes, KC: kuffer cells, CV: central vein, BD: bile duct, HPV : portal vein, SS : sinusoids.

V. Conclusion and Summary

Liver is known as important site for the storage of ester form of retinoids. The kufer cells are the site for the storage of retinoid (De Ruyter 1934). Hypovitaminosis A has been found to enhance number of kuffer cells. Dilution of bile duct and pathological changes in the portal veins in rat , guina pig(uotila and simola 1938). These changes were reversed when Vitamin A given to deficient rat and pig as supplement diet (De Ruyter, 1934).The only pathological changes were reported in hypervitaminosis, A condition in rat and pig.Fats were deposited in kuffer cells of liver.The results of present study have clearly indicated that 2mg and 4 mg RA when administered orally to pregnant female on 5^{th}, 8^{th}, 11^{th} and 14^{th}, day of gestation are stage and dose dependent. 8^{th} day gestation stage was found to be most sensitive period as far as effect of RA is concerned of liver. Formation of liver takes place as a result of differentiation of endoderm in a specific manner.Since the process of organogenesis of liver starts after 7^{th} day and liver diverticulum developed on 9^{th} day gestation(Rugh, 1990).11^{th} and 14^{th} day gestation stages indicate that RA induced teratogenicity but 14^{th} day gestation stage showed almost normal morphogenesis of liver. 8^{th} day gestation stage showed separated liver lobes, reduction in number of kuffer cells abnormal shape in the hepatic portal vein wide sinusoids. The normal liver lobules consist of small parenchymal mass that is irregular in size and shape arranged around excess of terminal portal vanule, hepatic vascular and bile ductile.This lobule has central vein with its vascular biliary axis. There is no physical separation between two lobules (Ritkind et al 1969). In the present study 8^{th} day gestation treated females liver lobules are abnormally arranged
around the central vein and hepatic portal vein. Hepatic arteries are deformed and enlarged , this type of pathological changes are produced by other toxicants ie methylmercury, alcohol, thalidomide. Teratological effect was produced by RA in the developing liver. RA induced teratogenicity has resulted by changing the pathway differentiation and organogenesis of developing liver.

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