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EFFECTS OF VITAMIN A EXCESS ON POSTNATAL DEVELOPMENT OF THYMUS IN ALBINO RATS

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ABSTRACT

Objective: To determine the effects of Vitamin A excess on postnatal thymic development in albino rats.
Study design: Experimental
Place and Duration of Study: This study was conducted in the Department of Anatomy/ Histopathology, Shaikh Zayed Postgraduate Medical Institute, Lahore from September 2008 to September 2009.
Materials and Methods: Study was conducted with 36 rat pups, of Sprague dawley variety. They were randomly separated into two equal groups A and B (n=18) on postnatal day 1. Vitamin A was used in the form of isotretinoin (13-cis retinoic acid). Group A was control group while group B was experimental group. Oral dose of isotretinoin, 2.5 mg/ kg body weight, was given to the pups on every mid-day of the week, in subsequent four weeks of postnatal period (days 4, 11, 18 & 25). On 30th postnatal day all pups were euthanized and weighed. Their thymuses were then dissected for morphological study.
Results: Pups of group B showed reduction in mean body weight when compared with control group ($P<0.01$). The mean thymocyte population of experimental group was found to decrease as compared to the pups in the control group ($P<0.01$). The mean percentage of immature thymoblasts of experimental group B was also found to decrease ($P<0.05$).
Conclusion: Postnatal development of thymus in albino rats can be interrupted with frequent use of Vitamin A in terms of lymphocytes depletion.

KEY WORDS: Vitamin A, Thymus, Postnatal, Development

INTRODUCTION

It has been claimed that prevention of Vitamin A deficiency by Vitamin A supplements reduces infant and early childhood mortality from infectious diseases.[1,2]As a result, Vitamin A supplementation programmes are widely implemented in areas of the world where Vitamin A deficiency is common.[3] Provision of such supplements presumably corrects deficits in immune function caused by Vitamin A deficiency and thereby improves recovery from serious infections. Thymus provides an optimal cellular and humoral micro-environment for the development of immunocompetent T lymphocytes.[4] It has been proved that excessive intake of Vitamin A by pregnant women can affect the developing organs of foetus.[5,6,7]Optimum level of Vitamin A is necessary for the development of thymus [8,9,10]and abnormal development of this organ is associated with impaired immunity in neonates.[11] As growth and development of thymus is not completed at birth, and it continues to grow till puberty,[12]it might be possible that Vitamin A excess may interfere with the postnatal development of thymus as well. Previous studies did not provide sufficient evidence regarding the effects of Vitamin A on postnatal development of thymus.
Due to the known teratogenic effect of retenoids, therapeutic doses are contra indicated

during pregnancy.[13]Although hypervitaminosis A can be due to excessive dietary intakes, the condition is usually a result of consuming too much preformed vitamin A from supplements or therapeutic retinoids.[14]

This study was therefore designed to observe the effects of Isotretinoin, on thymus, in a dose of 2.5mg/kg (therapeutic dose) in postnatal period in albino rat pups.

Isotretenoin is a synthetic retinoid (13-Cis-retinoic acid) and it closely resembles naturally accuring Vitamin A. It was released in the United States and Europe in 1982.[15]

MATERIALS AND METHODS

This experimental study was conducted in the Department of Anatomy/ Histopathology, Shaikh Zayed Postgraduate Medical Institute, Lahore. 36 rat pups, of Sprague-dawley variety of albino rats were used. These pups were selected randomly on the first day of their life.

Study Design: Rat pups were randomly separated into two equal groups, A and B (n=18). Isotretinoin (13-cis retinoic acid) in oral form was used in this study, given to the rats by nasogastric tube (N/G Tube). Group A was the control group containing 18 rat pups. They were given 1ml of olive oil through N/G Tube as vehicle. Group B was an experimental group consisting of 18 rat pups, received single oral dose of Isotretinoin, 2.5 mg/kg body weight, dissolved in olive oil, on mid day of the week, in subsequent four weeks of postnatal period (days 4, 11, 18 & 25).

Dosage of Isotretinoin

Isotretinoin was administered orally by N/G Tube to each rat. The oral solution in the given dose of 2.5 mg/kg body weight was prepared by evacuation of 10 mg capsular content of Roaccutane (Roche) into an insulin syringe. Its volume was 1 ml and then it was diluted in olive oil to make 100 ml. So each 1 ml of prepared solution constituted 0.1 mg, which was administered as 2.5mg per kg body weight to rats of each group.

All pups were euthanized on 30th postnatal day (procedure was approved by Institutional Review Board IRB, a research ethical committee). These animals were weighed. Then their thymuses were dissected and weighed. External morphological study of thymus was carried out. Zenker's solution was used to fix the thymus.After fixation thymus was embedded in paraffin. Serial 5µm sections were cut and stained with hematoxylin /eosin stain for detailed histological study of thymus.

The diameter of thymic lobules was measured with the help of ocular micrometer in µm in both groups. This measurement was done under 40x objective lens.

Thymocyte population was counted with the help of ocular squared graticule in per mm^2 area. Apoptotic cells and immature thymoblasts were counted in same area as that of thymocytes.

Statistical Analysis : Qualitative data was analyzed statistically by Chi-square (X^2) method, while quantitative data was analyzed by t-test using Statistical Package for Social Sciences (SPSS) Version 16. All the quantitative variables were described by Mean, ± SD and all qualitative variables were described by frequency and percentages. P-value of < 0.05 was considered significant.

RESULTS

The thymus tissues of the pups of experimental group B were normal looking and their appearance was like control group A at the time of dissection (30^{th} postnatal day). However, a reduction in mean weight of thymus was noted in pups of experimental group B but the difference had no statistical significance (P>0.05). Pups of group B showed reduction in mean body weight when compared with control group. This reduction in weight was highly significant statistically (P<0.01) No statistically significant difference was noted in relative

TABLE 1

Effects on Body weight, Weight of thymus and RTWI of 30 days old rat pups exposed to Vitamin A during postnatal period

	Group B				Group A				
	Mean	**S.D**	**Min.**	**Max.**	**Mean**	**S.D**	**Min.**	**Max.**	**P-value**
Body weight of offspring (g)	77.50	±3.09	70.00	80.00	82.39	±4.38	75.00	90.00	<0.001**
Weight of thymus (mg)	189.72	±13.34	160.00	210.0	215.0	±4.54	205.0	220.0	0.110++
(RTWI)	0.245	±0.91	0.38	0.44	0.261	±2.09	0.36	0.42	0.101++

A Control Group
B Experimental Group
SD Standard Deviation
Min. Minimum
Max. Maximum
** Highly significant difference(P<0.01)
++ Non significant difference (P>0.05)
Based on t-test

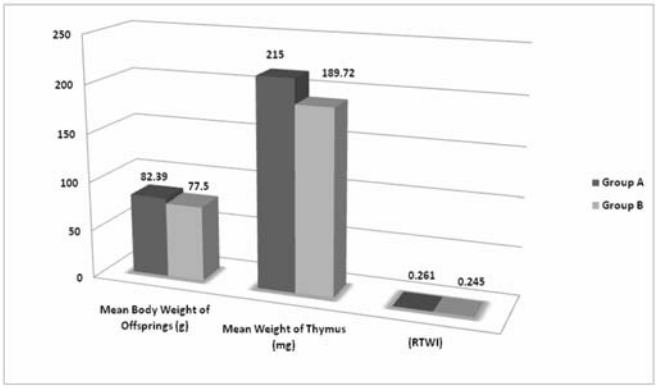
TABLE 2

Effects of Vitamin A on Thymocyte Population, Apoptosis, Immature thymoblasts and Diameter of thymic lobules

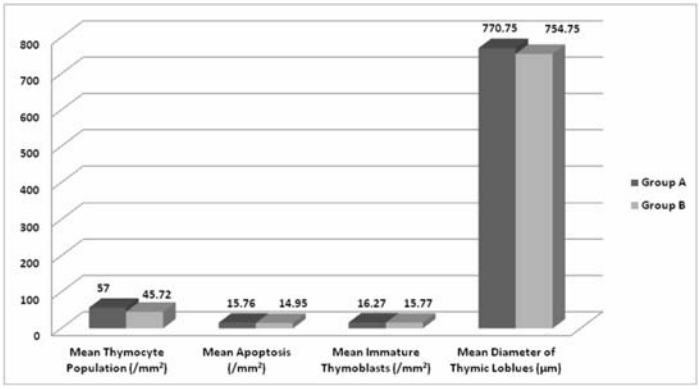
	Group B				Group A				
	Mean	**S.D**	**Min.**	**Max.**	**Mean**	**S.D**	**Min.**	**Max.**	**P-value**
Thymocyte Population / mm^2	45.72	±0.89	44.00	47.00	57.00	±2.38	54.00	62.00	<0.001**
Apoptotic cells / mm^2	14.95	±2.01	10.60	16.80	15.76	±0.50	14.50	16.50	0.115++
Immature thymoblasts / mm^2	15.77	±0.744	14.40	16.90	16.27	±0.45	15.20	16.90	0.023*
Diameter of thymic loblues (um)	754.75	±3.35	678	836.25	770.75	±1.55	687.5	850	0.070++

A Control Group
B Experimental Group
S.D Standard Deviation
Min. Minimum
Max. Maximum
** Highly significant difference(P<0.01)
* Significant difference (P<0.05)
++ Non significant difference (P>0.05)
Based on t-test

Effects on Body weight, Weight of thymus and RTWI of 30 days old rat pups exposed to Vitamin A during postnatal period



Effects of Vitamin A on Thymocyte Population, Apoptosis, Immature thymoblasts and Diameter of thymic lobules



tissue weight index of both groups. (Table 1)
Adipocytes were not found in control and experimental groups. Hassall's corpuscles were not noted in any group. The mean thymocyte population of experimental group was found to decrease as compared to the pups in the control group. Difference was significant statistically (P<0.01). No statistically significant difference was found in mean apoptosis. The mean percentage of immature thymoblasts of experimental group B was found to decrease. Difference was significant statistically (P<0.05).Mean diameter of thymic lobules showed no statistically significant change in experimental group. (Table 2)

DISCUSSION

The present study was intended to assess the effects of Vitamin A on postnatal development of thymus in albino rats exposed to the drug during postnatal period up to 30 days. No difference in gross appearance of thymus was noted in group B as compared to control group. A non significant reduction in mean weight of thymus was noted in pups of experimental group B. However, in experimental group B, pups showed statistically significant reduction in their weight after frequent exposure to 4 doses of Vitamin A. Possibly this decrease in the weight is due to the fact that Hypervitaminosis A can causes bone demineralization in children.[6] Relative tissue weight index of both groups was not affected.
In the pups of experimental group significant decrease in mean lymphocyte population was noted. It should be noted that this group had received four doses of isotretinoin in postnatal period. Result indicates that single dose of isotretinoin may not cause the significant lymphocyte depletion but frequent doses of

Vitamin A are responsible for significant lymphocyte depletion on 30th postnatal day.[16]

In experimental group the percentage of immature thymoblasts was also found to decrease significantly. This shows a decrease proliferation rate which was responsible for the decreased thymocyte population of this experimental group.

Apoptosis is a programmed physiological necrosis of cells. It regulates the atrophy of completely developed organ, e.g. thymus and can be developed by metabolites of Vitamin A.[17]In this study no significant change in apoptosis was noted in experimental pups.

Apoptosis has an essential role in shaping the T-Lymphocyte repertoire of the body.[18] Majority of the T-cells express functionally unacceptable TCR, they cannot interact with the antigen presenting cells (APC), and will enter the apoptotic programme. Retinoic acid can induce a significant rate of this type of thymocyte cell death.[19]Both all-trans RA and 9-Cis RA (two forms of retinoic acid) directly suppress T-helper cells 1 (Th_1) development and enhances T-helpers cell 2 (Th_2) development in naïve (newly manufactured) T-cells.[20]

With the reference of above mentioned studies it is obvious that Vitamin A can induce apoptosis in one type of thymocytes and can suppress in other type. It is therefore not possible to determine the exact ratio of apoptosis. In this study no change in apoptosis was observed.

Diameter of the thymic lobules appears to be the least effective parameter of this study.

This study suggests that frequent use of Vitamin A in new born can cause an insult to the immune system by decreasing the thymocyte population, predisposing the newborn to infections.[21]

CONCLUSION

The result of this research work indicates that postnatal development of thymus in albino rats can be interrupted with frequent use of Vitamin A in terms of lymphocyte depletion.

Recommendations: Caution must be taken while administrating Vitamin A to newborns in human.

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