ORIGINAL ARTICLE

Effect of Prenatal Administration of Retinoic Acid on the Thymus of Chick Embryo- A Histological Study

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Author`s Contribution

[1]Conceived the idea, conceptualized the study design, article writing,
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ABSTRACT

Objective: This study was designed to see the effects of retinoic acid exposure on the histological development of the chick thymus.

Study Design: Experimental study.

Place and Duration: Department of Anatomy, Regional Centre, College of Physicians and Surgeons Pakistan, Islamabad, from February 2009 to February 2010

Material and methods: A defective model of chick thymus was induced by exposing chicken eggs to retinoic acid during the early stages of neural crest migration. The retinoic acid exposed experimental groups were compared with the age matched control groups at embryonic day 15 and at hatching. **A statistical comparison of differences among groups was evaluated by the student's t test. Values of < 0.05 were considered significant.**

Results: The mean number of lymphocytes was significantly reduced in the retinoic acid exposed experimental groups. Preferential cortical lymphodepletion was noted at the embryonic stage. There was a diminution of connective tissue septa in the experimental groups at both stages of development.

Conclusion: Retinoic acid exposure caused hypo-proliferation of lymphocytes and diminished connective tissue septa in the chick thymus.

Key words: Lymphocytes, Thymus, Neural crest, retinoic acid.

Introduction

A primary site of T-lymphocyte development, the thymus is an epithelial organ and contains developing lymphocytes. Histologically, it is surrounded by a mesenchymal capsule and is divided into a cortex and a medulla, each consisting of a distinct population of lymphocytes.[1] Embryologically, the epithelial primordium of the thymus is derived from the endoderm of the 3rd pharyngeal pouch. Lymphoid stem cells, from the yolksac and fetal liver, invade the primordial epithelium and proliferate under the inductive effect of neural crest cells.[2] Classical ablation studies have shown that cranial neural crest cells are critical for thymus organogenesis, giving rise to the mesenchyme and epithelial components of the thymus. Notably, neural crest ablation led to meagre lymphoid cell development.[2]

Retinoic acid (RA) is an oxidative metabolite of vitamin A, with teratogenic potential. Among the complex mechanisms of RA teratogenesis, severe disturbances of the neural crest pathway play a leading role.[3] The initial patterning and development of neural crest-derived mesenchyme in the thymus involves signalling peptides. These comprise endothelin-1 (ET-1), dHAND and Msx-1.[4] RA impairs thymic development by targeting neural

crest and suppressing the expression of these signalling molecules.[5]

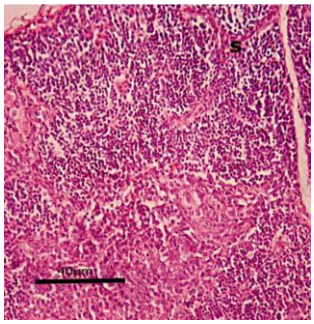
Since the specialized microenvironment provided by the neural crest derived epithelial reticulum is essential for normal thymic development, defects in the thymus are a consistent feature of retinoic acid teratogenesis targeting neural crest cells. [6] The defect predominantly manifests itself as thymic aplasia or hypoplasia and is widely reported. [2,7]

Although the detrimental effects of retinoic acid on the morphological development of thymus are well documented, literature reporting effects on its cellular population, including lymphocytes is scarce. Keeping this in view, this study was designed to study the quantitative effect on thymic lymphocytes through exogenous RA administration during prenatal period.

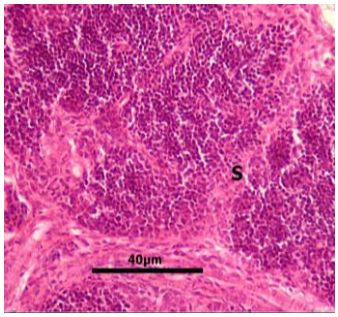
There is adequate concordant data from chick embryotoxicity screening test and mammals.[8] Therefore chick can serve as an effective experimental model and any findings can well be expected in humans.

Methodology

This was an experimental study which was carried out at the Department of Anatomy, Regional Centre, College of Physicians and Surgeons Pakistan, Islamabad, from February 2009 to February 2010. Fertilized, Egyptian Fayoumi chicken eggs were purchased from the Poultry Research Institute, Rawalpindi. The eggs were processed without storage to ensure viability and quality. Randomly selected subjects were divided into two main groups, experimental group A and a control group C, each comprising 60 eggs. Each group was subdivided into two subgroups, 1 and 2, based on the day of sacrifice. All-trans retinoic acid (Sigma) was dissolved in 95% ethanol to make a stock solution of 3mg/ml. Subsequent dilutions were prepared in normal saline. All solutions were prepared under subdued light to minimize photo-oxidation and were immediately used. A volume of 50µl containing 0.3µl of RA in saline was administered to group A via yolk sac, on day 0 of incubation[9] during the early stages of neural crest migration (H&H stage 9-10). Matched control received the same volume of saline. The eggs were placed in the incubator under standard incubation conditions. Experimental subgroups A1 and the matched control group C1 were incubated until embryonic day (ED) 15 of the (21 day) incubation period. Subgroups A2, and C2 were incubated till hatching or day 22 (whichever was earlier). Dead embryos or chicks were excluded at the time of sampling. The embryos were removed from their shells through the method described by Kamran et al.[10] Embryos (ED 15) and chicks (hatched) were fixed in a 10% neutral buffered formalin solution for 24 hours. After fixation, the lobes of the thymus were carefully removed along with the surrounding connective tissue. All lobes from one animal were processed in a sealed permeable packet until they were embeded. After staining, sections were observed under an oil immersion lens. Lymphocytes were recognized and the number of nuclei was counted in a unit area of 4800µm^2 in the cortex and medulla.



Thymic section from experimental group (A2) showing diminished septa(S) Stained by haemotoxylin and eosin



Thymic section from control group (C2), showing well developed interlobular septa(S). Stained by haemotoxylin and eosin.

Figure-I: Photomicrographs comparing the development of connective tissue derived septa in the experimental group A2 and its age matched control C2.

A statistical comparison of differences among groups was evaluated by the student's t test. Values of < 0.05 were considered significant.

Results

There were 120 specimens divided into four subgroups, each comprising 30 eggs. Due to the teratogenesis of RA, 5 chick embryos belonging to subgroup A1 and 5 chicks belonging to subgroup A2 did not survive.

Histologically, defects in connective tissue development were seen. Notably, connective tissue septa were diminished in the thymuses from the animals exposed to retinoic acid, as compared to those from the control animals (Figure-I).

Table I: Comparison of the number of lymphocytes of retinoic acid exposed embryonic and fully hatched groups with their age matched controls.

Subgroup	n	Number of lymphocytes in cortex/UA Mean±SE	P-value	Number of lymphocytes in medulla/UA Mean±SE	P-value
A1	25	101.60±0.27080	0.001*	52.0400±0.26128	0.428
C1	30	106.00±0.72397		61.866±0.33126	
A2	24	929.33±10.89752	.011*	755.38±8.843	0.002*
C2	30	1031.11±3.3207		847.77±4.17455	

Key:* Significant N = Number of specimens
UA=Unit area SE = Standard error of the mean

In the experimental subgroup, A1 the number of lymphocytes/ unit area in the cortex (101.60±0.27080) was significantly less than that in the age-matched control group C1 (106.00±0.72397). The number of lymphocytes/ unit area in medulla (52.0400±0.26128) was not significantly different from that in the control (61.866±0.33126) (Table -I).

Regarding the fully hatched group, significantly less number of lymphocytes/unit area was present in the experimental subgroup A2 in the cortex, (929.33±10.89752) than the control C2 (1031.11±3.3207). The decrease in the number of lymphocytes was replicated in the medulla and the number of lymphocytes/unit in subgroup A2(755.38±8.843) was significantly less than in the control group C2 (847.77±4.17455).

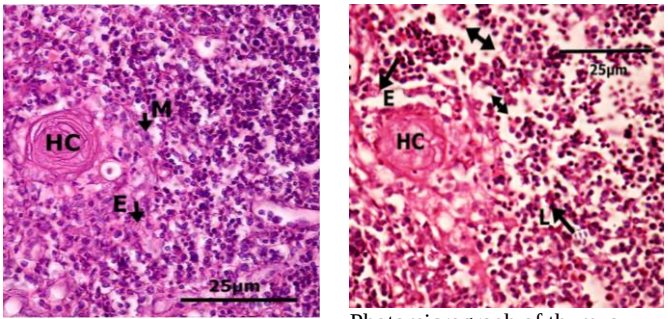
Results show that histological deformation was consistently encountered in the experimental groups. Defective development was seen at the embryonic stage as well as in the hatched chicks. Additionally this derangement progressed with the time of incubation.

Discussion

In the present study, we created a defective model of chick thymus by administering RA to disrupt the signaling molecular cascade, essential for normal development of branchial arches.[11] The defective development manifested as decreased number of lymphocytes and deficient connective tissue septa in thymus. The thymic lymphodepletion encountered in this study has been described previously[2.] Possibly the mechanism leading to defective development was through disruption of ET-1 secretion. This led to failure of dHAND expression in the distal mesenchyme. In the absence of dHAND, the expression of Msx1 in the distal branchial arch could not be regulated, leading to abnormal development of the branchial arch, disturbed cellular proliferation, differentiation and cell death.

At the embryonic stage, lymphocytic depletion was found mainly in the cortex, in the retinoic acid exposed group A1, and it was significantly lower than the control (p=0.001). This preferential lymphodepletion in the cortex[12] has been reported previously but its association with RA exposure has not been described. This predominantly cortical depletion of lymphocytes was probably because the cortex is the site of most active proliferation. Moreover, the cortex is more vulnerable to damage due to its high concentration of densely populated, small, immature and rapidly dividing cells.[13]

In the fully hatched group there was a significant lymphocytic depletion in both cortex and medulla of the experimental group A2 (Figure: II).



Thymic section of chick belonging to control group C2, showing well developed Hassall's corpuscles (HC), Myoid cell (M) and Epithelioreticular cells (E) masked by lymphocytes. Stained by haemotoxylin and eosin

Photomicrograph of thymus chick belonging to experimental group(A2) showing areas of atrophy marked by double headed arrow. Hassall's corpuscle(HC) Epithelioreticular cells(E) masked by lymphocytes(L). Stained with hematoxylin and eosin

Figure II: Photomicrographs of thymus comparing the lymphocyte development in experimental group A2 and the age matched group C2.

The uniform involvement of cortex and medulla in the latter stages of incubation shows the progression of damage as cellular density rapidly increases in medulla making it equally susceptible. Scientists have shown this previously.[12]

The diminution of connective tissue septa encountered in experimental groups A1 and A2 can be explained by the development of defective mesenchyme as a result of failed neural crest induction. It is well documented that neural crest induced mesenchyme invades thymus early in development and forms the connective tissue septa dividing thymus into lobules.[14]

In this study retinoic acid exposure led to disruption of neural crest induced mesenchyme. Diminished septa formation in this study appears to be one of the manifestations of RA teratogenesis. RA mainly affecting neural crest migration to the branchial arch mesenchyme which manifested as diminished connective tissue septa between the thymic lobules. (Figure: I)

Conclusion

Exogenous retinoic acid exposure disrupted the neural crest derived mesenchyme. Defective mesenchyme failed to provide the microenvironment essential for normal cellular proliferation and differentiation in chick thymus.

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