

Does folinic acid ameliorates retinoic acid induced teratogenicity in chick embryo?

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Abstract

Objective: To investigate the protective effect of folinic acid on the hatching ability and developmental defects in a retinoic acid-induced teratogenic model of chick embryo.

Methods: The experimental study was conducted at the Department of Anatomy, Regional Centre of the College of Physicians and Surgeons Pakistan, Islamabad, from February 2009 to February 2010. Chicken eggs were divided into two experimental groups and a control group. The first experimental group was injected with retinoic acid to induce a defective model, while the second experimental group was concomitantly injected folinic acid to observe its protective effects on retinoic acid-induced defects in the development and hatching process. Both groups were compared with the age-matched control group.

Results: A total of 90 fertilised eggs were divided into three groups. The experimental groups had significantly more delayed and assisted hatchings compared to the control group ($p < 0.05$) but the difference between the experimental groups regarding the mode and day of hatching was insignificant ($p > 0.05$).

Conclusion: Irrespective of the presence of folinic acid, prenatal retinoic acid exposure significantly altered the hatchability characteristics in the experimental groups compared to the control.

Keywords: Retinoic acid, Chick embryo, Folinic acid, Hatching. (JPMA 66: 302; 2016)

Introduction

Retinoic acid (RA), a metabolite of vitamin A, is a well-known teratogen. A distinctive constellation of defects labelled as RA embryopathy involves craniofacial, thymic and cranial structures.¹

RA exerts its actions mainly through retinoid acid receptors (RARs) and rexinoid receptors (RXRs). These receptors bind to RA response elements (RAREs) in the regulatory regions of direct target genes. RA thus acts by gene transcription. Due to its potential to inhibit growth and promote differentiation, RA holds great therapeutic promise and its clinical role is expanding. In spite of the clinical benefits, there is a severe teratogenic risk associated with its use during pregnancy and despite continued warnings, the risk of foetal exposure to RA remains high.^{2,3} Animal studies along with recent epidemiological reports on human teratogenicity raise concerns about the potential for induction of defects in offspring of women of child-bearing age.

Recently a number of studies have demonstrated the protective role of folinic acid (FA), a metabolically active form of folic acid, against RA embryopathy. Memon and

Pratten⁴ described its potential to prevent RA-induced cardiac defects in chick embryo, while Naseer et al.⁵ demonstrated its cardio-protective role against RA-induced teratogenicity in albino mice. Firat et al.⁶ concluded that prenatal administration of FA counters RA-induced defects in maxillofacial defective model in rats. Experimental work by Zhang et al.⁷ showed that FA supplementation can prevent RA-induced craniofacial abnormalities. Since there is considerable interest regarding the role of FA to counter the teratogenic effects of RA, the current study was planned to confirm the teratogenic potential of RA and determine the role of FA in countering teratogenesis imparted by RA in the form of visible defects and ability to hatch naturally.

Materials and Methods

The experimental study was carried out at the Department of Anatomy, Regional Centre, College of Physicians and Surgeons of Pakistan (CPSP), Islamabad, and comprised fertilised eggs belonging to Egyptian Fayoumi breed of *Gallus domesticus* which were obtained from the Poultry Research Institute, Punjab, Murree Road, Rawalpindi. The eggs were divided into 3 groups; two experimental and one control. The specimen was randomly selected for each group by using the random selection table. Further, 0.3 µg of RA (Sigma) was dissolved in ethanol and subsequently dispensed in 0.5 ml of saline. It was then injected in the experimental group A2 via yolk sac on day 0 of incubation.⁸ Experimental group B2 was

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injected with 5µg of FA in addition to RA via the same route and on the same day.⁹ Control group C2 was sham-injected with saline. The eggs were placed in the incubator and that day was considered day 1 of incubation. Temperature in the incubator was maintained at 102°F and relative humidity was kept between 70% and 80%. The eggs were turned periodically during the day.

The eggs were incubated until hatching or on day 22 of the incubation, whichever was earlier. If the chicks were unable to hatch by day 22, they were taken out of the shells manually.

The day and mode of hatching were noted. The mode was labelled as normal or assisted. Newly-hatched chicks were examined for gross abnormalities, an abnormal gait or behavioural abnormalities.

Fisher's exact test was employed to analyse any significant difference regarding the day and mode of hatching between the groups. Experimental groups A2 and B2 were compared in relation to developmental anomalies by applying chi-square test, and $p < 0.05$ was considered statistically significant.

Results

There were 90 eggs in the study that were divided equally into three groups of 30(33.3%) each. In experimental group A2, the number of alive chicks was 24(80%) and in B2, it was 23(76.6%).

All the 30(100%) chicks belonging to the control group C2 cracked open the shell unassisted on day 21 of incubation. In group A2, 13(54.1%) of the alive chicks were able to crack open the egg shell unassisted on day 21, while 11(45.8%) had to be assisted after waiting till day 22. Thus, hatching was significantly delayed compared to the

Table-2: Comparison of the developmental anomalies between groups A2 and B2.

Variable	Group		Total alive chicks	p-value
	A2 (n=24)	B2 (n=23)		
Apathy	11 (45.83%)	10 (43.47%)	21(44.68%)	0.871
Limb deformities	9(37.5%)	7(30.43%)	16(34.04%)	0.609
Abd. wall defects	11(45.83%)	9(39.1%)	20 (42.55%)	0.642

Key: n= number of alive chicks.



Figure-1: Newly-hatched chicks belonging to experimental groups A2, B2 and control group C2. The chicks belonging to the experimental groups A2 and B2 were unable to maintain erect posture and displayed diminished mobility.

controls ($p < 0.000$). In experimental group B2, 19(82.6%) of the alive chicks managed to hatch out of the shell

Table-1: Comparison of the day and mode of hatching between the groups A2 & B2, A2&C2 and B2 & C2.

Group	Day& mode of hatching of alive chicks		Total alive chicks	P-value
	Day 21(natural)	Day 22 (assisted)		
A2	13 (54.1%)	11(45.8%)	24	0.059
B2	19 (82.6%)	4 (17.39%)	23	
Total	32 (68.0%)	15(31.91%)	47	
A2	13 (54.1%)	11(45.8%)	24	<0.0001*
C2	30 (100%)	0	30	
Total	43 (79.6%)	11(20.37%)	54	
B2	19 (82.6%)	4 (17.39%)	23	0.030*
C2	30 (100%)	0	30	
Total	49 (92.4%)	4(7.54%)	53	

Key: * Significant

A2= Experimental group exposed to retinoic acid.

B2= Experimental group exposed to retinoic acid and folinic acid.

C2= Sham injected age matched control group.

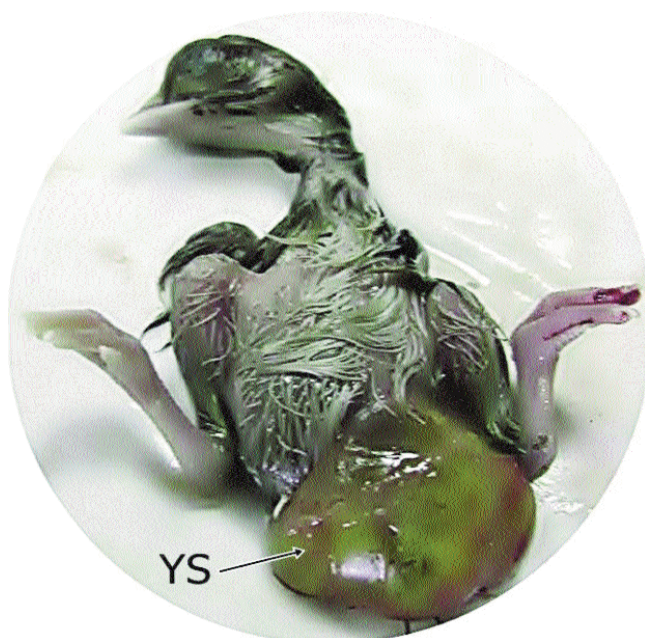


Figure-2: A retinoic acid-exposed chick belonging to experimental group A2, with failed retraction of the yolk sac (YS).

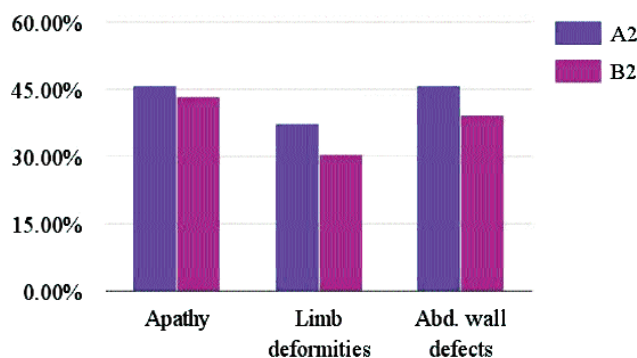


Figure-3: Comparison of the experimental groups regarding developmental anomalies.

without assistance on day 21, which was also significantly less than the controls ($p=0.030$). However, hatching was slightly improved compared to group A2 ($p=0.591$) (Table-1). Apathetic behaviour was assessed by diminished mobility and lack of interest in consuming food and water. Such behaviour was demonstrated by 11(45.83%) of the alive chicks in group A2 and 10(43.47%) in group B2. Contrastingly, C2 chicks were actively jumping and consuming food (Figure-1). The apathy demonstrated by group A2 was not significantly improved by co-administration of FA in group B2 ($p=0.871$).

Limb deformity, manifested by an inability to straighten the toes leading to limping on attempted walking, was demonstrated by 9(37.5%) chicks in group A2 and by 7(30.43%) in group B2 ($p=0.609$).

No gait abnormalities were shown by the controls.

In group A2, the anterior abdominal wall failed to close with failure of retraction of the yolk sac in 11(45.83%) chicks (Figure-2), while it was present in 9(39.1%) chicks of group B2 ($p=0.642$). All the controls showed fully formed anterior abdominal wall without any evisceration.

Discussion

Study results indicate that hatching was significantly delayed in the experimental groups, exposed prenatally to RA or RA with FA compared to the control group. Although there were slightly more unassisted hatchings on day 21 in B2 compared to A2, but the protection offered by FA was not enough to rescue the detrimental effects of RA.

The cause of delayed hatchings demonstrated by the experimental groups could have been the hypoactivity and motor dysfunction induced by central nervous system damage. This is in accordance with the findings of previous studies.^{10,11} Scientists have shown that prenatal RA exposure affects cortico-cerebellar connections and induces several structural abnormalities, including reduced cerebellar size and impaired foliation profile.¹²

The slight improvement in the ability to hatch naturally in B2 compared to A2 ($p=0.591$) could be because of the partial protective effect of FA. This can be explained by its role as 1-carbon donor for metabolism and methylation.¹³ RA acts by modulating the activity of transport systems for amino acid uptake, including methionine, which serves as methyl donor for deoxyribonucleic acid (DNA) synthesis.^{14,15} This decreased protein synthesis might have been prevented by supplemental FA, maintaining the optimal concentration of methyl donor for DNA synthesis.¹³

Regarding the behavioural abnormalities, the apathy demonstrated by the chicks belonging to the RA-injected group can be explained by the previously documented effect of RA on the brain in the form of decreased hypothalamic cells.¹⁶ This reduction in the hypothalamic cellular population can lead to increased depression-related behaviour in mice. This depression has been assessed by impaired locomotion by a number of studies.¹⁷⁻¹⁹ In this study, apathetic behaviour was not significantly improved by FA administration. This was probably because the damage induced by RA was

irreversible.

Abnormal limb development is a well-known effect of exogenous RA, and the results of our study were commensurate with previous scientific work.²⁰⁻²² Although the difference was not statistically significant, but limb deformities decreased from 37.5% in A2 to 30.43% in B2, indicating minimal protection imparted by FA. Many scientists have shown the capability of FA to prevent congenital anomalies. RA can interfere with DNA synthesis leading to apoptosis, while FA could interfere with apoptosis and preserve chondrocyte proliferation.²³

Our study also showed a predominant failure of retraction of yolk sac into the abdominal wall. Normally yolk sac starts retracting at 19th day and completely retracts on day 20 of incubation.²⁴ Researchers have shown that abdominal wall defects, particularly omphalocele, are associated with prenatal RA administration in animals.²⁵ DNA mutations are implicated as the cause.²⁶ The incidence of failed yolk sac retraction was seen in the FA-injected group as well. Thus FA administration could only partially rescue the developmental defects induced by RA (Figure-3).

Conclusion

RA significantly delayed hatching and induced developmental defects, irrespective of the presence or absence of FA. Chicks exposed to RA had considerably more assisted hatchings and exhibited apathetic behaviour, limb deformities and abdominal wall defects. Further studies are essential to ensure better understanding of the extent of protection offered by FA against teratogenesis.

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