

EFFECTS OF ADMINISTRATION OF VITAMIN A TO PREGNANT DAMS ON THE SKIN OF THE FETUSES OF ALBINO MICE

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ABSTRACT

Introduction: For differentiation and maintenance of epithelial cells *in vitro* and *in vivo* under the influence of retinoic acid treatment, skin seems to be a major target organ for both the normal and pathological states. The current study was conducted to evaluate the effects of retinoic acid on fetal skin if given to albino mice during pregnancy.

Materials and Methods: Twelve pregnant albino mice were divided into two groups of 6 each; the experimental group was given 60 mg/kg/day of retinoic acid (R.A) dissolved in 0.1ml of olive oil orally on 7, 8 and 9 day of gestation. Fetuses were delivered and dissected on 18th day of gestation; skin samples were removed and processed for microscopic study.

Results and Conclusion: Histological examination of fetal skin in RA treated group showed increased keratinocyte proliferation resulting in increased number of epidermal cell layers and increased epidermal thickness. It also inhibited the development of hair follicles which are seen in the form of rudimentary buds in the dermis. So its usage during pregnancy should be warranted. Given the essential role of retinoids in epidermal differentiation and their effectiveness in the treatment of several skin disorders it is important to see its histological effects on skin.

Key words: Retinoic acid, skin, dams, fetus, proliferation.

INTRODUCTION

Retinoids are naturally occurring and synthetic analogues of vitamin A which is essential for embryogenesis, growth and epithelial differentiation.¹

Maternal vitamin A deficiency or retinoic acid (RA) excess results in a spectrum of congenital malformations in a dose and developmental stage dependent manner.^{2,3} Thus, RA levels must be strictly controlled and should be consumed with caution during pregnancy.

The effects of RA are mediated by two major groups of peptides, the nuclear receptor proteins and the cytoplasmic binding proteins. There are two members of retinoic acid receptors: retinoic acid receptors (RAR) and retinoic X receptors (RXR).⁴ Each receptor family has at least three subtypes (α , β & γ). Another study demonstrated expression of RAR – gamma in human epidermis, and suggested that RAR – gamma is a molecular target of RA action in adult human skin.⁵⁻⁷

RA – RAR complexes bind to short cis – acting DNA sequences, hormone responsive elements near the promoters of target genes in order to regulate the target gene expression.⁸

Morphogenesis of embryonic tissue is greatly affected by retinoids. The pattern of mouse vibrissae follicles are modified to form mucus gland pattern and also

feather epidermis of the chick are reported to be transformed from the chick scales epidermis.⁹

In mammalian hair follicles development there is reciprocal induction between ectodermal epithelium and mesodermal mesenchyme. In mouse it begins at 14th day of gestation by local proliferation of epidermis, forming thickenings (placode stage) followed by hair germ stage then hair peg – stage (dermal papilla formation) and finally hair follicles are formed.¹⁰

For chemoprevention or treatment of skin disorders including malignant conditions, retinoids are found to be effective agents. Major role in growth and differentiation for a variety of malignant and normal cells, retinoids are regarded to have an important function.

They are known to play a major role in regulating growth and differentiation of a variety of normal and malignant cells.¹¹

Topical all – trans RA treatment caused an increase in the number of living cell layers in the epidermis of both human and mouse.^{12,13}

It has been reported that retinoic acid treatment results in expression of post-transcriptional elevation in epidermal growth factor receptors (EGF – R).¹⁴

The epidermal growth factor receptor (EGFR) is the cell surface receptor for members of the epidermal growth factor family (EGF family) of extracellular

protein ligands. Upon activation by their ligands there is stimulation of tyrosine kinase pathways that ultimately initiate several signaling pathways involving cellular proliferation, differentiation and apoptosis.^{15,16}

RA alters expression of epithelial growth factor receptor (EGFR) signaling pathways as in kidney epithelial and fetal lung cell lines; it was also found to modulate the action of EGF in developing skin of mouse embryos.¹⁷ On this basis, we have tried to see the histological effects of administration of retinoic acid to pregnant dams on developing skin of mouse fetuses to substantiate the earlier observations.

MATERIALS AND METHODS

Sixteen albino mice (twelve female and four males) weighing 25 – 30 gm and 6 – 8 weeks old were procured from National Institute of Health, Islamabad. Animals were housed in the Research laboratory of University of Health Sciences, Lahore under controlled conditions of temperature ($22 \pm 0.5^\circ\text{C}$), humidity ($50 \pm 10\%$) and light and dark cycle of 12 hours each. They were given mouse chow and water ad libitum.

The experimental animals were randomly divided into two groups of eight animals each, six female and two males. Female mice were caged overnight with normal males in the ratio of 1male: 3 females. Mating was confirmed by the presence of a vaginal plug on the following morning and was considered gestational day 0 (zero).¹⁸

Animals of control group were given 0.1ml of olive oil orally on 7th, 8th and 9th day of pregnancy whereas experimental group received 60 mg/kg/day of retinoic acid dissolved in 0.1ml of olive oil orally on comparable days of pregnancy. Pregnant mice were sacrificed and dissected on the 18th day of gestation to obtain the fetuses. The fetal skin samples were taken from limb and dorsal aspect of trunk for histological preparation; these were fixed in 10% formalin for 48 hours and later processed for preparation of paraffin blocks. Sections 5 μ thick were obtained using Leica rotatory microtome (RM 2125); stained with Haematoxylin and Eosin for light microscopic examination.

Micrometry

The thickness of epidermis was measured using ocular micrometer at X40 objective after calibrating it with linear stage micrometer. The number of hair follicles per mm was counted under 40X magnification using an ocular reticule. The hair follicles were counted at four sites and then their mean was taken.

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 regar-

ded statistically significant if the 'p' value was ≤ 0.05 .

RESULTS AND OBSERVATIONS

At gestational day18, histological examination of fetal skin from the control group revealed a well organized epidermis with distinct layers of closely packed cells. It was characterized with prominent stratum basal and corneum (Fig. 1).

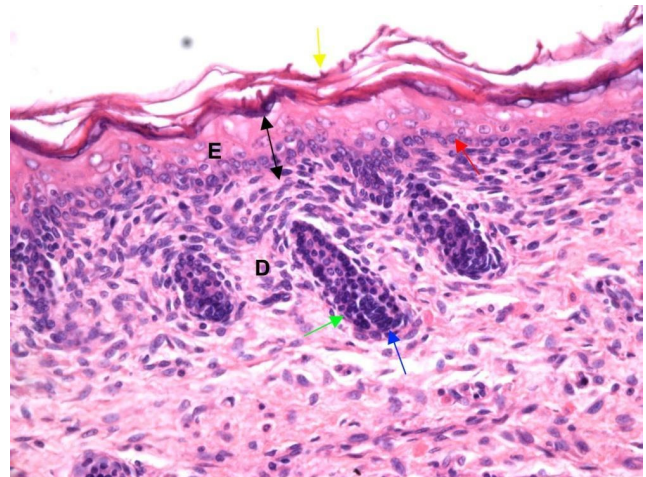


Fig. 1: Photomicrograph of fetal skin from control group showing well defined epidermis (E) with prominent stratum basal (red arrow) and stratum corneum (yellow arrow). Dermis (D) shows numerous hair follicles at different stages of differentiation with its different parts; hair bulb (green arrow) and dermal papilla (blue arrow). H and E stain X 400.

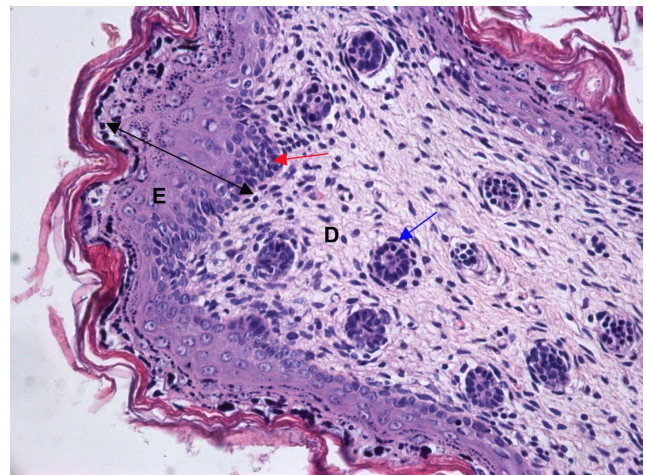


Fig. 2: Photomicrograph of fetal skin from treated group shows significant thickening of epidermis (E) with large intercellular spaces. Dermis (D) shows hair follicles comprising a few premature buds (blue arrow and red arrow). H and E stain X 400.

In treated group, epidermal layers were not evidently discernible and there appeared to be gaps between

the cells which were presumably due to accumulation of inter cellular matrix. Epidermis was discernibly thicker than that of the control group; this was possibly due to an increase in the number of cells resulting from rapid proliferation of those in stratum basal (Fig. 2). There was no change in dermal connective tissue of the skin of both groups. Mean epidermis thickness of treated group was higher when compared to that of the control groups, the difference was statistically significant (p -value < 0.001).

In addition, hair follicle morphogenesis was also altered in the treated group; they were reduced in number and their growth was arrested at germ stage of hair follicle development. They did not invaginate deeply into dermis and rudimentary buds were found.

The mean hair follicle count of treated group was lower than that of the control and the difference was statistically significant, p -value < 0.001 .

DISCUSSION

Growth and differentiation of epithelial tissues are greatly affected by retinoids. Changes in keratinocyte morphology when treated with different doses of various retinoids in earlier cell culture studies had been reported. Retinoic acid receptor-gamma (RAR – gamma) is one of the variety of retinoic acid receptors and is reported to be present exclusively in skin.¹⁹ RAR- α and RAR- γ play a role in epidermal – dermal interactions that lead to hair follicle morphogenesis.²⁰

In present study, pregnant female mice were given 60 mg/kg/day of retinoic acid dissolved in 0.1 ml of olive oil orally on 7th, 8th and 9th day of pregnancy; significant concentrations of all – trans RA in the plasma and embryo were reached after oral treatment of pregnant dams in mouse, rat and rabbit; administration of RA in high concentration had in the past been reported to produce teratogenic effects on account of its metabolites filtering through placental barrier.^{21,22}

Histological structure of fetal skin in the control group showed stratified epidermis with distinct layers of closely packed cells whereas fetal skin from the treated group showed increased thickness of epidermis due to increased proliferation of cells in stratum basal induced by RA. Similar findings were reported earlier, in which single topical application of all-trans retinoic acid for 4 days stimulated keratinocyte proliferation, increasing the number of epidermal cell layers and increasing epidermal thickness.²³

Another study demonstrated aromatic retinoid induced epidermal hyper-proliferation and numerical hyperplasia after oral administration of the drug in the animal model of the hairless mouse.²⁴

Increasing EGF binding capacity in several cell types after RA treatment also has been found.²⁵ Mechanism of action of epidermal growth factor (EGF) is pre-

Table 1: Showing comparison between parameters of fetal skin when compared among groups.

Parameter	Control Group **(n = 45) Mean (\pm S.D)	Treated Group **(n = 42) Mean (\pm S.D)	p-value
Epidermal thickness (μ m)	131.20 \pm 24.50	102.09 \pm 25.95	0.001*
No of hair follicles / mm ²	38.72 \pm 5.93	61.07 \pm 12.49	0.001*

* $p \leq 0.05$ is statistically significant

**n = number of fetuses born to pregnant females in control and treated groups

sumed to occur through binding with EGFR resulting in an increase in the tyrosine kinase activity of receptor. It subsequently triggers a cascade of intracellular events leading to increased DNA synthesis and cell proliferation.²⁶

In another study it was found that RA activated the transcription of two different nuclear receptors RAR and PPAR β/δ ; the activation of PPAR β/δ lead to expression of genes producing cell proliferation.²⁷

Fetal skin in treated group also showed that layers of the epidermis were not characteristically structured and the large gaps between the cells were observed. These findings are consistent with another study in which RA lead to accumulation of Hyaluronate (HA), producing gaps between the cells in the superficial layers of epidermis; this was produced by increased activity of keratinocytes.²⁸

Hair follicle morphogenesis and hair cycling are controlled by complex bidirectional ectodermal – mesenchymal interactions between epidermal keratinocytes and a specialized population of fibroblasts with inductive, morphogenic properties, which mature into dermal papilla of the hair follicle.²⁹ In our study number of hair follicles were reduced in treated group and most of them were arrested at germ stage. Similar findings were reported earlier by Okano et al (2012) in which increased RA levels reduced the density of hair follicles and arrested hair follicle growth: further, in retinoic acid treated dams in mouse the fetuses were ablated on account of degradation of enzyme, Cyp_{26b1} (cytochrome P₄₅₀, family 26, subfamily b, polypeptide 1).³⁰

Vitamin A treated cultures, altered differentiation and increased proliferation of human keratinocytes as reported by Chopra;³¹ however, this did not offer an explanation to its therapeutic effect seen in various skin diseases of hyperkeratosis. Additional work is suggested in this direction.

ACKNOWLEDGEMENTS

Facilities and financial support provided by the Uni-

versity of Health Sciences, Lahore and the help by the technical staff is greatly appreciated.

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