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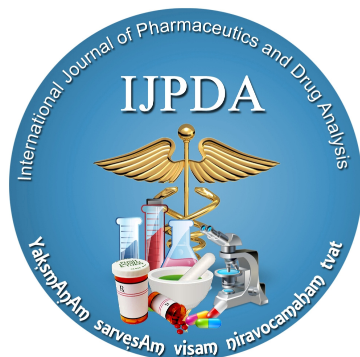
RESEARCH ARTICLE



INFLUENCE OF VITAMIN-A ON CORNEAL REGENERATION IN TADPOLES

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Abstract:
This study comprises of regeneration of cells of cornea. This study supports the possibility of their clinical use as a cell source for reconstruction of the damaged corneal surface. For this experimental study, tadpoles were reared from eggs collected from ponds in two developmental stages that are 3 toe stage and 5 toe stages. Tadpoles were anaesthetized. The tadpoles were reared in conditioned tap water for the control group and exposed to 5 IU/ml vitamin A for experimental groups tadpoles. Tadpoles were then subjected to operate their cornea and reared in vit-A solution. The amputated part or tissue is then dehydrated, cleared and infiltrated with embedding material. The final embedding was done into wooden block and then section was cut by microtomy. Slides were prepared and stained. Examinations of slides were done under inverted microscope. Tadpoles treated with vit-A showed more corneal regeneration as compared to tadpoles reared in water as control group. Therefore, vitamin –A has proved to be significant chemical model to induce and accelerate the corneal regeneration.

Keywords: Microtomy, cornea, anaesthetized, vit-A

Introduction

A clear cornea is essential to visual acuity and depends on stromal avascularity and epithelial integrity (Wagoner MD. 1997) The cornea is responsible for protecting the eye against insults such as injury and infection. It also provides the majority (two thirds) of the total refractive power of the eye and is therefore the major refracting lens. The cornea of the human eye is composed of three main components: the epithelium, stroma, and endothelium. The corneal epithelium contains stem cells* that are responsible for metabolism. If these stem cells are depleted as a result of autoimmune diseases, burns, or other causes, the corneal epithelium on the ocular surface will no longer regenerate easily. The defective epithelial area and eventually the entire surface will be gradually covered with conjunctival and vascular tissue, leading to visual disturbance and other disorders.

These disorders are collectively known as limbal stem cell deficiency. Ocular burns cause depletion of limbal stem cells, which leads to corneal opacification and visual loss. This process leads to neovascularization, chronic inflammation, and stromal scarring, with corneal opacity and loss of vision(Dua and Azuara 2000).

Limbal stem cell deficiencies cause conjunctival epithelial in growth, neovascularization, chronic inflammation, recurrent epithelial erosions and defects, destruction of the basement membrane, and fibrous tissue in growth, leading to severe functional impairment (Tsai et al 1990; Tsubota et al 1995; Kruse and Reinhard 2001). The pathology includes Stevens-Johnson syndrome, ocular cicatricial pemphigoid, chemicals and thermal burns and radiation injury. Proper visual function requires an intact ocular surface.

The integrity of the corneal surface is maintained by two specialized epithelia, the conjunctival epithelium and the limbal corneal epithelium. Corneal epithelial stem cells reside in the palisades of Vogt, located in the basal layer of the limbus, coinciding with the transitional zone between the cornea and the bulbar conjunctiva (Schermer et. al 1986). Limbal stem cells produce undifferentiated progeny with limited proliferative potential that migrate centripetally from the periphery of the corneal epithelium to replace cells desquamating during normal cell turnover. (Kinoshita et al., 1981)

At the corneo-scleral junction in an area known as the limbus, there is a population of limbal epithelial stem cells (LESCs). LESCs share common features with other adult somatic stem cells including small size (Romano *et al*., 2003) and high nuclear to cytoplasmic ratio (Barrandon and Green, 1987).

Autologous cultured epithelial cells can restore damaged corneas, but this technology is still developing. We sought to establish a culture system that allows preservation of limbal stem cells and preparation of manageable epithelial sheets and to investigate whether such cultures can permanently restore total limbal stem cell deficiency. Therefore, healthy limbal cells can be extracted from limbus region and cryopreserved for the corneal damage. Therefore corneal renewal and repair are mediated by stem cells of the limbus, the narrow zone between the cornea and the bulbar conjunctiva

MATERIALS AND METHODS

Collection Tadpoles (Rana cyanophylictis) and Amputation of cornea

Tadpoles were reared from eggs collected from ponds. Two developmental stages viz 3 toe stage (young) and 5 toe stage (mature) tadpoles were employed for experiments. All the ethical parameters are followed for using the tadpoles experiments performed in accordance with bioethilcal regulation for the use laboratory animals. Tadpoles were anaesthetized with 1: 2000 MS222 solutions for 3-5 minutes before the operation. For control group, tadpoles were reared in conditioned tap water. While tadpoles for experimental groups were exposed to 5 IU/ml vitamin A for 3 days and then transferred to conditioned tap water.

Series I consists of those tadpoles which were subjected to operate their cornea and reared in vitamin A solution (treated).

Preparation of explants: Experiments are preceded in different manners along with same parameter.

EMBEDDING

In this the tissue samples are placed into molds along with liquid embedding material (such as agar, gelatin, or wax) which is then hardened. This is achieved by cooling in the case of paraffin wax and heating (curing) in the case of the epoxy resins. Embedding is done by putting the tissue into wax1,wax 2,wax3, followed by putting in wooden blocks.

The hardened blocks containing the tissue samples are then ready to be sectioned. Thicker sections (0.35µm to 5µm) of resin-embedded tissue can also be cut for light microscopy.

After embedding the material into the Wax III form wooden block. After wooden block formation cut the section of the material. After cutting the section form a slide with the help of adhesive material (egg albumin + glycerol in equal quantity).Then spread the section with the help of hot water. After this give a 5 to 10 minutes oven treatment at 70 ºC then turn towards staining.

STAINING

Staining is employed to give both contrasts to the tissue as well as highlighting particular features of interest. Hematoxylin and eosin (H&E stain) is the most commonly used light microscopical stain in histology and histopathology. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. Uranyl acetate and lead citrate are commonly used to impart contrast to tissue in the electron microscope. Then slide is dipped in xylene and finally dpx is put onto the slide.

After completion of the staining procedure slides are examined under the microscope. Three types of microscopes are used for this purpose. 1st is light microscope, used for superficial studies of slides followed by binocular microscope for marking of selected slides Which are further followed by Inverted microscope to get high resolution of selected slides along with clear photographs

RESULTS AND DISCUSSIONS

The results presented in this study clearly demonstrate an ability of toad tadpoles to heal corneal injuries and extend the utility of the amphibian model of regeneration.(Majo etal 2008; Pellegrini et al 2001; Di Iorio et al 2005). Vitamin A has proved to be significant chemical model to induce and accelerate corneal regeneration in both modes of experiments(Kruse FE. 1994) The percentage of corneal regeneration in vivo it was 65% in Vit-A treated cases in comparison to untreated control tadpoles where it was 40% in 3 toe stage tadpoles and in 5 toe stage tadpoles percentage of corneal regeneration is 35% in untreated control and 60% in Vit. A treated cases in first mode of experiment but in second mode of experiment, the percentage of corneal regeneration in vivo but ex situ it was low in

Table 1: "Corneal regeneration from various stages of tadpole"

Stage of experimental animals	**Group (After operation animals reared in)**	**Day of preservation**	**No. of preserved animals**	**Percentage of corneal regeneration**
3 toe stage young tadpoles	Control IA(after operation animals were reared in water)	3 7 15	5 5 10	**40%**
	Vit. A treated IB (after operation animals were reared in vit A sol)	3 7 15	5 5 10	**65%**
5 toe stage mature tadpoles	Control IC(after operation animals were reared in water)	3 7 15	5 5 10	**30%**
	Vit.A treated ID(after operation animals were reared in vit. A sol)	3 7 15	5 5 10	**55%**

Fig1: It shows microphotograph of a section passing through operated eye showing amputated area of cornea of vit –A treated tadpoles.

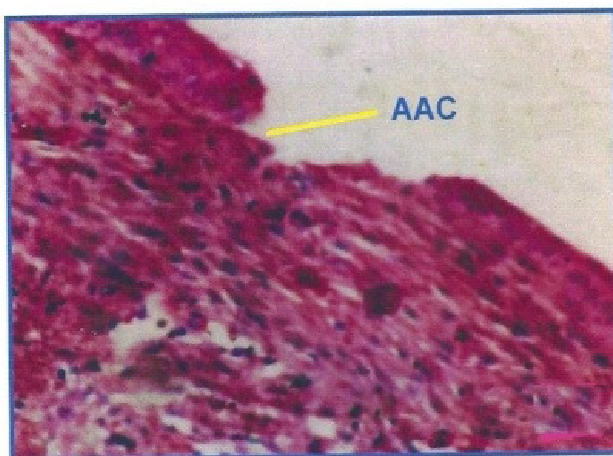

Fig.1: AAC- Amputated area of cornea

Fig 2 and 3: It shows microphotograph of sections through injured cornea of 3 day's vit- A treated young tadpoles showing proliferation of cells in corneal epithelium and stroma part

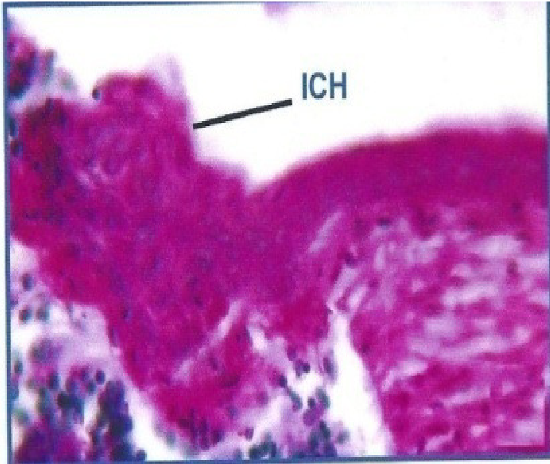

Fig.2 ICH- Initial stage of corneal healing

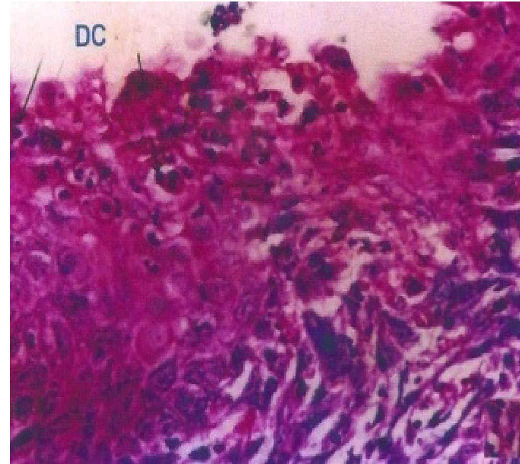

fig.3: DC- Dividing cell

Fig.4: It shows microphotograph at wound edges passing through the operated cornea of day-7, vit-A treated tadpole showing fibroblasts appear

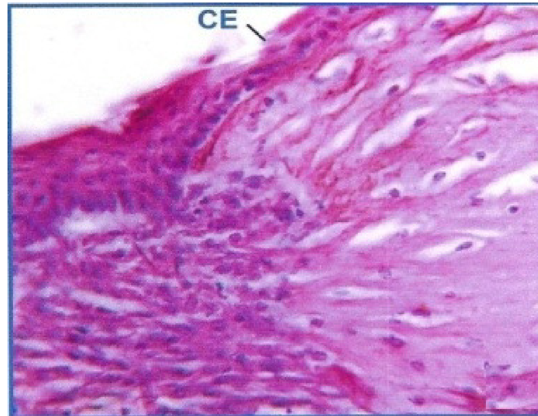

Fig. 4: CE-Corneal epithelium

Fig.5 and 6: It shows microphotograph passing through the operated cornea of days-15, vit-A treated tadpole showing well differentiated fibres which are arranged parallel to each other similiarnto intact cornea,showing scarless healing

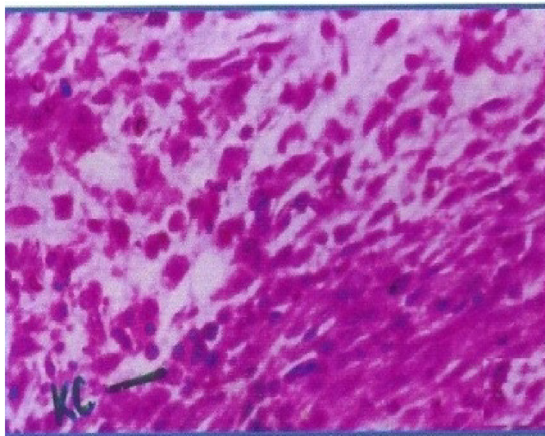

Fig .5 : KC:- Karyocytes cells

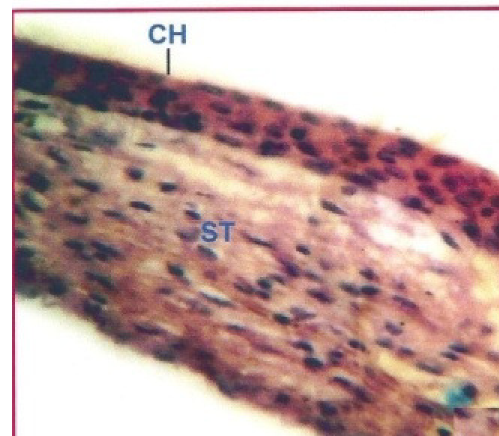

fig.6 : CH- Corneal healing, ST- Stroma

untreated 30% in control and high in Vit-A treated cases 50% in 3 toe stage tadpoles but in 5 toe stage tadpoles the percentage of regeneration is 20% in untreated control tadpoles and 45% in Vit. A treated tadpoles as shown in table.1

DISCUSSION

Amputation was done in corneal cells or part of an eye of young tadpoles, followed by the treatment in vit-A as well as water as control group. After the few days, initial stages of corneal healing was seen as in fig.1. In amputated part cells started dividing and was filled by cells. Therefore, regeneration in more percentage was seen I tadpoles treated in vit-A. The observation was simultaneously seen in 7th and 15th day tadpole. In 7th day tadpoles, fibroblasts were seen as in fig. 4. The result of 15th day vit-A treated tadpole was well differentiated fibres which are arranged parallel to each other similar to intact cornea, showing scarless healing as seen in fig. 5 and 6. Thus study clearly demonstrate an ability of toad tadpoles to heal corneal injuries and extend the utility of the amphibian model of regeneration. The introduction of limbal epithelial cell transplantation was a major advance in the therapeutic techniques for reconstruction of the corneal surface. Limbal epithelial cell transplantation is clinically conducted when cultured allografts as well as autografts are available; however, allografts have a risk of immunologic rejection and autografts are hardly available for patients with bilateral ocular surface disorders. We induced corneal epithelial cells from ES cells by culturing them on type IV collagen or alternatively, by introduction of the pax6 gene into ES cells. Recent advances in our study supports the possibility of their clinical use as a cell source for reconstruction of the damaged corneal surface

CONCLUSIONS

In conclusion, our study shows that Cultures of limbal stem cells thus represent a source of cells for transplantation in the treatment of burn-induced destruction of the human cornea. Complete blindness can be cured by corneal transplantation by making the use of limbal stem cell. The best advantage of limbal stem cells is it can be stored by cryopreservation for later use in corneal damage for transplantations.

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