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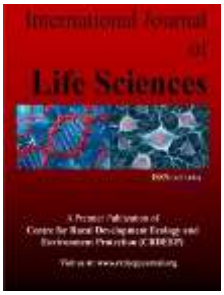

Full Length Research Paper

A Histological Study of Lens Regeneration in Aphakic Swiss Albino Mice under the Influence of Turmeric

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ABSTRACT

The present study revealed that pigmented epithelial cell of dorsal iris are capable of regenerating a new lens when old lens is removed from eye. Lentectomy was done on 120 swiss albino mice. Two different developmental stages were taken for experimental purpose. One stage was consisted of 7 days young swiss albino mice and another was of 60 days old sexually mature swiss albino mice .Half of the animal were taken in control groups prepared from both the developmental stages and another half were placed in turmeric treated groups. Mother tincher of turmeric was used for experimental purpose .Doses were prepared by dissolving a known quantity of mother tincher in distilled water. Injections were given intra peritonially to both the developmental stages of swiss albino mice of treated groups. Effects of turmeric on lens regeneration were observed by histological studies. Purpose of this study was to understand the process of lens regeneration through dorsal iris and investigating the effect of turmeric on it .Present study revealed that lens regeneration is a multistep process. PECS of iris plays main role in lens regeneration. It has been deduced that regenerating lens was an age dependent process, declined with the advancement of age. Turmeric which is known for its healing properties has the capacity of accelerating the process of cell proliferation and inducing differentiation in pigmented epithelial cells (PECS).

Introduction

The process of lens regeneration is a very fascinating process of biology. In lens regeneration the non-lens ocular tissues like retinal, Iris and corneal tissue get Trans-differentiate in to lens tissues. So lens regeneration is a clear case of trans differentiation in which one differentiated cellular type which is structurally and functionally distinct lose their identity and de-differentiate and then re-differentiate into another cellular type by acquiring different structure and function which is the case studied in lens regeneration in which pigmented epithelial cells of dorsal Iris or retina lose their pigments and trans differentiate into lens cells. The process of lens regeneration is a very complex process which consists of many events requiring integrated function of multiple tissue, cell lineages and growth factors. After injury or removal of lens these factors actively participates in complex response in order to restore and recreate lens structure in vertebrates. The lens regeneration is seen in few species of amphibians mainly in salamanders, in some fishes. In mammals studies were done on rats, mice, pigs, rabbits and guinea pigs. General events of lens regeneration in mammals are similar to amphibians. PECs from dorsal Iris trans differentiated into lens in amphibian as well as mammals. It has been found that these pigmented cells have a dual capacity to get converted into either retinal tissue or occasionally lenses when transferred into lentactomized eye. This unique phenomenon offers valuable insights into the process of cellular differentiation, genetic reprogramming and may help understand why other animals lack the ability to regenerate their lenses (Tsonis et al., 2000; Tsonis, 2000, 2004, 2006. Del Rio Tsonis and Tsonis, 2003; Jangir and Sharma 2005, Alvardao and Tsonis 2006, Tsonis et al. 2006). In present paper effect of Turmeric was studied in lens regeneration.

Charles Bonnet in 1781 studied lens regeneration in Salamander. In this experimental studies found that a complete lens regenerate after the removal of old natural native lens. In 1891 histological studies made by Vincenzo copucci described lens regeneration in newts from dorsal iris. Turmeric scientifically known as Curcuma longa has been used for centuries for, its medicinal properties particularly in wound healing, lightening scars and reducing inflammation. Apart of its wound healing properties its compound, curcumin in well known for its pro-angiogenic properties mean it promotes new blood vessels formation by inducing the production of

transforming growth factor beta, which in turn stimulate both angiogenesis (formation of new blood vessels) and accumulation of extracellular matrix which contributes in overall repair of wounds Nezhad reported curcumin led to significant increase in cell proliferation and progression during wound healing.

Material and Method:

Experiment was conducted on two different development stage of Swiss albino mice consist of 7 days old Swiss albino mice and another consist of 60 days old sexually mature Swiss albino mice operation was carried out on 120 animals and Lentectomy was done after pre-treatment of animals with local anesthesia (2% xylocaine). A longitudinal slit was made in the cornea of right eye of mice. Whole operation is carried out under a stereoscopic Binocular microscope. Fine syringe, needles, curved forceps and ophthalmic crossway scissor were used to extract the whole lens with lens capsule. Operation was carried out only on right eyes of animals and left eyes remained untreated. Turmeric solution was prepared from mother tincture of curcuma longa. Dose for Intraperitoneal injection was prepared by diluting mother tincture in distilled water.

Fig 1 : Showing the colony of swiss albino mice.

Fig 2 : Showing the removal of complete lens with lens capsule through lentectomy**.**

Animals were divided into two groups A and B. A group was taken as control groups without turmeric treatment and another groups, Group B was treated with turmeric. These two groups were further divide into subgroups. A_1, A_2 and B_1, B_2, A_1 and B_1 group were control group of different development and stages and A_2 and B_2 were turmeric treated groups of these development stages. Immediately after lentectomy the turmeric treated groups A_2 and B_2 were treated with diluted turmeric solution by putting directly a drop into operated right eye. Control groups were not given any treatment until the end of experiment. Treated groups were given dose of turmeric on alternate days up to termination of experiment and sham injections were given to control groups until the end of experiment. Animals of both groups were preserved in Bouin's solutions for 24 hours 30 hours for various interval of time (3,5,9,25,45). For preservation animals were sacrificed with cervical dislocation experiments were carried out for 45 days. The eyes were removed and transferred in 70% alcohol for histological study. After then pass to 90% and 100% alcohol twice for 15 minutes each and then transferred to xylene for 15 minutes. Paraffin block were prepared by embedding eye balls in wax. Thin sections of eyeball were cut by microtome and stained with Haematoxylin and counter stained with eosin and examine under microscope.

Results

During Histological study it has been found that lentectomy induces Iris cells to de-differentiate and trans differentiate in to lens forming cells. It has been seen that the border of dorsal region of Iris begin to thicken, and cleft start forming between inner and outer lamella Amoeboid cells migrate from stroma on to surface of the epithelium and into the cleft. The thickened dorsal Iris changed into double layer. Nuclei of Iris cells began to change their shapes. The pupillary margin of Iris became knob like and this structure continuously develop until the free margin take the shape of a swollen loop. Intercellular communication between cells of pigmented epithelial cells reduced rapidly as a result of inflammatory reaction. During initial phase, the condensed chromatin of PECs nuclei became progressively dispersed, nuclear volume increased and melanosomes of cells moved toward the peripheral region of each PECs at dorsal margin or Iris and then threw out of cells and engulfed by macrophages which appeared around dorsal margin of Iris. After the discharge of melanosome these PECs started to de-differentiate and then started mitotic division. The pupillary margin of dorsal Iris became knob like and this structure continuously to develop until the free margin takes the shape of swollen loop. Scattered mitotic figures were also seen. These changes continued upto to 8^{th} day after operation in turmeric treated animals. These de-pigmented PECs of iris trans-differentiate in to vesicle like appearance which further transform in to a new regenerated lens. Dorsal

iris cells ceased division Lens fibres formation was seen in inner surface of vesicular. Lens Lumen of vesicle was completely filled with primary lens fibres. Later on secondary lens fibre began to form, differentiate and grow around the central nuclei. Regenerated lens got converted in to well define structure. At last regenerated lens got detached from dorsal iris and take the position of natural lens. Secondary lens fibre disappeared progressively. Similar processes were seen in both the control group and turmeric treated groups of Swiss albino mice.

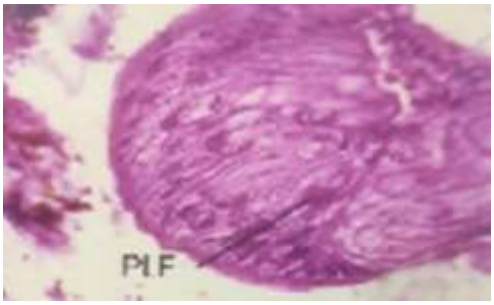

Fig 3 : Microphotograph through eye section of 7 days old Swiss albino mice after 7 days of operation showing primary lens fibres.

A complete lens was regenerated from pigmented epithelial cells of dorsal Iris after the termination of experiment 45th day after operation. Newly regenerated lens was found morphologically resembling with natural intact lens. Size, shape and transparency of regenerated lens were exactly similar to natural lens. It seems very difficult to discriminate between regenerated lens of and original natural lens. The newly formed lens was surrounded with lens epithelium. Cells of epithelial layer were cubodial and slightly taller than before. When experimentally tested with black tape method regenerated lens were found normal in function Lens regeneration was seen in control group of 7 days young mice, Turmeric treated both groups of 7 days young mice and 60 days old sexually mature Swiss albino mice. 60 days old control group didn't show any regeneration. In the experiment as show in figure 1 control group of 7 days old Swiss albino mice show 43% lens regeneration. While control group of 60 days old Swiss albino mice shows 10% lens regeneration. Very less 10% lens regeneration are noticed. In the turmeric treated 7 days old Swiss albino mice group 80% regeneration were seen and turmeric treated 60 days old sexually mature group 60% lens regeneration occurred. The declining trend of regeneration was noticed with the advancement of age which proves that regeneration is age dependent process. Thus, seen more in animals of young group then the advanced age animals. Some lentoid structure were also seen in place of a complete lens in 60 day old sexually mature animals histological study revealed that:

Table 1. Influence of Turmeric on different developmental stages of Swiss albino mice

Developmental Stage	Group	Sub Group	Day of preservation	No. of animal employed	No. of operated animal preserved	Normal regeneration	Non regeneration	Lentoid	Regeneration (%)
7 days young Swiss albino mice	A	A_1 (Control)	3	30	5	-	5	-	
			5		5	1	4	-	
			9		5	2	3	-	43%
			25		5	4	1	-	
			45		10	6	4	-	
		A_2 (Turmeric Treated)	3	30	5	3	2	-	
			5		5	4	1	-	
			9		5	4	1	-	80%
			25		5	4	1	-	
			45		10	9	1	-	
60 days old Sexually mature Swiss albino mice	B	B_1 (Control)	3	30	5	0	5	-	
			5		5	0	5	-	
			9		5	1	4	-	10%
			25		5	1	4	-	
			45		10	2	8	-	
		B_2 (Turmeric Treated)	3	30	5	3	2	-	
			5		5	3	2	-	
			9		5	4	1	-	60%
			25		5	2	2	1	
			45		10	6	3	1	

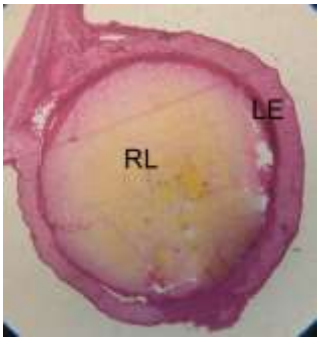

Fig 4 : Microphotograph through eye section of 7 days old Swiss albino mice after 45 days of operation. Showing a complete regenerated lens with single layer lens epithelium.

Discussion

The result of present study showed that turmeric induces lens regeneration not only in young Swiss albino mice but also in sexually mature mice. It also revealed that this regeneration process is age dependent. Young group whether it is control group or treated groups shows high rate of lens regeneration. Which declines with the advancement of age i.e. less regeneration is seen in 60 days old Swiss albino mice in turmeric treated animals in 60 days control group lesser regeneration were noticed. Turmeric is extract from roots of curcuma longa. It contains a chemical agent called curcumin, which have good healing property and reduces swelling. It enhances proliferation and de-differentiation of Iris pigmented epithelial cells. The accelerating effect of turmeric on lens regeneration was seen in both to developmental stage of Swiss albino mice. Lens regeneration from non-ocular tissue (dorsal Iris) has been well known in urodele amphibians, some fishes and mammals like rat, pigs, rabbits and mice. Just like dorsal Iris the retinal pigment epithelial cells have the ability to trans-differentiate into lens which is also widely conserved in amphibians and vertebrates. Good lens regeneration from PECs in vivo has occurred in some fishes and mammals. Turmeric have positive accelerating and proliferating effect on lens regeneration as well as cardiac muscle regeneration. In cell culture study, almost all vertebrates' pigmented epithelial cells (PECs) can switch trans-differentiation to acquire the characteristics of lens. In the similar way, the pigmented epithelial cells extracted from a fully grown human eye trans-differentiated in to lens phenotypes in culture medium. Removal of lens from eye, stimulate pigmented epithelial cell of iris in Swiss albino mice to re-enter in to cell cycle to undergo DNA synthesis and proliferation. It is a multiple event process in which pigmented epithelial cell loses their pigment and undergoes de-differentiation. All the events have been reported in the present experiment. It has been noticed that after de- differentiation some cell escape from cell cycle and trans-differentiate in to lens fibres which get converted in to lens. Lens regeneration is the clear case of metaplasia or trans-differentiation where iris cells transform in to lens. It is also found that turmeric have the power to enhance de-differentiation of PECs cells of dorsal Iris in Swiss albino mice.

Conclusion

From the histological observation, it has been revealed that turmeric has proliferative as well as accelerative effects on pigmented epithelial cells of dorsal iris. Lens regeneration is distinct from simple regeneration as it is the clear case of metaplasia in which pigmented epithelial cells of dorsal iris Trans-differentiate in to lens. The dosages of turmeric utilized in this study were determined to be effective and can be regarded as optimal, because no mortality was seen after the administration of these dosages. Lens regeneration were seen in both young and adult animals .But percentage of regeneration were seen more in turmeric treated groups as compared to control group and adult turmeric treated group. In future, lens regeneration technology will prove a boon to blinds and lens associated diseases.

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