Original Article

Optic Nerve Astrocytosis (Astrogliosis), Induced By Ethambutol

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Purpose: To determine astrogliosis in optic nerve of rabbit induced by ethambutol. **Materials and Methods:** Ten, New Zealand white, albino rabbits of either sex, 6-12 months old; weighing 1- 2 kg were randomly divided into two group comprising five animals in each. Group A served as control, while Group B was given ethambutol 100 mg/kg/day for four weeks. At the end of experimental period, each animal was sacrificed by using chloroform and both optic nerves were dissected out, preserved and processed; in this way, twenty optic nerve specimens Maximum stone size included in our study was 3 cm. All patients included in our study were treated by rigid URS and stone fragmentation with Pneumatic Lithoclast. (ten for each group) were collected from ten albino rabbits. **Results:** Mean number of astrocytes, calculated in group B was 27.94 ± 2.94, that was significantly increased from those in group A, showing mean number of astrocytes as 09.66 ± 2.16 (p value = 0.001). **Conclusion:** Ethambutol, in toxic doses, induces the proliferation of astrocytes in optic nerve, a part of astrogliosis, indicating the repair process in damaged optic nerve. **Key Words:** Ethambutol (EMB), optic nerve, astrogliosis, Endothelin 1 (ET-1)

INTRODUCTION

Ethambutol (EMB) is a well-known antituberculous drug[1]. Its bacteriostatic and bactericidal effect at daily doses of 15 mg/kg and 25 mg/kg respectively, are achieved due to its property of chelating the essential metals in the enzyme systems of bacteria, thus stopping the bacterial cell multiplication leading to their death.[2,3]

EMB has been reported to damage both retina and optic nerve (OPN), the latter being more common, is manifested either as an axial or paraxial type.[4] The axial type in which central fibers of OPN are reported to be extensively involved, is more common. It has the characteristics of gradual loss of vision, decreased visual acuity, central scotoma and color vision disorders [5,6] .

It has been reported that toxic doses of EMB produce axonal fragmentation, axonal dilatation (vacuole formation), mild demyelination, central necrosis with inflammatory changes and macrophagic response in the optic nerves of rats, dogs, monkeys and rabbits.[7]

These, EMB induced histological changes observed in the retinal ganglion cell bodies and their axons were similar to those produced by excessive levels of excitatory amino acids especially glutamate (GTM), a neurotransmitter in the human central nervous system, seen in different types of neuropathies. [8,9] The central nervous system responds to these neuropathies with a vigorous activation of astrocytes resulting in reactive astrocytosis (an increase in the number and size of cells) and astrogliosis, (characterized by astrocytes proliferation and activation) followed by morphologic and cellular changes, hypertrophy, alteration of extracellular matrix profile, and formation of a glial scar.[10-12] In this way, the astrocytes not only protect nervous tissue but also preserve function as seen after spinal cord injury.[13]Astrogliosis occurs in human glaucomatous optic nerve heads[5] as well as in many animal models of glaucoma, including monkey[14] and rat.[15] The detrimental effects of astrogliosis on nerve cell repair are well established in different injury models, including white matter demyelination.[16]

As mechanism of EMB induced injury to optic nerve, is same as in all the pathophysiological conditions producing astrogliosis, therefore it was presumed that EMB can also cause astrogliosis as an additional feature of optic neuropathy. So the present study was aimed to verify astrogliotic changes in the optic nerve of rabbit by toxic doses of EMB.

MATERIAL AND MEHODS

Chemicals

Ethambutol dihydrochloride and all chemical materials used during processing and staining were produced by Merck (Germany).

Animals

Ten, New Zealand white, albino rabbits of either sex, 6-12 month old; weighing 1-2 kg were procured from Veterinary Research Institute, Lahore.

Each animal was housed in a separate cage and transferred to the animal house having standard conditions, at a temperature of 20 ± 0.5ºC, humidity (50 ± 10%) and 12 hours light and dark cycle. They were fed on standardized diet and water ad libitum. The experiment was started by randomly dividing the animals into two groups; all interventions were done through feeding tube once daily for 4-weeks.

GROUP A served as a control, and was fed with distilled water equal in volume given to experimental group B.

GROUP B was treated with EMB, 100 mg / kg body weight / day.

At the end of the experimental period, each animal was sacrified under anaesthesia The scalp was removed, skull cap detached free from the dura mater and the brain with its meninges was exposed by lifting the calvarium. Falx cerebri was detached from the crista galli and pulled posteriorly. The frontal lobes were lifted from the anterior cranial fossa and incised to expose the orbital plates before removing them. Both optic nerves along with chiasma were thus exposed and the nerves were severed from the eye ball.

Optic nerve was taken as a single piece by incising it proximal to the optic chiasma, washed with normal saline and fixed in 10 % formal saline for 24 hours; the tissue pieces were processed, dehydrated by passing through ascending grades of ethanol, cleared in xylene and infiltrated with molten paraffin wax (melting point 56-58°C). The tissue pieces were placed vertically in Leuckhart's moulds, to obtain transverse sections of the intracranial part of optic nerve, embedded in molten paraffin; tissue blocks were solidified, trimmed at chiasmatic end; sections cut at 5 µm thickness, were shifted to water bath at 45-50° C. Sections were then transferred on the surface of albumenized glass slide, dewaxed in xylene, hydrated and stained with hematoxylin and eosin in a usual way. To demonstrate the myelin sheath, luxol fast blue and cresyl violet stains were used in separate preparations.

The prepared slides were examined under the light microscope and observations were recorded in term of counting the number of astrocytes. Astrocytes were counted per 0.0625 mm^2 randomly selected three areas per slide, after calibrating the ocular graticule with stage linear micrometer, using X40 objective. Three slides were taken from optic nerve of each animal of each group and mean number of astrocytes were calculated for each animal and each group.

STATISTICAL ANALYSIS

Statistical analysis was conducted using the computer software, Statistical Package for Social Sciences (SPSS version 15.0). The difference, in number of astrocytes between control and the experimental group was analyzed by using the independent sample t-test.

RESULTS

Cross sections of the optic nerves of all rabbits in the experimental and control groups were observed under light microscope. It was found that three meninges surround each nerve and fine branching connective tissur septa arising from pia mater, extending transversely across the substance of nerve.

Figure-1

Cross section of optic nerve from group A showing dura mater (A), arachnoid mater (B), pia mater (C), subdural space (D), subarachnoid space (E), blood vessles (BV) and connective tissue septa (CTS). H & E stain, X 50.

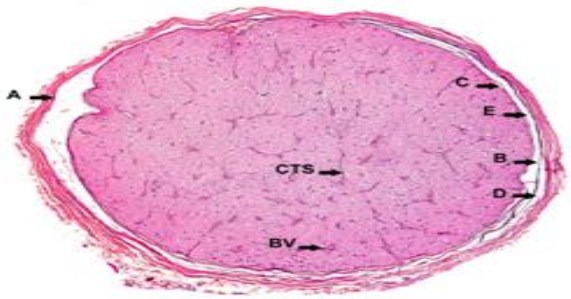
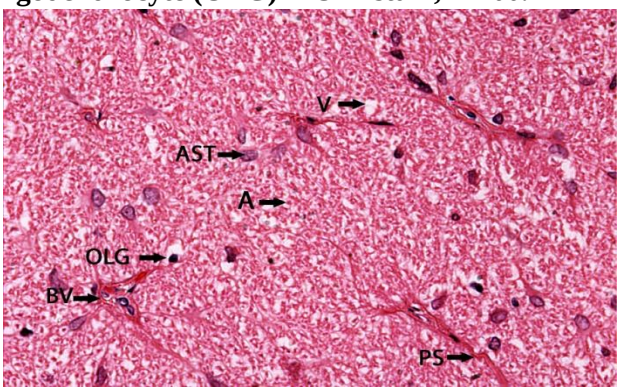


Figure-2

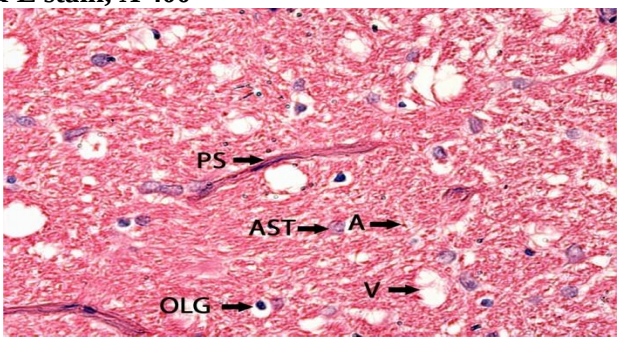
Optic nerve from group A showing astrocytes (AST) pial septa (PS), blood vessels (BV) and oligodendrocyte (OLG) H & E stain, X 400.



The nerve itself was composed of axons of ganglion cells, supported by astrocytes and oligodendrocytes. Invariably all sections showed branches of central retinal artery inside the pial septa of the optic nerve (Fig. 1).

Figure-3

Optic nerve section from group B, showing a large number of astrocytes (AST), vacuoles of different sizes in the neurites of OPN (V), pial septa (PS), oligodendrocytes (OLG) and blood vessels (BV) H & E stain, X 400



ASTROCYTOSIS

All sections of the two groups showed variable number of astrocytes per 0.0625 mm^2 area of each optic nerve preparation. Number of astrocytes in preparations from almost every rabbit of group B was immensely increased (Figure. 3 and 6) as compared to those in group A (Figure. 2 and 5).

It showed proliferation of astrocytes in term of astrocytosis which is a part of astroglyosis, in response to the injury to the optic nerves of animals of group B induced by EMB (Fig. 4).The statistical analysis of the changes in number of astrocytes, using independent sample t-test showed that mean number of astrocytes calculated in group B was 27.94 ± 2.94, that was significantly increased from those in group A, showing mean number of astrocytes as 09.66 ± 2.16 (p value = 0.001) as shown in Table-1.

Figure-4

Bar graph showing mean and standard error of number of astrocytes in groups A and B

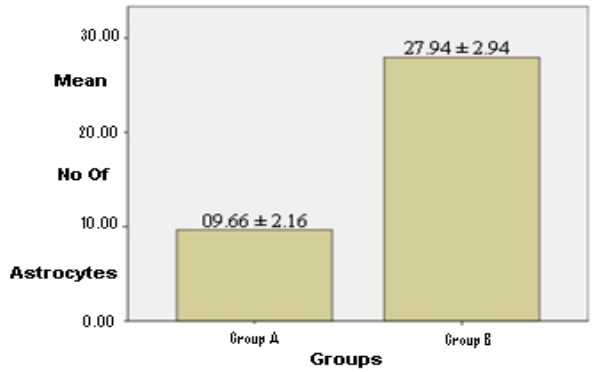


Figure-5

Photomicrographs of cross section of rabbit optic nerve from group A, showing astrocytes. (AST), oligodendrocytes (OLG), blood vessels (BV) and pial septa (PS). Luxol Fast bluE & Cresyl Violet stain. X 200

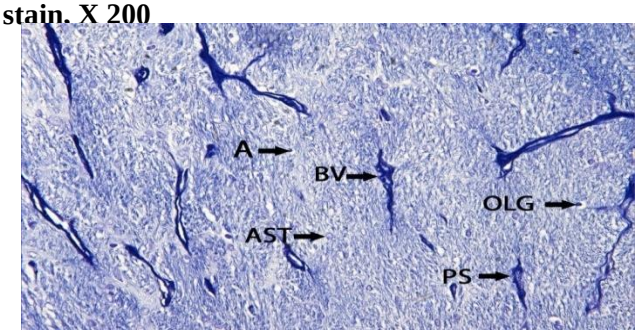


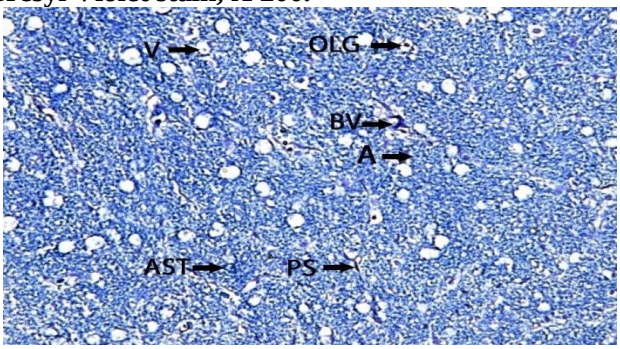
Table-1

The mean of Number of astrocytes in control group A and experimental group B

Variable	Group A M±SE	Group B M±SE	Mean Difference	Degree of Freedom	t-score	p-value (2-tailed)
Numer of Astrocytes	09.66 ±2.16	27.94±2.94	0.49	8	-5.00	0.001*

Figure-6

Photomicrographs of cross section of rabbit optic nerve from group B, showing vacuoles in OPN (V), astrocytes (AST), oligodendrocytes (OLG), blood vessels (BV) and pial septa (PS). Luxol Fast bluE & Cresyl Violet stain, X 200.



DISCUSSION

In this study, we have investigated the astrogliotic response of optic nerve of rabbit against the chemical insult produced by toxic doses of ethambutol. It was observed that a significant number of vacuoles, representing the damaged axons of retinal ganglion cells, have appeared in the preparations from experimental group B.

Development of intense vacuolations in almost same experimental conditions applied over different animal models has been previously reported in different studies.[7, 17] These vacuoles are produced, probably, by EMB mediated increased Ca^{+2} influx which activates certain intracellular enzymes including phospholipases, proteases, endonucleases, and ATPases resulting in destruction of retinal ganglion cells and axons that microscopically appear like vacuoles due to processing techniques.[18] Alternatively vacuolation may, probably, be the result of metabolites of EMB forming a complex with Zn and Cu which enters the axons and enhance the axonal dilatation (vacuolation) process.[19]

It was noticed that the number of astrocytes had been considerably increased in all slides of our experimental groups. The mean number of astrocytes in experimental group B (27.94 ± 2.94) was markedly increased when compared to that in control group A (09.66 ± 2.16); this difference was statistically significant (p-value = 0.001) It represented the reaction of optic nerve in attempt to repair the damage produced by ethambutol referred as astrocytosis which is a part of astrogliosis, characterized as astrocyte proliferation and activation followed by morphologic and cellular changes, hypertrophy, alteration of extracellular matrix profile, and formation of a glial scar.[10,11] No comparable study is available yet, in which ETB induced optic nerve astrogliosis was directly observed, however, it has been reported as a common feature of all pathological and experimental injuries including glaucoma, ischemic nerve injury, neurotrauma and Alzheimer disease,[10-12] produced by the same mechanism associated with sustained release of excessive glutamate which also works in pathogenesis of ETB induced optic neuropathy. [20-21]

Astrocytes play a major role in the remodeling of the extracellular matrix of the optic nerve head, synthesize growth factors and other cellular mediators that may affect directly, or indirectly, the axons of the retinal ganglion cells.[10] Astrocytes also play an essential role in protecting neurons and oligodendrocytes, restricting inflammation and helping to limit tissue degeneration.[22,23]

Endothelin (ET)-1 is a potent vasoactive vasoconstricting peptide isolated from vascular endothelial cells and can be released from its intraocular sources, including ciliary epithelium, retinal pigmented epithelium, and optic nerve head astrocytes in association with neuronal injury.[24-26] Its level, has been observed, to become elevated in optic nerve transection, experimental or clinical neurotrauma and neuropathies (including glaucoma) which cause mitogenesis of astrocytes resulting in their proliferation.[25, 27-33]

ET-induced astrogliosis has also been found in other neuropathies such as Alzheimer's disease, subarachnoid hemorrhage and ischemia.[28,29,34] ET-1 and its G-protein–coupled endothelin A (ETA) and endothelin B (ETB) receptors, are numerously present in optic nerve, retina, astrocytes of optic nerve.

These have been reported to become activated and upregulated in almost each case of ET-1–induced proliferation of astrocytes.[25,29,34] To verify, this proliferative effect was successfully prevented experimentally, by blocking the both receptors with ETA and ETB antagonists BQ610 and BQ788 respectively[12,29,33]. Endogenous ET-1, thus produced, could influence astrocyte morphology and behavior by autocrine or paracrine actions and promote astrogliosis through functional ET receptors.

CONCLUSION

Ethambutol, in toxic doses, induces the proliferation of astrocytes in optic nerve, a part of astrogliosis, indicating the repair process in damaged optic nerve.

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