

Optic Nerve Oligodendropenia, induced by Ethambutol

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ABSTRACT

Aim: To determine oligodendropenia in optic nerve of rabbit, induced by ethambutol

Method: Ten, New Zealand white, albino rabbits of either sex, 6-12 month old; weighing 1- 2kg were randomly divided in to two groups 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 (ten for each group) were collected from ten albino rabbits.

Results: Mean number of oligodendrocytes was significantly decreased in group B (04.31 ± 0.40) as compared to group A (10.41 ± 0.34); $p = 0.002$.

Conclusion: Ethambutol, in toxic doses, induces the death of oligodendrocytes in optic nerve, resulting into demyelination of optic nerve.

Key words: Ethambutol (EMB), optic nerve, oligodendrocytes, N-methyl-D-aspartic acid (NMDA) receptors and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors

INTRODUCTION

Ethambutol, a water soluble, white crystalline powder, is a second drug of choice, after rifampicin, in the treatment of pulmonary tuberculosis, especially for INH-resistant and streptomycin-resistant tubercle bacilli. It may produce undesirable effects as seen in all drugs¹.

It is reported that EMB induces toxic bulbar and retrobulbar neuritis, that manifests clinically as painless, symmetrical and progressive loss of vision, central or caecocentral scotoma and dyschromatopsia^{2,3,4}.

Histopathologically, EMB produces degeneration and loss of optic nerve axons having diameter of 1 μ m or less.⁵ Different experimental animals including rats, dogs, monkeys and rabbits, when treated with toxic doses of EMB, revealed multiple dilated axons (vacuoles), axonal fragmentation, inflammatory changes, mild demyelination and myelin like structure in the axoplasm along with central necrosis in the optic nerve comprising of the axons of retinal ganglion cells⁶.

Mild demyelination in the optic nerve of experimental animals as a result of toxic doses of EMB is possibly due to the death of oligodendrocytes

which belong to one of the important glial cell type of the vertebrate CNS. Oligodendrocytes are the chief variety of glia cells in CNS, and their main function is the deposition of myelin sheath to axons which facilitate the process of nerve conduction in the CNS. These cells play an important role in the regulation of outgrowth and regeneration of central neurons. These are, also, involved in many pathological conditions including demyelination, multiple sclerosis, white matter injury in stroke and periventricular leukomalacia⁷.

These glial cells develop soon after the majority of neurons have appeared and have extended their axons. Their proliferation depends on the electrical activity of neighboring axons because the axonal signaling induces the formation of required number of oligodendrocytes to myelinate the necessary axons⁸.

Despite their significant role in the brain, little is known about their cellular biology, however the developmental sequence leading to their formation from multipotent precursors as well as the presence of ion channels in their plasma membranes, have been demonstrated in the recent studies⁷.

Oligodendrocytes and their precursors have been reported to express NMDA receptors, and activation of these receptors by the glutamates in an experimental study has led to the death of oligodendrocytes and the loss of myelin^{7,9}.

The present study was designed in the light of hypothesis that the mechanism of EMB induced injury to the optic nerve and retinal ganglion cells was just the same as seen by the excessive levels of glutamate. It was therefore presumed that

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ethambutol, in toxic doses, might also cause death of oligodendrocytes responsible for deposition of myelin sheath around the optic nerve fibers.

MATERIAL AND METHODS

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 months old and weighing 1-2kg 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 $20\pm 0.5^{\circ}\text{C}$, humidity ($50\pm 10\%$) 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 control, and was fed with distilled water equal in volume as given to experimental group B.

Group B was fed with EMB, 100mg/kg body weight in water daily for four weeks.

At the end of the experimental period, each animal was sacrificed under anesthesia. The scalp was removed, skull cap detached free from the dura mater and the brain with its meninges 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 cleanly dissected and removed from the eyeball. Optic nerve was taken as a single piece by excising it proximal to the optic chiasma, washed with normal saline and fixed in 10% formal saline for 24 hours. The tissues were processed, dehydrated by passing through ascending grades of ethanol, cleared in xylene and infiltrated with molten paraffin wax (melting point $56-58^{\circ}\text{C}$). The tissues were placed vertically in Leuckhart's moulds in a manner to obtain transverse sections of the intracranial part of optic nerve, embedded in molten paraffin. The tissue blocks were solidified, trimmed at chiasmatic end and sectioned at $5\mu\text{m}$ thickness. The sections were shifted to water bath at $45-50^{\circ}\text{C}$, transferred on the surface of albumenized glass slides, dewaxed in xylene, hydrated and stained with hematoxylin and eosin in a usual way. The prepared slides were examined under the light microscope to record the number of oligodendrocytes. These were counted per 0.0625mm^2 randomly selected three areas per slide, after calibrating the ocular graticule

with stage linear micrometer, using X40 objective. Three slides from optic nerve of each animal of each group were studied and mean number of oligodendrocytes were calculated for each animal as well as each group.

Statistical analysis: Statistical analysis was conducted using the computer software, Statistical Package for Social Sciences (SPSS version-15.0). The statistical difference, in the number of oligodendrocytes between control and the experimental groups was analyzed by using the independent sample t-test at $p\leq 0.05$.

RESULTS

Cross sections of the optic nerves of all rabbits in the experimental and control groups were observed under light microscope. At low magnification, It was found that three meninges surrounded each nerve and fine branching connective tissue septa arising from pia mater, extended transversely across the substance of nerve (Fig. 1). At higher magnification in slides of group A, the nerve 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).

The microscopic appearance in group A, under high power microscope revealed fine branching pial septa, blood vessels, Optic nerve fibres were visible as an empty space with a central axis cylinder. These were supported by astrocytes and oligodendrocytes; the latter were identified as small cells with a central dark nucleus and a rim of unstained cytoplasm; astrocytes were identified by their large lightly stained vesicular nuclei. Each of these sections also showed two to four vacuoles as shown in Fig. 2.

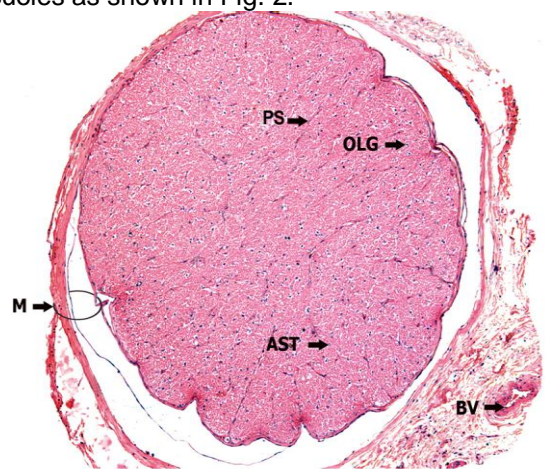


Fig.1: Photomicrograph of cross section of optic nerve from group A showing meninges (M), oligodendrocytes (OLG), astrocytes (AST), blood vessels (BV) and pial septa (PS). H & E stain, X 50.

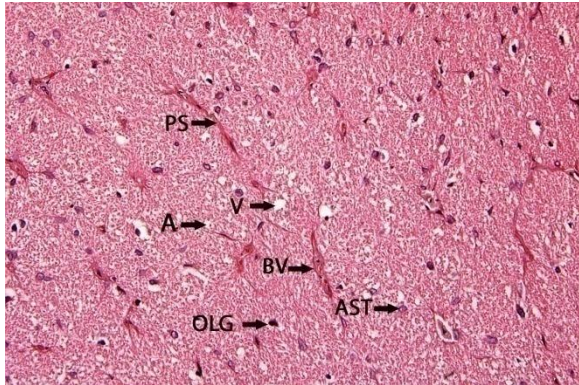


Fig 2: Photomicrograph of optic nerve from group A showing astrocytes (AST) pial septa (PS), blood vessels (BV), optic nerve fibres (A), vacuoles (V) and oligodendrocyte (OLG) H & E stain, X 200.

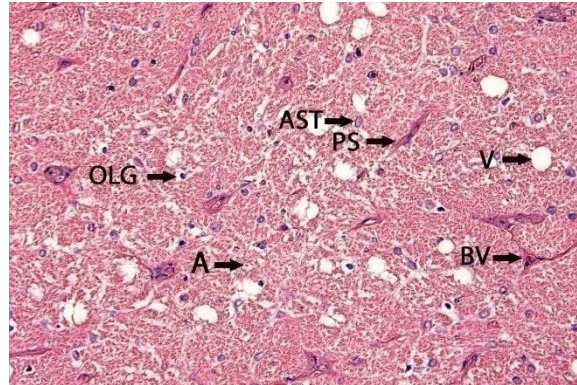


Fig. 3: Photomicrograph of optic nerve section from group B, showing a reduced number of astrocytes (AST), vacuoles of different sizes in the neurites of OPN (V), pial septa (PS), oligodendrocytes (OLG) optic nerve fibres (A) and blood vessels (BV) H & E stain, X 200

The microscopic slides in group B at higher magnification also revealed the presence of pial septa, blood vessels and axons of ganglion cells, supported by astrocytes and oligodendrocytes (Fig. 3). The neurites of ganglion cells exhibited vacuoles of variable sizes. The oligodendrocytes were found to be relatively reduced in number.

Oligodendropenia: All microscopic sections of both the groups showed variable number of oligodendrocytes per 0.0625mm² area of each optic nerve preparation. The number of oligodendrocytes in preparations from almost every rabbit of group B was significantly decreased (Fig. 3) as compared to those in group A (Fig. 2). It showed the death of most of the oligodendrocytes resulting into oligodendropenia in the optic nerves of experimental group B, in response to the toxic doses of EMB given to these animals (Fig. 4).

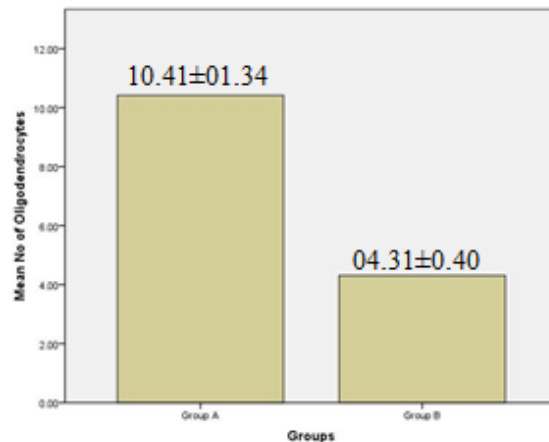


Fig.4: Bar graph showing mean number with standard error of oligodendrocytes in groups A and B.

Table1: The mean Number of Oligodendrocytes in control group A and experimental group B

Variable	Group A	Group B	Mean Difference	Degree of Freedom	t-score	P-value (2-tailed)
Number of Oligodendrocytes	10.41±01.34	04.31±0.40	06.10	08	04.34	0.002*

*Significant

The statistical analysis of the changes in number of oligodendrocytes, using independent sample t-test showed that mean number of oligodendrocytes, calculated in group B was 4.31±0.40 that was significantly decreased from those in group A, with mean number of oligodendrocytes as 10.41±1.34 (p=0.002) as shown in Table1.

DISCUSSION

In the present study, we have investigated the effect of toxic doses of ethambutol on the optic nerve oligodendrocytes of rabbits. The results reported here

support our hypothesis that these are vulnerable to ethambutol toxicity, which in turn is related with the excessive levels of excitatory amino acids like glutamates. It was observed that the number of oligodendrocytes (4.31±0.40) in the preparations from optic nerves of experimental group B rabbits was markedly decreased when compared to that in group A (10.41±1.34). The difference was statistically significant (p=0.002) which indicates the death of oligodendrocytes induced by EMB.

The pattern of loss of oligodendrocytes and ganglion cell bodies with their axons, seen with EMB toxicity, was strikingly similar to that seen with excessive levels of excitatory amino acids, especially

glutamate (GTM).¹⁰ GTM acts as a neurotransmitter in the human CNS, is stored in presynaptic vesicles and released in response to presynaptic neuronal membrane depolarization. Normally, GTMs interact with specific receptors like N -methyl-D-aspartic acid (NMDA) and α -amino-3-hydroxy-5-methyl-4-soxazolepropionic acid (AMPA) on the neurons and the retinal ganglion cells, to mediate many normal neurological and physiological functions; however, excessive activation of these receptors results in injury or death of receptors bearing cells¹¹⁻¹⁷

Oligodendrocytes expressed both NMDA and AMPA receptors activated by the, glutamate. EMB did not directly stimulate these receptors. It was thought that the endogenous glutamates were necessary to express its toxic effects in the retinal ganglion cells which became sensitive due to certain mechanisms.

The endogenous GTM binds initially to α -amino-3-hydroxy-5-methyl-4-soxazolepropionic acid (AMPA), then to NMDA receptor resulting in free entry of Ca^{2+} into the neuronal and glial cells. Intracellular calcium is important for initiating a number of enzymatic processes necessary for physiological learning, cognition, movement, sensation and memory processes; however, during certain pathological processes like stroke, epilepsy, glaucoma, traumatic and ischemic neuronal injury, Parkinson and Alzheimer, Huntington's diseases, there is a sustained release of excessive GTMs, allowing the influx of a high concentration of Ca^{2+} through the receptors channels; it changes the permeability of mitochondrial membrane to buffer the calcium level to some extent as there is a decrease in cytosolic and an increase in mitochondrial calcium. It contributes to the overstimulation of normal enzymatic processes that initiate a cascade of neuronal and glial cell degeneration^{10,8-25}.

Alternately EMB toxicity may be mediated through the endogenous glutamates by the sustained activation of AMPA and NMDA receptors. It induces the calcium influx through the receptor channel which alters Ca^{2+} homeostasis and induces a change in the mitochondrial membrane, leading to release of proapoptotic molecules such as cytochrome c and apoptosis-inducing factor. This results in oligodendrocyte death through caspase-dependent and -independent pathways^{8,9,26}.

EMB induced oligodendrocytes death through the endogenous glutamate also appears to involve a mechanism that ultimately renders the cell vulnerable to oxidative stress, probably because of depletion of cystine leading to depletion of glutathione. It was confirmed by addition of cystine that totally prevented the glutamate toxicity to oligodendroglia⁷.

In humans, oligodendrocytes and their precursors have recently been shown to express NMDA receptors, and activation of these receptors by the glutamates, for example, in brain ischaemia or leucodystrophies, leads to the death of oligodendrocyte-precursors and the loss of myelin⁹. It has been reported that human adult oligodendrocytes express low levels of AMPA receptor in vitro and are resistant to excitotoxicity even with prolonged activation of these receptors, in striking contrast to rat oligodendrocytes which show strong AMPA receptor expression and are susceptible to excitotoxicity, It has, also, been observed that oligodendrocytes in the human brain sections do not express AMPA receptors in situ and that glial expression of AMPA receptors is only limited to astrocytes²⁷.

It has been reported that oligodendrocytes have a role in preventing the sprouting and stabilizing the number of optic nerve fibers in a pathway during development. It indicates that in case of death of the oligodendrocytes, the optic nerve become demyelinated and its fibers are able to form the sprouts²⁸.

CONCLUSION

The results reported here provide evidence that the optic nerve oligodendrocytes are vulnerable to the toxic doses of Ethambutol due to excessive levels of endogenous glutamate that triggers calcium influx.

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