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Original Article

Vigabatrin toxicity-effects on optic nerve

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ABSTRACT

Introduction: Vigabatrin is an antiepileptic drugwhich is used as the drug of choice in resistant epilepsy and infantile spasms. Cases of visual field constriction have been frequently reported following the use of this drug indicating some involvement of the visual system. Studies have been done on the effects on retina but few are available stating the effects on optic nerves. Hence the present study was designed to study the histopathological effects of vigabatrinadministrationon the optic nerves of albino rats.
Material and Methods: Rats were divided into control and experimental groups. Vigabatrin was administered intraperitoneally to the experimental group in three graded doses for a period of 4 weeks, after which the rats were sacrificed. Brains were dissected out, followed by dissection of the eyeballs along with optic nerves. Slides of optic nervewere prepared for histological examination.
Results: Atrophy of optic nerve and signs of intramyelinicoedema in the form of vacuolation were seen. Features of demyelination were not found in any slide. Severity of the findings increased with increasing doses.
Discussion: Vigabatrin may be toxic to the visual system, especially the optic nerves. We suggest that it should be used with caution and only if required, keeping doses as low as possible. Tests for assessing visual function should be performed during treatment and doses should be adjusted accordingly.

1. Introduction

Vigabatrin is an antiepileptic drug (AED) which is indicated for adjunctive therapy in adults and children >10 years of age with refractory complex partial seizures. It is also used in those cases of infantile spasms which fail to respond to the standard treatment with adrenocorticotropic hormone (ACTH).In such cases, 40–70% improvement has been noticed in seizure control after treatment with Vigabatrin. The decision of using Vigabatrin for infantile spasms varies from physician to physician. Most commonly, it is considered as a drug of choice in infantile spasms associated with tuberous sclerosis while some prefer to use it in all cases of infantile spasms.[1] Vigabatrin, along with ACTH and prednisolone have been considered as the best first-line medications for infantile spasms.[2]

Vigabatrin is an analogue of gamma amino butyric acid (GABA).It is an irreversible inhibitor of 4-aminobutyrate transaminase (GABA-T). GABA-T is the enzyme involved in catabolism of GABA. Low levels of GABA in the brain may be responsible for seizure precipitation in epileptic patients. Vigabatrin administration elevates brain GABA levels thus reducing the likelihood of a seizure.[3]

Vigabatrin was initially approved by the FDA in 2009 but remained controversial as its use was associated with visual field defects (VFDs). Cases of bilateral concentric visual field constriction, including tunnel vision and decreased visual acuity were reported after its use. It was approved with a risk evaluation and mitigation strategy as the benefits outweighed the risks involved in its usage.[4] Animal toxicity studies revealed that the use of Vigabatrin was shown to induce intramyelinic edema (IME) in different parts of brain in some animals.[5,6] However, benefits of treating infantile spasms with Vigabatrin seem to outweigh the risk, but further prospective studies are needed to evaluate the place of Vigabatrin in this indication.The visual field defects observed after usage must have some histopatholgical basis, efforts of exploration of which have been made time and often. Studies are available which elaborate the histopathological effects of this drug on the retina.[5,7,8] Keeping in mind, the clinical importance of this drug as an antiepileptic, the pathogenesis of Vigabatrin induced VFDs and other adverse effects needs to be understood in detail in other parts of the visual system like the optic nerves too. Histopathological studies are difficult to perform in humans and thus, animals are usually preferred. The present study was conducted to understand the histopathological effects of Vigabatrin usage in graded doses on optic nerves of albino rats.

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2. Material and methods

This study was conducted in the Department of Anatomy, Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun. Sixty albino rats (RattusNorwegius, Wistar strain) of both sexes weighing 150–200 g m were obtained from the Central Animal House of the Institute after taking permission from the Institutional Animal Ethical Committee. The research work followed ARRIVE guidelines and was carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines. Rats were kept under the same environmental conditions which included 12 h light/dark cycles. The rats were healthy and free from any disease or disability at the time of procurement. They were given standard rat feed and allowed water ad libitum. Daily monitoring of the body weight was done. The experimental animals were divided into two main groups:

Group 1- Control group of 15 rats which were given intraperitoneal injection of 0.9% normal saline

Group 2- Experimental group of 45 rats divided into 3 subgroups: 2a–c with 15 rats in each subgroup.

Each of the three subgroups (2a–c) were given intraperitonealVigabatrin in doses of 125 mg/kg body weight, 250 mg/kg body weight and 500 mg/kg body weight respectively as a single dose once daily for four weeks. All the doses were calculated according to the standard guidelines for drug administration to rats in animal studies. After four weeks, the rats were sacrificed after giving deep anaesthesia by ether inhalation. Both the eye balls were carefully dissected out along with optic nerves and fixed in Davidson's fixative. Paraffin blocks were prepared and transverse sections of optic nerve were prepared and stained with routine Haematoxylin and Eosin stain. Special staining was done using Modified Luxol Fast Blue stain (specific for myelin sheath) to look for any associated demyelination. Slides were observed under the low and high powers of light microscope. In each section of optic nerve, the optic nerve fibre bundle and the surrounding meninges were identified. For each section, diameter of the optic nerve bundle and width of the subdural space surrounding the optic nerve fibre bundle were measured at ten random fields and the average dimensionswith standard deviations were recorded.

3. Results

Decreased mean diameter of optic nerve as compared with the control was found in the experimental group (Group 2).Diameter was found to decrease with increasing doses. There was shrinkage of the optic nerve and increase in thickness of subdural space in 67% rats of subgroup 2b and 67% rats of subgroup 2c while subgroup 2a showed this change in 40% of rats (Table 1, Figs. 1–8).

With H&E staining, the pale area around axons (which is negligible in a normal nerve section)was found to be increased as compared with control which indicates vacuole formation. This feature increased with increasing doses and was further confirmed by Modified LuxolFast Blue Staining as this stain has affinity for myelin and vacuolation was evident by the increased space between axon and myelin sheath. Vacuolationwas found in 20% rats of subgroup 2a, 60% rats of subgroup 2b and 67% rats of subgroup 2c (Figs. 1–8).

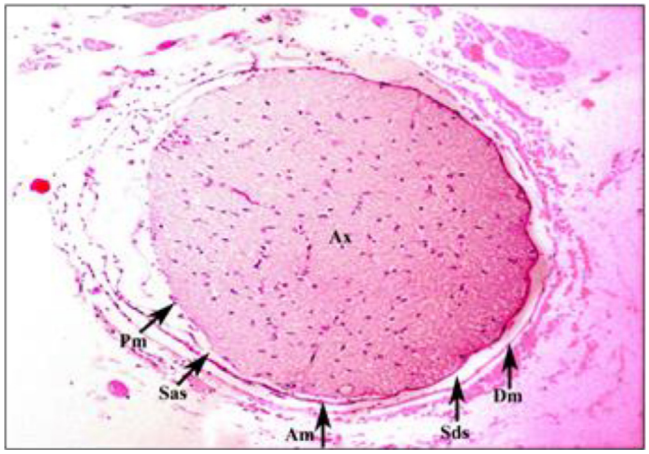

Fig. 1. Photomicrograph of control optic nerve. Duramater (Dm). subdural space (Sds), arachnoid mater (Am), subarachnoid space (Sas). pia mater (Pm) & axons of nerve fibres (Ax). (H&E X100).

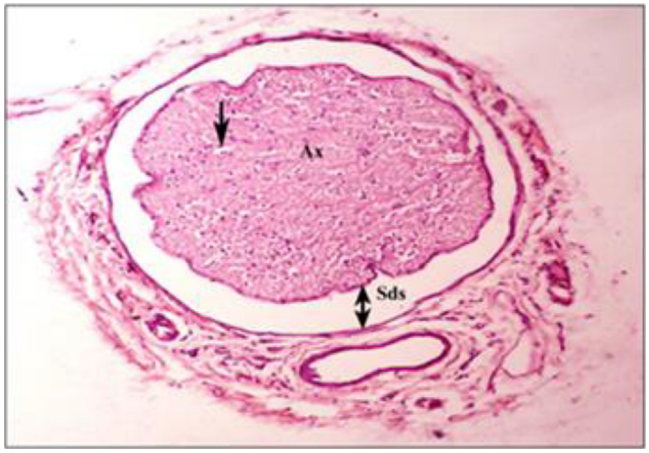

Fig. 2. Photomicrograph of mild dose treated optic nerve showing slight increase in the pale area (arrow) around the axons (Ax), increased subduralspace (Sds) and shrinkage optic nerve. (H&E 100).

Uniform staining was observed with Modified Luxol Fast Blue in all dose groups as compared with control showing absence of demyelination (Figs. 5–8).

4. Discussion

Decreased number of nerve fibres and shrinkage of optic nerve as evidenced by a decrease in mean diameter of optic nerve and increase in mean thickness of subdural space with increasing doses, were the main findings in the optic nerve in the present study. These features may indicate signs of atrophy in the nerve. Previous studies have shown a reduced number of ganglion cells in the retina following administration of similar graded doses of Vigabatrin.[8] As optic nerve is made up of myelinated axons of ganglion cells, this finding also indicates to a certain extent that the

Table 1
Mean diameter of the optic nerve (in μm) and thickness of subdural space (in μm) in control group and experimental group.

Parameter	Group 1(Control)	Group 2 (Experimental)		
		Subgroup 2a	Subgroup 2b	Subgroup 2c
Mean diameter of optic nerve (μm)	19.5 ± 1.0377	17 ± 1.0233	14 ± 1.002	13 ± 1.232
Mean thickness of subdural space(μm)	1.54 ± 0.774	2 ± 0.784	4.92 ± 0.997	8.85 ± 0.735

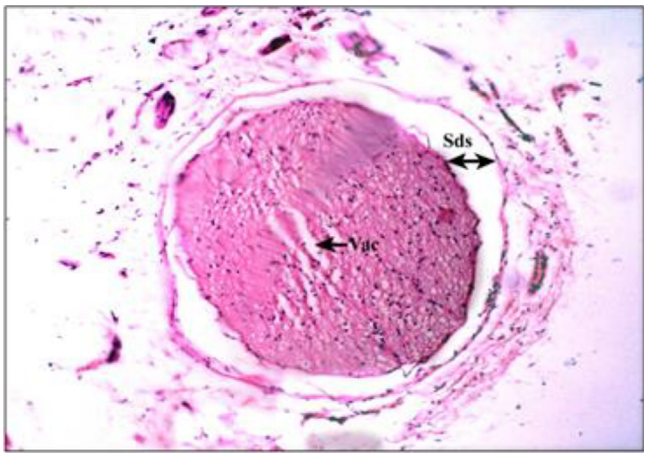

Fig. 3. Photomicrograph ofmoderatedose treated optic nerve showing vacuoles (Vac), increased subduralspace (Sds) and shrinkage of optic nerve. (H&E 100).

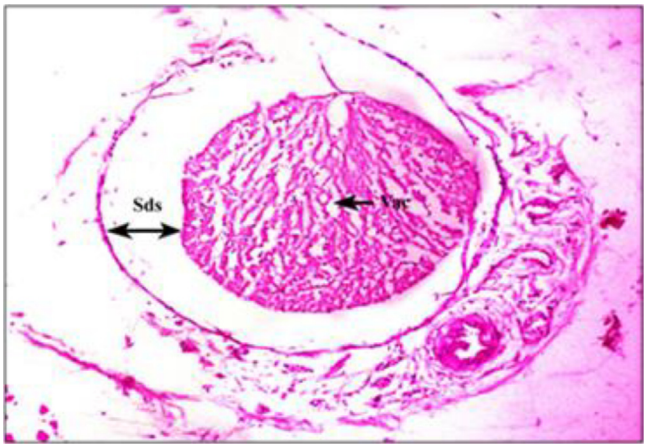

Fig. 4. Photomicrograph ofhigh dose treated optic nerve showing marked vacuolation(Vac), increased subduralspace (Sds) and shrinkage of optic nerve. (H&E 100).

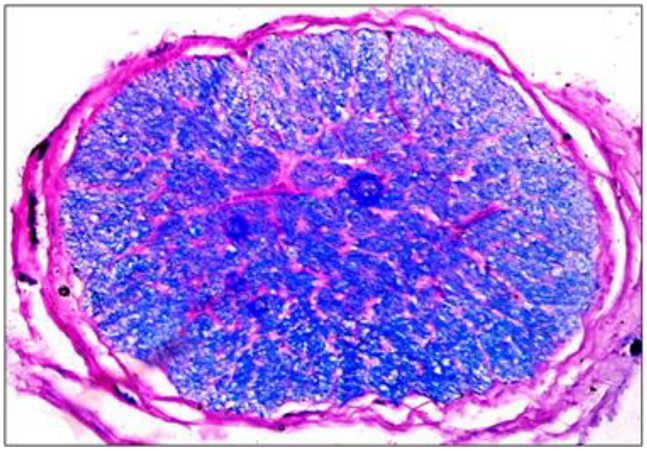

Fig. 5. Photomicrograph of control optic nerve. (Modified Luxol Fast Blue X100).

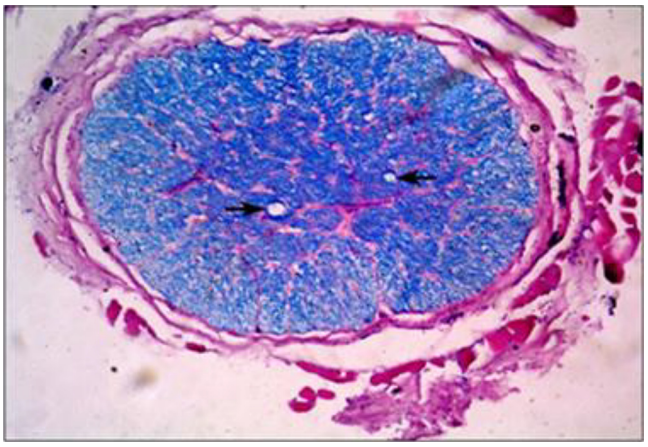

Fig. 6. Photomicrograph of mild dose treated optic nerve showing vacuolopathy (arrows). (Modified Luxol Fast Blue X100).

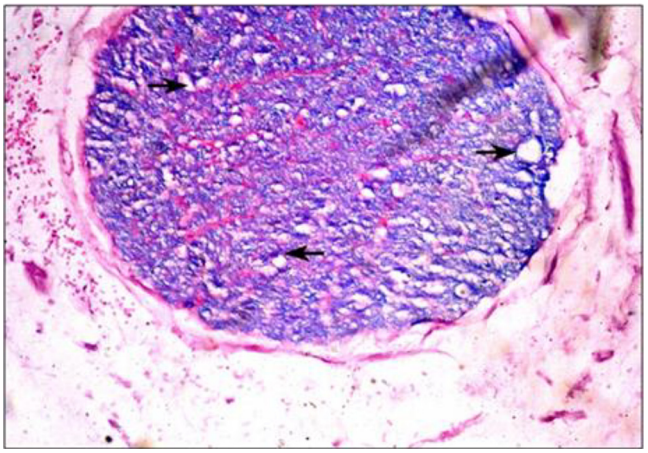

Fig. 7. Photomicrograph of moderate dose treated optic nerve showing myelin vacuolopathy (arrows). (Modified Luxol Fast Blue X100).

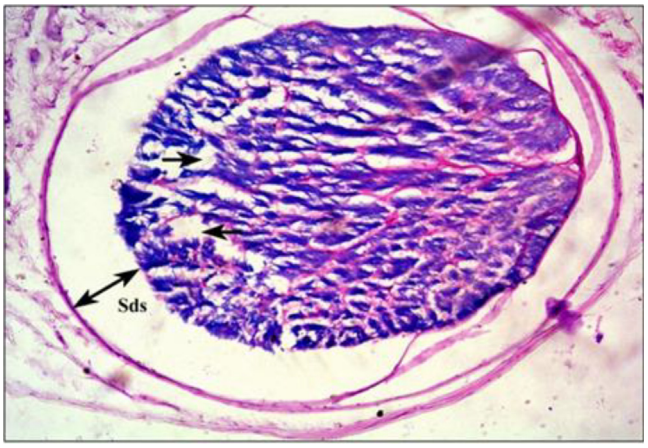

Fig. 8. Photomicrograph of high dose treated optic nerve showing myelin vacuolopathy (arrows) and shrinkage of optic nerve with increased subdural (Sds). (Modified Luxol Fast Blue X100).

decrease in number of fibres and shrinkage of optic nervecould have been secondary to reduction in ganglion cells in the retina.

Vacuolation(increased space between the axon and myelin sheath), a feature indicative ofIME,was observed in the experimental group and increased in severity with increasing doses of Vigabatrin. Similar findings have been previously reported.[9,10,11] The appearance of IME may be considered a form of exaggerated pharmacology of Vigabatrin, as formation of

microvacuoleshave been seen following administration of other GABA-T inhibitorsalso in rats.[12] It has been observed that young rats are more sensitive to Vigabatrin toxicity and vacuoles usually resolve after stopping drug treatment.[13] Ultra-structural studies have demonstrated that microvacuoles form as a result of fluid accumulation and separation of the outer layers of myelin at the intraperiod line, a condition known as IME. Such vacuolation may also represent fixation artifacts and immunohistochemistry must ideally be used to differentiate between the two. In typical IME, there isanassociated reactive astrocytosis and microglial activation, which can be demonstrated with immunohistochemical markers for astrocytes (glial fibrillaryassociated protein) and microglia (ED4).[14] However, it has been seen that features of IME are usually bilateral in presence as were found in the present study and their consistent presence in all dose groups further strengthens their categorization as IME.

There was no evidence of demyelination as all slides were uniformly stained by Luxol Fast Blue stain which is specific for myelin staining. Similar absence of demyelination has been previously reported [11] although studies do suggest that oligodendrocytesresponsible for myelination are affected by Vigabatrin administration but more so in young rats during initial developmental stages.[13]

The present study shows the effects of Vigabatrin administration in rats. Ravindran J. et al, in their study, performed serial step sections in postmortem specimensof the optic nervesof a 41 year old man with complex partial seizures who presented with visual field constriction. They found severe atrophy (shrinkage to a half to a third normal size) of the optic nerves although features of IME were not seen.[15] Such studies further re-establish the fact that pathological changes similar to animals have been observed in human beings also.

5. Conclusion

Vigabatrin is a valuable AED as far as treatment of refractory epilepsy and infantile spasms is concerned but effects on optic nerve in the form of atrophy and features of intramyelinic edema cannot be ruled out. So, it must be used if the benefits seem to outweigh the risks. We suggest that limited use and low dosages along with regular assessment of the visual function should be practiced while using this drug. Dose modifications and treatment duration adjustments may be done accordingly. Most of the studies available have been performed on animals.Further studies are suggested in humans to study pathological findings and decide safe doses and duration.

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