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Original Article

Vigabatrin induced intramyelinic oedema in cerebellum of albino rats

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ABSTRACT

Introduction: Vigabatrin (an antiepileptic drug) is used as the drug of choice in resistant epilepsy and infantile spasms. Ataxia, tremors and abnormal gait have been frequently reported following the use of this drug indicating an involvement of the cerebellum. Hence the present study was designed to study the histopathological effects of Vigabatrin on the cerebellum of albino rats.

Methods: Rats were divided into control group and experimental group. Vigabatrin was administered intraperitoneally to the experimental group in three graded doses for a period of 4 weeks. At the end of the treatment period, rats were sacrificed, brains dissected out, the cerebellum was separated and fixed. Slides were prepared for histological examination.

Results: Signs of intramyelinic oedema in the form of vacuolation were seen. Severity of the findings increased with increasing doses.

Discussion: Vigabatrin may be neurotoxic and should be used with caution, weighing the benefits against the risks. Tests for assessing cerebellar function should be performed during treatment with Vigabatrin and doses should be adjusted accordingly.

1. Introduction

Vigabatrin (VGB) is an Antiepileptic Drug (AED) which has been used in refractory epilepsy and Infantile Spasms (IS) for more than 10 years but had a restricted usage due to some concern about its safety profile, as it can cause retinal toxicity.[1,2] However, VGB has now been approved by the US Food and Drug Administration as monotherapy for IS. A number of studies supported the use of VGB in the symptomatic treatment of IS, making it the drug of choice in this condition.[3,4] The success is more in cases of tuberous sclerosis complex where the drug controls spasms in up to 95% of patients.[5,6] The response is better with higher doses of VGB as compared with low-dose.[7] When VGB is compared with hormonal treatment (with corticosteroids) for IS, patients treated with VGB have shown a better control of spasms and better cognitive outcome.[8]

VGB acts as a selective inhibitor of gamma amino butyric acid transaminase (GABA-T), an enzyme that degrades gamma amino butyric acid (GABA) in the brain. Thus, VGB administration results in elevated brain GABA levels. It is thought that epileptic seizures may be due to low levels of GABA. By increasing the amount of GABA, Vigabatrin reduces the likelihood of an epileptic seizure.[9] VGB has a favourable pharmacokinetic profile since, it is not metabolized by the liver (does not induce the hepatic cytochrome p 450 system), is

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excreted by the kidney, has minimal protein binding, and has a long effective half-life, allowing once- or twice-daily dosing. Interaction with other antiepileptic drugs is minimal.[10]

Clinical experience in humans shows that Vigabatrin provides effective seizure control and has excellent tolerability. However, long term usage and higher doses of this drug usually leads to certain visual field defects. These have been extensively studied but other side effects including ataxia, tremors and abnormal gait have received little attention.[11] These effects indicate involvement of the cerebellum but the pathogenesis remains poorly understood.

The aim of the present study was to see the histopathological effects of this drug on the cerebellar tissue of albino rats. The findings may help in guiding the physician to include certain tests to assess cerebellar function apart from monitoring the visual system during the course of treatment.

2. Material and methods

Sixty albino rats (*Rattus norvegicus*, Wistar strain) of both sexes, weighing 150–200 g were taken from the central animal house of the institute, after obtaining permission from the Institutional Animal Ethical Committee. When procured, the rats were healthy and free from any disease or disability.

The rats were housed in cages and kept under the same environmental conditions which included 12 h light/dark cycles. They were fed with standard rat feed and allowed water ad libitum with daily monitoring of the body weight.

The animals were divided into two main groups:

- Group 1 – Control group of 15 rats.
- Group 2 – Experimental group of 45 rats.

The experimental group was further divided into 3 subgroups (as per the administered dose) – Subgroup 2a, 2b & 2c having 15 rats each.

Each of the three subgroups was given intraperitoneal Vigabatrin in the following doses:

- Subgroup 2a –125 mg/kg body weight.
- Subgroup 2b –250 mg/kg body weight.
- Subgroup 2c –500 mg/kg body weight.

The drug was administered as a single dose daily for a period of 28 days. All the doses were calculated according to the standard guidelines for drug administration to rats in animal studies. Simultaneously, the control group was given an equivalent dose of intraperitoneal normal saline – 0.9%. All the animals were weighed on a weighing machine prior to initiation of drug therapy and thereafter daily before each dose administration.

After four weeks of treatment for each group, the rats were sacrificed after giving deep anaesthesia by ether inhalation. A mid line incision was given, starting from the dorsal aspect of upper part of the neck, extending it to the head. After reflecting the scalp, the dorsal calvarium was removed in small chips, taking care not to damage the underlying brain tissue. The brain was carefully dissected out. The cerebellum was slightly lifted up and the cerebellar peduncles were cut, thus separating the cerebellum from the brainstem. The cerebellum was separated from the rest of the brain. The two cerebellar hemispheres were separated by a sagittal cut passing through the vermis. The tissues were washed with normal saline and fixed in the fixative (10% formal saline containing 100 ml of formalin, 8.5 g of sodium chloride and 900 ml of water) contained in glass bottles with glass stoppers which had been properly labelled beforehand. Tissue processing was done. Sagittal histological sections of 4–5 μm thickness were taken and stained with routine Haematoxylin and Eosin stain. Special stains (Beilschowsky's Silver Stain and Modified Luxol Fast Blue Stain) were also used to look for any axonal degeneration and demyelination. Slides were observed under the light microscope in low and high magnifications.

3. Results

The presence of scattered vacuoles was the most prominent and significant finding in the experimental group. Vacuolation was found in 67%, 87% and 93% of rats of the mild dose, moderate dose and high dose groups respectively (Figs. 1–6).

The severity of vacuolation was assessed in two ways:

(a) According to different regions.
(b) Grading of vacuolation.

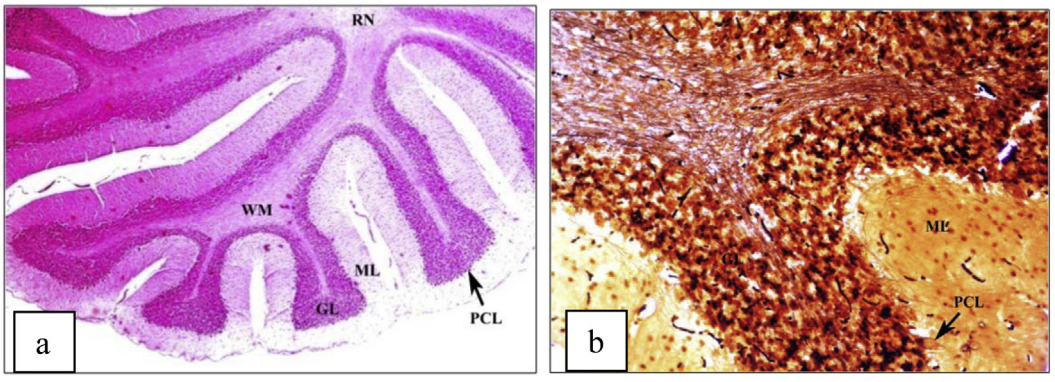

Fig. 1 – Photomicrograph of control cerebellum showing (a) Molecular layer (ML), Purkinje Cell Layer (PCL) and Granular Layer (GL) of the cerebellar cortex, White Matter (WM) and Roof Nuclei (RN). (H&E ×50) (b) Beilschowsky's Silver stain at higher magnification ×200.

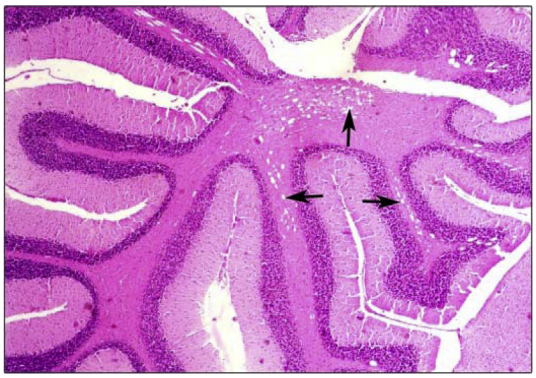

Fig. 2 – Photomicrograph of cerebellum of subgroup 2a showing Vacuolation (arrows) in the region of roof nuclei and white matter of folia (H&E ×50).

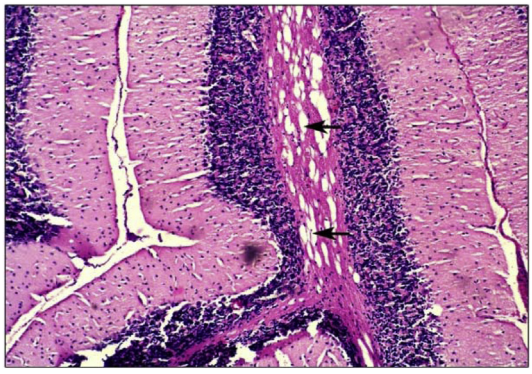

Fig. 3 – Photomicrograph of cerebellum of subgroup 2b showing Vacuolation (arrows) in the region of white matter of a folium (H&E ×100).

3.1. *(a) According to different regions (Table 1)*

Vacuolation was observed in the following regions:

1. **Roof nuclei** – Vacuolation was seen near roof nuclei equally in mild and moderate groups (67% rats of each) and 47% rats of high dose group (Fig. 2).

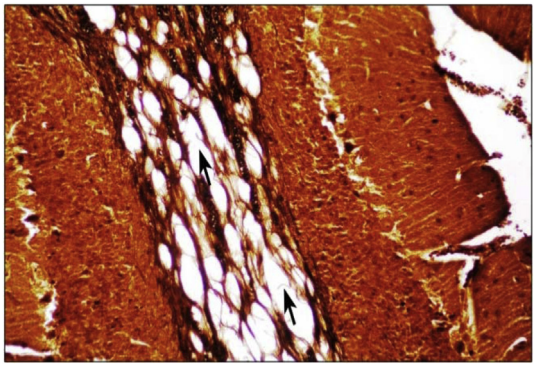

Fig. 4 – Photomicrograph of cerebellum of subgroup 2c showing marked Vacuolation (arrows) in the white matter of a folium (Beilschowsky's Silver ×100).

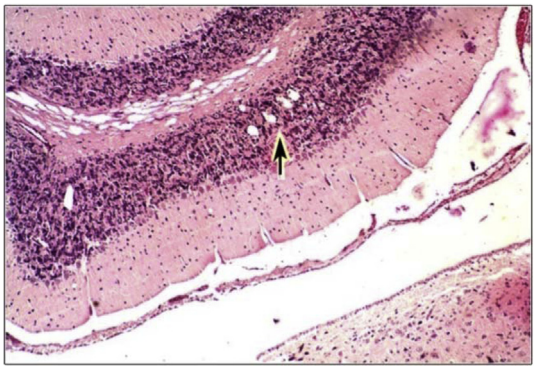

Fig. 5 – Photomicrograph of cerebellum of subgroup 2b showing Vacuolation (arrow) in the Granular layer (H&E ×100).

2. **White matter of cerebellar folia** – Vacuolation was seen in white matter of cerebellar folia significantly in 80% rats of high dose group (Figs. 2–4).
3. **Granular layer** – Vacuolation was significantly noted in the granular layer in 53% rats of moderate dose group (Fig. 5).
4. **Molecular layer** – Vacuolation was significantly observed in 40% rats of high dose group (Fig. 6).

3.2. *(b) Grading of Vacuolation*

This was done according to the area involved (Table 2):

- Grade 0: No vacuolation.
- Grade 1 (+): Vacuoles in <25% of the total area seen under low magnification (×50) **Mild vacuolation**.
- Grade 2 (++): Vacuoles in 25–50% of the total area seen under low magnification (×50) **Moderate vacuolation**.
- Grade 3 (+++): Vacuoles in >50% of the total area seen under low magnification (×50) **Marked vacuolation**.

Grade 0 was observed in the control group.
Grade 1 was seen in all groups, mostly in mild dose group.
Grade 2 was seen in moderate dose group.
Grade 3 was seen in high dose group.

4. Discussion

In the present study, vacuolation was the main feature in the treated group which increased with increasing doses of Vigabatrin. Vacuoles were seen mainly in the white matter near roof nuclei in the lower dose group while in the higher dose group, vacuolation mainly involved the white matter of the folia. Apart from white matter, granular and molecular layers of cerebellar cortex were also involved in moderate and high dose groups respectively. Similar neuropathology has been reported with other GABA-T inhibitors such as ethanolamine-o-sulphate,[12] γ-allenyl GABA and BW357U,[13] suggesting that elevated GABA levels secondary to inhibition of GABA-T may be involved in the pathogenesis as they are toxic to the neurons and neuroglia in the brain. Damage particularly involves the myelinated axons of the white matter where separation of

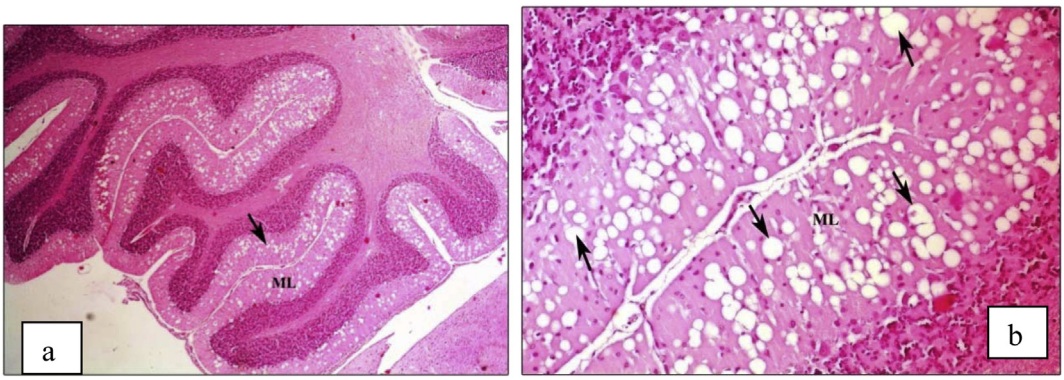

Fig. 6 – Photomicrograph of cerebellum of subgroup 2c showing marked Vacuolation (arrow) in the Molecular Layer (ML) (a) (H&E ×100) (b) (H&E ×200).

layers of myelin sheath (Intramyelinic oedema) occurs, leading to vacuolation.[14] Presence of vacuolation in cortex in the present study suggests that elevated GABA levels not only affect the myelinated axons, but also affect the neuropil consisting of dendritic processes, unmyelinated fibres and glial fibres.

The toxicity of Vigabatrin can be a result of direct injury by GABA or an indirect injury due to its metabolite, glutamate. Glutamate is the dominant excitatory amino acid and the primary neurotransmitter in about one-half of all the synapses in the brain.[15] It is responsible for many important neurologic functions including cognition, memory, movement, and sensation. In pathologic conditions, glutamate mediates neuronal injury or neuronal death, particularly through activation of the *N*-methyl-D-aspartate (NMDA) receptor subtype of the glutamate receptors. Neuronal glutamate is released from the presynaptic terminal of neuronal axons into the synaptic cleft and then works as a neurotransmitter. Reuptake of extracellular glutamate takes place at the presynaptic terminals and adjacent glial cells. Mitochondria provide energy for the reuptake of glutamate. The excessive glutamate binding to NMDA receptors allows entry of Ca^{2+} into the postsynaptic neuron, causing necrotic cell death or apoptosis, whereas the excessive glutamate binding to non-NMDA receptors allows entry of Na^{+} into the postsynaptic neuron, resulting in cytotoxic oedema. Because glial cells also have these receptors, the excessive glutamate leads to glial cell swelling.[16] This is the underlying mechanism behind the IME and generalized oedema which was found in different parts of the brain in the present study.

Table 1 – Regions of the cerebellum involved in vacuolation in different dose groups.

Regions	Subgroup 2a No. of rats (%)	Subgroup 2b No. of rats (%)	Subgroup 2c No. of rats (%)
Roof nuclei	10 (67%)	10 (67%)	7 (47%)
White matter of cerebellar folia	10 (67%)	10 (67%)	12 (80%)
Granular layer	4 (27%)	8 (53%)	5 (33%)
Molecular layer	0	2 (13%)	6 (40%)

Table 2 – Grades of vacuolation seen in different dose groups.

Grades	Subgroup 2a No. of rats & %	Subgroup 2b No. of rats & %	Subgroup 2c No. of rats & %
0	5 (33%)	2 (13%)	1 (7%)
1 (+)	7 (47%)	8 (13%)	2 (13%)
2 (++)	2 (13%)	9 (60%)	4 (27%)
3 (+++)	1 (7%)	2 (13%)	8 (53%)

Butler et al observed the effects of three different doses of Vigabatrin (30, 100 and 300 mg/kg/day) given for 90 days on the central nervous system of albino rats. Rats treated with lower doses showed vacuolation in the white matter near roof nuclei but those treated with higher doses showed vacuolation in folia. Vacuoles were restricted to myelinated tracts in the white matter. Electron microscopic studies done by them revealed that the vacuolation was due to separation of outer lamella of myelin sheath at the intraperiod line, known as IME.[17] Findings of the present study correlate well with those of this study except for the involvement of the cerebellar cortex also along with white matter in the present study which indicates that other additional mechanisms may be involved in formation of vacuoles apart from IME and require further evaluation. Further studies like immunohistochemistry and electron microscopy are needed to establish the facts of pathogenesis.

Demyelination of white fibres near roof nuclei and in folia was also a finding in the present study. Toxic damage to oligodendroglia by increased levels of GABA could be a cause for the demyelination. Qiao et al observed the neuropathological findings & did biochemical assays in the brain of rats after giving injectable Vigabatrin in doses of 25–40 mg/kg/day for 2 weeks. They found a reduction in myelination, reduced activities of myelin and oligodendrocyte associated enzymes and decrease in myelin basic protein on Western Blots, confirming toxic damage to the oligodendrocytes which are the cells responsible for myelination in the central nervous system.[18]

Phillip L. et al reviewed the MRIs of patients treated with Vigabatrin. Patients were assessed for age at the time of MRI, diagnosis, duration and dose of VGB treatment, MRI findings

pre-, on, and post-Vigabatrin, concomitant medications, and clinical correlation. These findings were compared to MRI in patients with IS who did not receive Vigabatrin. Their observations strongly suggest that the oedema and microvacuolization, seen in radiographic and histopathological animal studies may have a correlate in patients treated with Vigabatrin. The affected patient group had abnormalities noted within a relatively short time frame, comparable to the therapy duration used in the animal studies. Their data suggests that side effects have been found in humans too, infants being a high risk group for this complication, and there also appears to be a dose-related effect with more neurotoxicity in patients on higher doses.[19]

5. Conclusion

Although Vigabatrin is well tolerated and has the most favourable improvement rates in epilepsy as compared with other newer AEDs, it may be neurotoxic. Therefore, we suggest that the dose and duration of treatment should preferably be kept to as minimum as possible, weighing the potential for seizure reduction against safety risks. A strict monitoring for the onset of the side effects should be done with reassessment at regular intervals (using MRI examination if required), particularly in infants and those receiving higher doses of VGB. Tests for assessing cerebellar function should be done at the beginning of treatment and thereafter at regular intervals as this can help in determining the onset of toxicity. Early assessment of efficacy and ongoing evaluation of the benefits and risks of Vigabatrin therapy should be done.

Conflicts of interest

All authors have none to declare.

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