Original Paper

The Vigabatrin Induced Retinal Toxicity is Associated with Photopic Exposure and Taurine Deficiency: An *In Vivo* Study

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Key Words

Vigabatrin • Retinal toxicity • *In vivo*

Abstract

Background/Aims: Retinal toxicity is one of the most commonly discussed and concerning adverse effects of vigabatrin (VGB). The present study explored the relationship between the VGB elicited retinal toxicity, photopic exposure, and taurine deficiency, aiming at screening for risk factors to minimize the adverse effects of VGB. **Methods:** The effects of VGB on function and morphology of mouse retinas were examined via a series of *in vivo* tests, including electroretinography (ERG), Spectral domain optical coherence tomography (SD-OCT), and optokinetic testing. Moreover, VGB-treated mice were in addition treated with taurine to verify possible protective effects against retinal toxicity. **Results:** A close relationship between VGB induced retinal toxicity and light exposure was observed. The VGB-treated mice which were reared in darkness preserved better visual function and retinal architectures as verified by the optokinetic tests, OCT and ERG examinations. The retinal taurine level of the VGB-treated mice which were exposed to light were significantly lower than that of the VGB mice reared in darkness. Furthermore, several *in vivo* evidence provided by our research confirmed that the VGB induced morphological and functional impairments could be partially alleviated by taurine treatment. The present study showed the retinal toxicity of VGB by *in vivo* measurements. **Conclusion:** The VGB induced retinal toxicity is closely associated with photopic exposure and taurine deficiency. Patients who are taking VGB might benefit from minimization of light exposure and dietetic taurine supplements.

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Introduction

Vigabatrin(VGB), an irreversible inhibitor of gamma aminobutyric acid (GABA) transaminase(GABA-T), is specifically designed as a suicide substrate for this enzyme and thereby augment brain GABA concentration[1, 2]. Hitherto, it has been used as the mainstream pharmacological treatment for infantile spasms, and represents an important therapeutic strategy for patients with refractory complex partial epilepsy [3, 4]. However, the VGB concentration is indeed five-fold greater in the retina than in the brain after systematic administration. Therefore, the VGB administration could result in a significant increase of retinal GABA concentrations [5]. A series of clinical literature have documented the retinal toxicity in up to 40% of VGB treated patients since 1997 [6-9]. Chronic administration of VGB is found to induce the bilateral constriction of visual field, abnormality in visual acuity, color discrimination, and contrast sensitivity. Moreover, abundant evidences suggest that the chronic administration of VGB induces impairments to the electroretinography (ERG) function, outer nuclear layer (ONL) disorganization, and retinal ganglion cell (RGCs) death[10-12].

Recently, an histological investigation based on the neonatal rats reported that the VGB induced retinal toxicity was caused by taurine deficiency[13]. Therefore, the researchers proposed that taurine should be administrated together with VGB for infantile spasms or epilepsy. Moreover, the VGB mediated retinal toxicity is related to the photopic exposure because the albino animals maintained in darkness experience no features of retinal toxicity[14]. The effects of taurine deficiency are consistent with the mechanism of phototoxicity because retinal lesions are known to be exacerbated by light in the taurine-deficient animals. However, these results are mainly harvested from histological and immunochemistry observations of fixed tissues, and hitherto, the detailed mode of VGB induced retinal toxicity and correlated risk factors are rarely touched by *in vivo* measurements. Furthermore, in clinical practice, most screening investigations on VGB induced retinal toxicity rely heavily on monitoring visual fields due to the lack of functional examinations[15]. Nevertheless, the patient's visual field always remains relatively stable over the early stage of VGB treatment [16].

The objective of this study is to investigate the relationship between the VGB induced retinal toxicity and photo exposure, aiming at screening for risk factors to minimize the adverse effect of this drug. The VGB induced impairments to the animal retina are explored via *in vivo* measurements and correlated with photopic exposure as well as taurine deficiency. Additionally, our research suggests that the VGB induced impairments could be prevented partially by taurine treatment. Herein, we provide an appropriate example to screen for and monitor the VGB induced retinal toxicity via *in vivo* measurements, including the spectral domain optical coherence tomography (SD-OCT) imaging, ERG examination, and optokinetic tests. These *in vivo* findings cast a snapshot into the complex world of retinal pathology which can be influenced by correlated risk factors. Future clinical trials are needed to determine whether the co-treatment of taurine and VGB can limit epileptic seizures without inducing visual impairments.

Material and Method

Animal Grouping and Treatments

All experiments were conducted in accordance with the ARVO Statements for the Use of Animals in Ophthalmic and Vision Research. All efforts were made to minimize the number of animals used and their suffering. The C57BL/6 mice used in this study were purchased from the animal center of General Hospital of Chinese PLA (8 weeks old at the beginning of the VGB treatment with both sexes) and were maintained under standard laboratory conditions (room temperature 18°C to 23°C, 40% to 65% humidity) with food and water available ad libitum. Light intensities of normal light luminance in the animal cages were between 70 and 80 lux during the day period of the 12h/12h light/dark cycle. The animals were divided into three groups: 1) the normal controls which were reared under normal laboratory light luminance and without any

pharmacological administration; 2) the VGB+Light mice which were administered with VGB and then were exposed to normal laboratory light luminance; 3) the VGB+Dark mice which were administered with VGB and then were reared in darkness. VGB dissolved in 0.9% NaCl was administered at 5.0 mg/day (100mg/ml, 0.05ml) to the C57BL/6 mice by daily intraperitoneal injection. Ten animals from each group were subject to visual function evaluation respectively at 15 days (P15), 30 days (P30), 45 days (P45), and 60days (P60) after the VGB administration. In the therapeutic test, the taurine supplementation was given in drinking water at the concentration of 0.1M for 60days. The average amount of taurine received by each mouse was approximately 120mg/day. The animal numbers and experimental groups are shown in Table 1.

Electroretinography (ERG) examination

The ERG recordings were performed according to the previously described method[17]. Briefly, animals were weighed and dark-adapted overnight before recording and then were anesthetized by an intraperitoneal injection of ketamine (80 mg/kg) and chlorpromazine (8 mg/kg) under dim red light conditions. Animals were lightly secured to the stage in the UTAS Visual Diagnostic System with a Big Shot Ganzfeld (LKC Technologies, Gaithersburg, MD, USA). Platinum circellus record electrodes were placed on each cornea and a reference electrode placed subcutaneously between the eyes. White flashes with the intensity of 0.5 log cd-s/m² were applied for stimulating the scotopic ERGs. Photopic ERG measurements were obtained for flash intensities at 1.48 log cd-s/m² in the presence of 30 cd/m² background light (the interstimulus interval was 0.4 seconds). Totally 60 photopic responses and 10 scotopic responses were recorded and averaged for a- and b-waves analysis. The amplitude of a-wave was measured as the distance from baseline to a-wave trough, while amplitude of b-wave was defined as the distance between trough and peak of each waveform. Signals were amplified and filtered by the band-pass (1 Hz to 300 Hz). Oscillatory potentials (Ops) were isolated from the averaged recording traces using a 75-300 Hz digital filter. The amplitude of the oscillatory potential was measured from the bottom of trough preceding each OP peak, to the top of that peak.

SD-OCT examination

Mouse pupils were dilated with 1% atropine and 2.5% phenylephrine hydrochloride and then were anesthetized as aforementioned. One drop of 2.5% hydroxypropyl methylcellulose was administrated to mouse eyes before examination. SD-OCT was performed noninvasively using a machine manufactured by Biophtigen (Durham, NC). Three lateral images (nasal to temporal) were collected, starting 3 mm above the meridian, crossing through the center of the Optic nerve head (ONH), and 3 mm below ONH meridian. A corresponding box centered on the ONH with eight measurement points separated by 3 mm from each other was created. Corresponding neural retinal thickness for examined eyes was compared at each point by measuring the distance from the vitreal face of the RGCs layer to the apical face of the retinal pigment epithelium. The SD-OCT cross-sectional images were analyzed with the InVivoVue™ DIVER 2.4 software (Biophtigen, Inc, NC, USA). The mean value of retinal thickness and retinal nerve fiber layer (RNFL) were averaged from the eight measurement points of each retina.

Table 1. Experiment design and animal numbers

Groups	Light Exposure	VGB Administration	Taurine Supplements	Animal Numbers
Test 1 (In vivo test)				
Normal control	+	-	-	10
VGB+Light	+	+	-	10
VGB+Dark	-	+	-	10
Test 2 (Time course observation)				
Normal control	+	-	-	30
VGB+Light	+	+	-	30
VGB+Dark	-	+	-	30
Test 3 (Therapeutic test under photopic condition)				
Normal control	+	-	-	10
Treated	+	+	+	10
Untreated	+	+	-	10
Test 4 (Therapeutic test under scotopic condition)				
Normal control	-	-	-	10
Treated	-	+	+	10
Untreated	-	+	-	10
Total				180

Optokinetic testing

Photopic visual acuities and contrast sensitivities of mice were measured using a two-alternative forced choice paradigm as described previously with minor modifications [18, 19]. Thresholds for each eye were determined simultaneously via stepwise functions for correct responses in both the clockwise and counter-clockwise direction. Visual acuity was defined as the highest spatial frequency (100% contrast) yielding a threshold response, and contrast sensitivity was defined as 100 divided by the lowest percent contrast yielding a threshold response (sinusoidal pattern at 0.256 cyc/deg). For acuity measurements, the initial stimulus was a 0.200 cyc/deg sinusoidal pattern with a fixed 100% contrast. For contrast sensitivity measurements, the initial pattern was presented at 100% contrast, with a fixed spatial frequency of 0.128 cyc/deg. All patterns were presented at a speed of 12 degrees/s. Photopic vision was measured at a mean luminance of 70 cd/m². For scotopic measurements, mice were dark-adapted overnight and light levels were attenuated to 3.5 × 10⁻⁵ cd/m² through the use of neutral density filters. Visual acuities and contrast sensitivities of each mouse were measured for four times over a period of 24 hours.

Taurine level assay

The concentration of retinal taurine was determined by the high performance liquid chromatography (HPLC). The HPLC system consisted of a Separation System, a fluorescent detector and an Alltima C18 column (Metachem Technologies, Inc., Torrance, CA). Each retina was homogenized in 300 µl 0.4 M potassium borate buffer (pH 10.4) and 50 µl of 20% sulfosalicylic acid. A specie sample was kept for protein analysis. Centrifugation of all tissue homogenates was carried out at 35 000 g for 20 min at 4°C. The supernatant fraction (25 µl) was kept at 80°C for HPLC analysis. 25 µl of the supernatants plus 175 µl of potassium borate buffer (pH 10.4) were derivatized by adding 200 µl of the mixture: 25 mg o-phthaldehyde, 500 µl methanol, 25 µl b-mercaptoethanol (1 g/ml), and 4.5 ml 0.4 M potassium borate buffer. Aliquots of the derivatized preparation were injected into the chromatographic system. The plasma taurine measurements were performed on each animal using formerly described technique [14]. Blood samples were collected in hemolysis tubes containing heparin (14 IU/ml) and centrifuged (2,200g, 15min). Plasmatic taurine was performed by ion-exchange chromatography with ninhydrin detection. The concentration of retinal taurine was quantified by the external standard and expressed as µmol/l.

Statistical analysis

A one-way ANOVA followed with Bonferroni post hoc test (in case of Gaussian distribution) or a Kruskal-Wallis analysis of variance followed Dunns test (in case of non-Gaussian distribution) was used to compare the means of each group. The Statistical Product and Service Solutions (SPSS) software was used (version 19.0, IBM, Chicago, IL, USA). Differences were considered statistically significant at $p < 0.05$, $**p < 0.01$ and $***p < 0.001$. The values were presented as mean ± standard deviation (SD) unless otherwise specified.

Results

Optokinetic testing of the VGB administered mice

The optokinetic behavioral analysis suggested that the mice in the VGB+Light group performed poorly equivalent to impaired cone-mediated visual function: the visual acuity in VGB+Light group (0.225 ± 0.033 cyc/deg, $n=10$) was significantly lower than the normal control group (0.533 ± 0.042 cyc/deg, $n=10$, $P < 0.001$; Fig. 1 A). Moreover, the contrast sensitivity in the VGB+Light group (4.865 ± 0.201 , $n=10$) was also significantly lower than the normal control group (11.571 ± 0.926 , $n=10$, $P < 0.001$; Fig. 1 B). Intriguingly, the eyes in the VGB+Dark group responded better than the VGB+Light group: both the visual acuity (0.411 ± 0.039 cyc/deg, $n=10$) and contrast sensitivity (7.193 ± 0.560 , $n=10$) in the VGB+Dark group were significantly higher than the VGB+Light group ($P < 0.001$). Meanwhile, both the visual acuity and contrast sensitivity in the VGB+Light group were significantly lower than the normal controls ($P < 0.001$).

Fig. 1. A. The visual acuity of the VGB+Light group was significantly lower than the normal controls. B. The contrast sensitivity of the VGB+Light group was also significantly lower than the normal controls. Both the visual acuity and contrast sensitivity of the VGB+Dark group were significantly higher than the VGB+Light group. Meanwhile, both the visual acuity and contrast sensitivity of the VGB+Light group were significantly lower than the normal controls. (Kruskall-Wallis analysis followed by Dunns post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

Fig. 2. A. The representative ERG wave forms of examined eyes. B. The amplitude of b wave in the VGB+Light group decreased substantially compared with normal controls. The amplitude of b wave in the VGB+Dark group was also significantly smaller than the normal controls. However, the amplitude of b wave in the VGB+Dark group was less impaired than the VGB+Light group. The amplitude of a wave in the VGB+Dark group was significantly smaller than the Normal group. However, it was significantly larger than the VGB+Light group. Moreover, the sum amplitude of the OPs in the VGB+Dark group was also significantly larger than the

VGB+Light group. (one-way ANOVA analysis followed by Bonferroni's post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

The ERG of the VGB administered mice

Representative ERG wave forms of the examined eyes were shown in the Fig. 2 A. The amplitude of b wave in the VGB+Light group (Photopic: $31.0 \pm 7.6 \mu$ V; Scotopic: $268.5 \pm 30.7 \mu$ V; $n=10$) substantially decreased than the normal controls (Photopic: $93.1 \pm 16.5 \mu$ V, $n=10$, $F_{2,27}=62.22$, $P<0.001$; Scotopic: $436.3 \pm 41.8 \mu$ V, $n=10$, $F_{2,27}=56.37$, $P<0.001$). The amplitude of b wave in the VGB+Dark group (Photopic: 58.5 ± 11.7 , $n=10$; Scotopic: $337.6 \pm 33.1 \mu$ V, $n=10$) was also significantly smaller than the normal controls ($P<0.001$; Fig. 2 B). However, it's noteworthy that the amplitude of b wave in the VGB+Dark group was less impaired than the

Fig. 3. A. The OCT examination revealed that the retinal organization had been remarkably disturbed by the VGB administration. B. The average retinal thickness of the VGB+Light group was significantly smaller than that of the normal control. The average retinal thickness of the VGB+ Dark group was also significantly smaller than that of the normal control. However, it was significantly larger than that of the VGB+ Light group. C. The RNFL thickness in the VGB+ Dark group was significantly smaller than that in the normal control group. Meanwhile, the RNFL thickness in the VGB+ Dark group was significantly larger than that in the VGB+ Light group. (one-way ANOVA analysis followed by Bonferroni's post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

VGB+ Light group, indicating that the visual function of these mice was partially preserved by darkness. The amplitude of a wave in the VGB+ Dark group ($70.5 \pm 15.1 \mu V$, $n = 10$) was significantly smaller than the normal controls ($94.1 \pm 17.2 \mu V$, $n = 10$, $F_{2,27}=17.31$, $P < 0.01$). However, it was significantly larger than the VGB+ Light group ($54.5 \pm 12.8 \mu V$, $n = 10$, $P < 0.05$). Moreover, the sum amplitude of the OPs in the VGB+ Dark group ($168.2 \pm 40.5 \mu V$, $n=10$) was also significantly larger than the VGB+ Light group ($126.1 \pm 35.8 \mu V$, $n = 10$, $F_{2,27}=21.59$, $P < 0.05$).

The retinal thickness of the VGB administered mice

The SD-OCT examination allows for the morphological assessment of mice retinas *in vivo*. After 60 days of VGB administration, the retinal organization was remarkably disturbed and the retinal thickness reduced significantly. The reduction of retinal thickness was clearly visible in a representative image taken 3 mm temporal of the optic nerve head (Fig. 3 A). In greater detail, the average retinal thickness of the VGB+Light group (0.163 ± 0.009 mm, $n = 10$) was significantly smaller than the normal control (0.215 ± 0.013 mm, $n=10$, $P<0.001$, $F_{2,27}=58.66$; Fig. 3 B). Meanwhile, the average retinal thickness of the VGB+ Dark group (0.184 ± 0.010 mm, $n = 10$) was also significantly smaller than the normal control group ($P<0.001$). However, it was significantly larger than the VGB+ Light group, indicating that the darkness was beneficial for preventing partially the VGB induced morphological disorganizations. Furthermore, the RNFL thickness of the VGB+ Dark group ($14.1 \pm 3.3 \mu m$, $n = 10$) was significantly smaller than the normal control group ($20.6 \pm 3.7 \mu m$, $n = 10$, $F_{2,27}=20.97$, $P < 0.001$; Fig. 3 C). Meanwhile, the RNFL thickness of the VGB+ Dark group was significantly larger than the VGB+ Light group ($10.6 \pm 3.5 \mu m$, $n = 10$, $P < 0.05$), indicating that the darkness could alleviate the VGB induced toxicity on RGCs.

Fig. 4. A. After VGB administration, the retinal taurine level of the VGB+ Light group substantially decreased than the normal controls. Meanwhile, the retinal taurine level of the VGB+ Dark group also remarkably decreased than the normal controls. However, it was significantly higher than the VGB+ Light group. B. The taurine plasma level of the VGB+ Light group decreased substantially than the normal controls. Meanwhile, the taurine plasma level in the VGB+ Dark group also remarkably decreased than the normal controls. However, it was significantly higher than the VGB+ Light group. (one-way ANOVA analysis followed by Bonferroni's post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

The taurine levels of the VGB administered mice

After VGB administration, the retinal taurine level of the VGB+ Light group ($26.5 \pm 6.1 \mu\text{mol/g}$, $n=10$) substantially decreased than the normal controls ($78.5 \pm 9.2 \mu\text{mol/g}$, $n=10$, $F_{2,27}=112.51$, $P<0.001$; Fig. 4 A). Meanwhile, the retinal taurine level of the VGB+ Dark group ($67.8 \pm 8.9 \mu\text{mol/g}$, $n=10$) also decreased remarkably compared with the normal controls ($P<0.01$). However, it was significantly higher than the VGB+ Light group, suggesting that the taurine deficiency should be closely correlated with the VGB induced retinal impairments. Furthermore, the plasma taurine level of the VGB+ Light group ($166.1 \pm 15.7 \mu\text{mol/l}$, $n=10$) substantially decreased than the normal controls ($288.3 \pm 18.4 \mu\text{mol/l}$, $n=10$, $F_{2,27}=131.31$, $P<0.001$; Fig. 4 B). Meanwhile, the taurine plasma level of the VGB+ Dark group ($219.6 \pm 16.5 \mu\text{mol/l}$, $n=10$) also decreased compared with the normal controls ($P<0.001$). However, it was significantly higher than the VGB+ Light group ($P<0.001$).

The time course of the VGB induced retinal impairments

Ten animals from each group were examined at the following time points respectively: 15 days (P15), 30 days (P30), 45 days (P45), and 60 days (P60) post the VGB administration. At the time point of P15, both the visual acuity and contrast sensitivity of the VGB+ Light group reduced significantly (visual acuity: VGB+ Light vs. normal control, $P<0.05$; visual acuity: VGB+ Light vs. normal control, $P<0.001$; $n=10$). The visual acuity and contrast sensitivity of the VGB+ Dark group were not different significantly from the normal control group (VGB+ Dark vs. normal control, $P>0.05$; $n=10$; Fig. 5.A and B). ERG examination suggested that the amplitudes of b wave and OPs of the VGB+ Light and VGB+ Dark group reduced, however, the difference was not statistically significant (VGB+ Light vs. normal control, $P>0.05$; VGB+ Dark vs. normal control, $P>0.05$; Fig. 5.C,D,E). Moreover, the ONL thickness of both VGB+ Dark and VGB+ Light group were not different significantly from the normal control group (VGB+ Light vs. normal control, $P>0.05$; VGB+ Dark vs. normal control, $P>0.05$; $n=10$; Fig. 5.F). Subsequently at P30, the reductions in the amplitudes of b wave and OPs progressed forward in the VGB+ Light group (photopic b wave: VGB+ Light vs. normal control, $P<0.01$; scotopic b wave and OPs: VGB+ Light vs. normal control, $P<0.001$; $n=10$; Table 2). The visual acuity, contrast sensitivity and ONL thickness in the VGB+ Light group also reduced progressively (VGB+ Light vs. normal control, $P<0.001$; $n=10$). Meanwhile, all these measured indicators

Fig. 5. A. At the time point of P15, the visual acuity and contrast sensitivity of the VGB+ Light group reduced significantly. B. Meanwhile, the visual acuity and contrast sensitivity of the VGB+ Dark group were not significantly different from the normal controls. The ERG examination suggested that the amplitudes of scotopic b wave (C), photopic b wave (D) and OPs (E) in the VGB+ Light and VGB+ Dark group reduced, however, the difference was not statistically significant. The ONL thickness in both VGB+ Dark and VGB+ Light group were not significantly different from the normal controls (F). Subsequently at P30, the reductions in the amplitudes of photopic b wave, scotopic b wave and OPs in the VGB+ Light group progressed forward. The visual acuity, contrast sensitivity and ONL thickness in the VGB+ Light group also reduced progressively. Meanwhile, all these measured indicators in the VGB+ dark group reduced significantly compared with normal controls. At the P45, the ERG amplitudes, visual acuity, contrast sensitivity and ONL thickness in both VGB+ dark and VGB+ Light group reduced significantly compared with normal controls. Eventually at P60, the reductions in all these measured indicators in the VGB+ Light and VGB+ dark group progressively reduced compared with normal controls. (Kruskal-Wallis analysis followed by Dunns post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

of the VGB+ dark group reduced significantly compared with normal controls (photopic b wave, OPs, visual acuity and ONL thickness: VGB+ dark vs. normal control, $P<0.05$; scotopic b wave and contrast sensitivity: VGB+ dark vs. normal control, $P<0.001$; $n=10$). At P45, the ERG amplitudes, visual acuity, contrast sensitivity and ONL thickness in both VGB+ dark and VGB+ Light group reduced more obviously compared with normal controls ($P<0.001$; $n=10$; Table 2). Eventually, at P60, the reductions in all these measured indicators of the VGB+ Light and VGB+ dark group reduced progressively compared with normal controls ($P<0.001$; $n=10$; Table 2).

The therapeutic effects of taurine supplements

Ten VGB administered mice received taurine supplements and were reared in normal light luminance with the 12h/12h light/dark cycle. Another ten VGB administered mice were left untreated (received normal diet) and reared under normal luminance condition. Ten normal mice receiving normal diet were reared under the same luminance condition (as controls). After 25 days of taurine treatment, the retinal taurine level of the taurine treated group ($70.8 \pm 8.5 \mu\text{mol/g}$, $n=10$) was substantially higher than the untreated group

Table 2. The time course of VGB induced toxicity in different experimental conditions(mean±SD; n=10). ^a $P < 0.05$ for difference compared with normal control group. ^b $P < 0.01$ for difference compared with normal control group. ^c $P < 0.001$ for difference compared with normal control group

Time Points	ERGs test								
	Scotopic b-wave(μ V)			Photopic b-wave(μ V)			OPs wave(μ V)		
	Normal control	VGB+ Light	VGB+ Dark	Normal control	VGB+ Light	VGB+ Dark	Normal control	VGB+ Light	VGB+ Dark
P15	439.0±42.7	401.9±42.5	408.9±44.6	95.7±14.2	83.5±11.6	84.8±13.9	249.4±37.0	231.3±39.8	233.2±38.7
P30	452.3±44.3	338.7±33.4 ^c	379.7±30.1 ^c	93.4±13.6	68.3±14.2 ^b	77.4±12.1 ^a	243.1±39.6	201.2±36.9 ^c	222.5±37.1 ^a
P45	446.2±45.1	299.1±32.0 ^c	353.2±31.3 ^c	97.8±12.4	53.1±12.9 ^c	71.0±10.2 ^c	248.1±32.3	163.8±37.5 ^c	198.6±38.4 ^c
P60	436.3±41.8	268.5±30.7 ^c	337.6±33.1 ^c	93.1±16.5	31.0±7.6 ^c	58.5±11.7 ^c	241.0±42.1	126.1±35.8 ^c	168.2±40.5 ^c
	Optokinetic test								
	Visual acuity(cyc/deg)			Contrast sensitivity			OCT test		
	Normal control	VGB+ Light	VGB+ Dark	Normal control	VGB+ Light	VGB+ Dark	Normal control	VGB+ Light	VGB+ Dark
P15	0.518±0.047	0.475±0.038 ^a	0.491±0.036	12.363±1.020	10.463±0.864 ^c	11.395±0.903	0.215±0.013	0.209±0.011	0.208±0.013
P30	0.522±0.045	0.410±0.031 ^c	0.477±0.032 ^a	11.838±0.954	8.851±0.590 ^c	9.790±0.662 ^c	0.222±0.013	0.193±0.014 ^c	0.201±0.015 ^a
P45	0.539±0.040	0.338±0.029 ^c	0.443±0.031 ^c	13.230±1.211	6.136±0.411 ^c	8.092±0.578 ^c	0.219±0.015	0.175±0.011 ^c	0.189±0.013 ^c
P60	0.533±0.042	0.225±0.033 ^c	0.411±0.039 ^c	11.571±0.926	4.865±0.201 ^c	7.193±0.560 ^c	0.223±0.018	0.163±0.009 ^c	0.184±0.010 ^c

Fig. 6. A. The taurine level of the taurine treated group was significantly higher than the untreated group. However, the retinal taurine level of the treated group was not significantly different from the normal controls. B and C. Both the visual acuity and the contrast sensitivity of the treated group were significantly higher than the untreated group. (one-way ANOVA analysis followed by Bonferroni's post-hoc test was used to examine the differences in terms of taurine concentration, Kruskal-Wallis analysis followed by Dunns post-hoc test was used to examine the differences in terms of visual acuity and the contrast sensitivity, n=10; $p < 0.05$, $**p < 0.01$ and $***p < 0.001$ for differences compared between the groups; All the values were presented as mean ±SD).

(25.0±8.6μmol/g, n= 10, F2 27=140.73 , $P < 0.001$; Fig. 6 A).However, the retinal taurine level of the treated group was not significantly different from the normal controls (75.3 ±9.5μmol/g, n= 10, $P > 0.05$). Moreover, both the visual acuity and the contrast sensitivity of the treated group were significantly higher than the untreated group (visual acuity: 0.131 ± 0.026 cyc/deg vs. 0.306 ± 0.031cyc/deg, n=10, $P < 0.001$; contrast sensitivity: 4.395 ± 0.258 vs.7.120 ± 0.479, n=10, $P < 0.001$; Fig. 6B and 6C). The amplitude of b wave in the treated group were significantly larger than the untreated group(Photopic b-wave: 25.0±8.6μV vs. 50.1±9.7μV, n = 10, F2 27=90.69, $P < 0.001$; Scotopic b-wave: 181.1±28.6μV vs. 279.6±31.2 μV, n = 10, F2 27=127.12, $P < 0.001$; Fig. 7A). The amplitude of a wave in the treated group(60.6±14.6μV, n = 10) was also significantly larger than the untreated control (44.8±12.1μV, n = 10, F2 27=35.16, $P < 0.05$; Fig. 7 B). The sum amplitude of the OPs in the treated group(173.0±41.1μV, n = 10) was also significantly larger than the untreated control (118.0±34.3μV, , n = 10, $P < 0.01$, F2 27=21.59), indicating that the taurine treatment could suppress the VGB induced visual functional impairments. Furthermore, the OCT examination suggested that the average retinal thickness of the taurine treated group(0.181 ± 0.010mm, n = 10) was significantly larger than the untreated group (0.153 ± 0.008 mm, n = 10, F2

Fig. 7. A. The representative ERG wave forms of the taurine treated, untreated, and normal control mice. B. The amplitude of b wave in the taurine treated group was significantly larger than the untreated group. The amplitude of a wave in the treated group was also significantly larger than the untreated control. The sum amplitude of the OPs in the treated group was significantly larger than the untreated group. (one-way ANOVA analysis followed by Bonferroni's post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

Fig. 8. A. The OCT examination suggested that the retinal organization had been preserved by the taurine treatment effectively. B. The average retinal thickness of the taurine treated group was significantly larger than the untreated group. C. The RNFL thickness of the treated group was significantly larger than the untreated control. (one-way ANOVA analysis followed by Bonferroni's post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

Fig. 9. A. The retinal taurine level of the treated group was significantly higher than the untreated group. The retinal taurine level of the treated group was not significantly different from the normal controls. B. and C. Both the visual acuity of and the contrast sensitivity of the treated group were significantly higher than the untreated group. D. Moreover, the amplitude of scotopic b wave in the treated group was significantly larger than the untreated group. E. The amplitude of photopic b wave in the treated group was also larger than the untreated group, however, the difference was not statistically significant. F. and G. The amplitudes of a wave and OPs in the treated group were not significantly different from the untreated control. H. The average retinal thickness of the the treated group was not significantly different from the untreated group. (one-way ANOVA analysis followed by Bonferroni's post-hoc test, $n=10$; $p<0.05$, $**p<0.01$ and $***p<0.001$ for differences compared between the groups; All the values were presented as mean $\pm$ SD).

27 = 78.38, $P < 0.001$, Fig. 8 A and 8B). In greater detail, the RFNL thickness of the treated group ($15.6 \pm 3.4 \mu\text{m}$, $n = 10$) was significantly larger than the untreated control ($11.1 \pm 3.2 \mu\text{m}$, $F_{2,27} = 15.32$, $P < 0.01$, $n = 10$; Fig. 8 C), indicating that the taurine treatment could effectively alleviate the VGB induced toxicity on RGCs.

Subsequently, we explored the effects of taurine treatments on the VGB administered retina under scotopic conditions. Ten VGB administered mice received taurine supplements and were reared in darkness. Another ten VGB administered mice received normal diet and were reared in darkness. Ten normal mice receiving normal diet were also reared in darkness (as controls). After 25 days of taurine treatment, the retinal taurine level of the taurine treated group ($73.9 \pm 8.8 \mu\text{mol/g}$, $n = 10$) was significantly higher than the untreated group ($65.0 \pm 7.6 \mu\text{mol/g}$, $n = 10$, $F_{2,27} = 3.94$, $P < 0.05$; Fig. 9A). Meanwhile, the retinal taurine level of the treated group was not significantly different from the normal controls ($74.6 \pm 9.1 \mu\text{mol/g}$, $n = 10$, $P > 0.05$). The taurine treated mice responded significantly better than the untreated mice in the optokinetic test: both the visual acuity of and contrast sensitivity of the treated group were significantly higher than the untreated group (visual acuity: $0.456 \pm 0.033 \text{ cyc/deg}$ vs. $0.401 \pm 0.036 \text{ cyc/deg}$, $n=10$, $P < 0.01$; contrast sensitivity: 8.120 ± 0.658 vs. 7.101 ± 0.605 , $n=10$, $P < 0.01$; Fig. 9B and 9C). Moreover, the amplitude of scotopic b wave in the treated group ($361.8 \pm 33.1 \mu\text{V}$, $n = 10$) was significantly larger than the untreated group ($325.6 \pm 35.7 \mu\text{V}$, $n = 10$, $F_{2,27} = 14.17$, $P < 0.05$; Fig. 9D). The photopic b wave in the treated group ($66.9 \pm 9.5 \mu\text{V}$, $n = 10$) was also larger than the untreated group, however, the difference was not statistically significant (57.9 ± 11.9 , $n = 10$, $F_{2,27} = 22.41$, $P > 0.05$; Fig. 9E). The amplitudes of a wave and OPs of the treated group were not significantly different from the untreated control (a wave: $77.6 \pm 15.2 \mu\text{V}$ vs. $69.3 \pm 13.0 \mu\text{V}$, $n = 10$, $F_{2,27} = 6.57$, $P > 0.05$; OPs wave: $171.8 \pm 36.9 \mu\text{V}$ vs. $162.5 \pm 38.1 \mu\text{V}$, $n = 10$, $F_{2,27} = 14.01$, $P > 0.05$; Fig. 9F and

9G). Furthermore, the OCT examination suggested that the average retinal thickness of the treated group was not significantly different from that in the untreated group (0.186 ± 0.012 mm vs. 0.180 ± 0.013 mm, $n = 10$, $F_{2, 27} = 20.80$, $P > 0.05$; Fig. 9H). These results suggested that the taurine treatment would be beneficial for the visual function of the VGB+dark mice. However, the VGB induced visual impairments could not be completely prevented by taurine supplementations.

Discussion

VGB, the only presently approved therapy for infantile spasms, has shown efficacy, tolerability, as well as relative safety in the treatment of catastrophic childhood epileptic encephalopathy [7, 20]. In the retina, the VGB administration can suppress the GABA-T activity to an undetectable level while increase the GABA concentration [5, 21]. One of the most concerning adverse effects of VGB is its retinal toxicity which leads to retinal impairments, including visual field constriction, visual acuity loss, impaired contrast sensitivity, and problems with color discrimination [13, 22-24]. Subsequent morphological studies have described the disorganized ONL, shortened inner and outer cone segments, as well as the disrupted RGCs layer [13, 14]. The VGB-elicited photoreceptor degeneration is reported to be light dependent [25, 26]. However, these results are mainly harvested from histological and immunochemistry observations of fixed tissues. Hitherto, the detailed mode of the VGB induced phototoxicity and underlying mechanisms are rarely touched by *in vivo* measurements. Therefore, the present study sought to explore the light dependence of VGB elicited toxicity via multiple *in vivo* examinations. The VGB administered mice were kept in darkness and their visual function as well as retinal architectures were compared with those reared under normal illuminant conditions. Our results suggested that the VGB induced retinal toxicity was closely correlated with light luminance. The VGB administered mice which were reared in darkness could preserve better visual function and retinal architectures. The optokinetic tests showed that the visual activities of the VGB administered mice were effectively preserved if they were kept in darkness. Subsequent ERG examinations also suggested that these mice had better photopic and scotopic visual function. Additionally, the OCT examination revealed that darkness was capable of preserving the retinal architecture of VGB administered mice. Taken together, these findings suggested that limiting light exposure could minimize the VGB induced cellular injury and partially prevent the development of retinal lesions. Further guidelines which are designed to monitor the VGB induced visual system toxicity should pay attention to the potential risk of photopic exposure for the treated patients. Wearing sunglasses during daytime and avoid bright illumination at night might be simple and safe recommendations which could limit efficiently the progression of the VGB induced retinal lesions. However, it is noteworthy that excessive limiting of visual stimuli in these infant patients may adversely affect the neural plasticity which leading to ocular cortical organization and amblyopia [27]. Consequently, there is no need to keep infant patients in darkness but rather reduce exposures to extreme light levels. A delicate balance between the phototoxicity and retinal development should be attained to minimize visual impairments.

Interestingly, it was found that the 6 weeks of VGB treatment via oral delivery did not induce retinal damage in the pigmented rats under normal facility illumination [28]. This discrepancy might be ascribed to the following reasons: (1) Animal species: that study was based on the Long Evans rats. The difference of species should give rise to the relatively different number and distribution of photoreceptor populations across mammalian retinas, as different photoreceptor subtypes show variable susceptibilities to VGB toxicity [29]. (2) Illumination levels: light is a significant enhancer of VGB toxicity and this is mediated, directly or indirectly, by phototransduction signaling in rod and cone photoreceptors [30]. Variations in caging and light intensities of animal rooms in the different laboratories would affect the outcome of VGB induced retinopathy [11]. Moreover, prolonged light exposure would

exacerbate the VGB-induced retinal toxicity[30]. The illumination level of the animal facility in that study (approximately 40 lx for 42 days) was considerably lower than the present study (approximately 80 lx for 60 days). (3) VGB dose and length of administration: there is a positive trend that the doses and treatment periods are correlated with the severity of VGB induced visual function damage[27, 31]. The dose of VGB administration in their study (initially at the level of 200 mg/kg/day and then reduced to 150 mg/kg/day) was significantly lower than the present study (300 mg/kg/day). Moreover, the length of VGB administration in the present study was longer (60 days) and it would lead more VGB accumulate in the retina. (4) Delivery mode: the delivery mode would affect both the incidence and severity of the VGB induced retinopathy[5]. Intraperitoneal injection can produce temporally higher plasma VGB levels than oral delivery, which may also be the underlying cause for the discrepancy.

In greater detail, we found that the retinal taurine level of the VGB mice exposed to light was significantly lower than that of the VGB mice reared in darkness, indicating that VGB might generate a taurine deficiency responsible for its retinal toxicity. The decrease in retinal taurine level appears to be inconsistent with previous findings showing no change in multiple taurine tissue levels after 17 days of VGB administration[32]. However, the shorter administration period and animal species difference may account for this discrepancy. Intriguingly, another research based on the VGB administered rat model found that the taurine plasma levels decreased significantly, which agreed well with our study[14]. In order to verify the possible correlation between the taurine deficiency and VGB induced phototoxicity, the taurine treatments were given to the VGB administered mice. The visual function of the taurine treated mice was preserved effectively as shown by the optokinetic tests and ERG examinations. The OCT examination also revealed more intact retinal structure in the treated mice under photopic conditions. The therapeutic effects of taurine were firstly demonstrated via *in vivo* means.

Most previous screening guidelines rely heavily on monitoring visual fields as evidence of the VGB induced retinal toxicity. Herein, we provided an appropriate example to monitor the retinal toxicity via *in vivo* measurements. More importantly, the efficiency of the taurine treatment could be directly quantified by these examinations without any disturbance from laboratory operations. The ability of *in vivo* methods to track the rescue outcome over time without killing animals greatly enhances the efficiency of long-term therapeutic studies and dramatically decreases the number of required animals. These *in vivo* methods might be able to detect the preliminary toxicity before the patient was visually symptomatic with several visual field losses.

As a vital amino acid, taurine is present in abundant amounts in the neural system [33, 34]. Taurine can promote the survival of adult RGCs in a pure culture under serum-deprivation condition[35, 36]. Taurine deficiency can trigger off photoreceptor damage in monkeys and cats which are fed with taurine-free diet[37, 38]. Moreover, it has been reported that the VGB induced lesions present close similarities to the retinal damage produced by taurine deficiency[14, 39]. Given the critical role of taurine in the retinal excitability, several researchers have proposed the supplements of taurine-rich foods to suppress the VGB -induced retinal lesions[40, 41]. As the anti-epileptic activity of taurine has also been described [42], it is conceivable that the combined use of taurine and VGB might allow for a reduced VGB dosage to minimize side effects such as the retinal toxicity. Herein, the successful preservation of visual function and retinal structure can be achieved in the VGB administered mice by taurine supplements. The profound protection in present study supports the co-administration of taurine supplements with VGB therapy in clinical practice. Generally, the VGB produces its antiepileptic effects by irreversibly inhibiting GABA-T, an enzyme responsible for GABA degradation, and thereby significantly enhances the GABA concentration in retina[1, 2]. On the other hand, the GABA is a known substrate for the taurine transporter and could limit the taurine uptake into the retina by competitive inhibition[43, 44]. This mechanism might be considered as a potential cause for the VGB induced taurine deficiency in retina.

In conclusion, our results suggest that the retinal toxicity is closely related to the photopic exposure in the VGB treated mice. Excessive photopic exposure for the VGB administered mice might generate the taurine deficiency. Therefore, it is especially promising to protect the VGB subjects from excessive light exposure. Moreover, amino acid plays an important role in the cellular homeostasis and pathologic mechanisms of various ocular diseases[45-47]. The taurine deficiency might be the correlated mechanism underlying the VGB induced retinal toxicity, and it is reasonable to supplement with taurine to minimize the visual impairments. Patients taking VGB might gain benefits from the restriction of light exposure and the dietetic advice on taurine-rich foods. Probably, a more robust taurine supplementation might be required to mitigate the VGB-induced retinal degeneration effectively.

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Disclosure Statement

The authors declare that there is no conflict of interests.

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