Original Article

Therapeutic potential of valproic acid in advanced glaucoma: A pilot study

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Purpose: Oral valproic acid (VPA) used as an anticonvulsant has been shown to improve contrast threshold sensitivities in patients receiving it on long-term. This study aimed to evaluate the efficacy of oral VPA in improving visual function in eyes with advanced stage glaucoma. **Methods:** In this prospective randomized study, 31 patients (n = 31 eyes) with advanced stage glaucoma (with an intraocular pressure <16 mmHg) in at least one eye received oral VPA 500 mg once a day for 3 months and 33 patients (n = 33 eyes) continued on glaucoma therapy. Patients were followed up at 3 and 12 months (to evaluate the legacy effect of the drug). Blood VPA concentrations were measured at 3 months. Following parameters were assessed at baseline, 3 months and 12 months: log of the minimum angle of resolution (LogMAR) visual acuity, mean deviation on visual fields, and multifocal electroretinogram (ERG). **Results:** Median LogMar visual acuity in the VPA group improved from 0.3 at baseline to 0.18 and 0.18 at 3 and 12 months, respectively (P < 0.01). In comparison, the median visual acuity in control group at baseline was 0.18 and showed neither worsening nor improvement over 3 and 12 months (P = 0.56). The improvement in VPA group was significant compared to the control group (P < 0.01; Wilcoxon Signed-rank test). An improvement in one line was experienced in 11 out of 31 eyes in the VPA group compared to 1 out of 33 eyes among controls (P = 0.003). No significant improvement was noted in the mean deviation, and the multifocal ERG (Latency and amplitudes) in the VPA-treated patients. The average blood VPA concentration measured at 3 months of therapy was 26 ± 8.9 μg/ml (range 8–55 μg/ml) which is much lower than that achieved during anticonvulsant therapy. None of the patients complained of any adverse effects that required stopping VPA therapy. **Conclusion:** A 3 months oral VPA therapy results in some improvement in visual acuity in a subgroup of eyes with advanced glaucoma and the effect was seen to persist 9 months after the drug was stopped.

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Patients with advanced glaucoma have been shown to have progression despite intraocular pressure (IOP) control.[1-3] Even in the absence of perimetric progression of the disease, patients may feel fluctuation in their visual function which impairs their quality of life. Neuroprotective agents have been tried in advanced glaucoma without significant benefit.[4-8] Few clinical studies have shown beneficial effect of oral valproic acid (VPA) in retinitis pigmentosa (RP).[9-12] Experimental studies in optic nerve crush injury and experimental glaucoma have shown neuroprotective effect of VPA.[13,14]

The neuroprotective effect of VPA is postulated through its suppression of pro-apopoptotic molecules such as caspase 3, caspase 8, caspase 9, Bax and induces the antiapoptotic factors such as Bcl-2 and Bcl-Xl.[15] VPA can directly upregulate cyclic AMP response element binding protein (CREB) at transcriptional level and reverse degeneration associated with histone deacetylation in neurons.[16] CREB is a transcription factor mediating stimulus dependent expression of genes critical to plasticity, growth, and survival of neurons. VPA reduces oxidative stress and stimulates cell survival signaling pathway associated with extracellular-signal-regulated kinases (ERK).[17] It has been shown to induce neuronal differentiation and promote neurite growth, however, there are no clinical studies evaluating the therapeutic potential of oral VPA in glaucoma.[18] This prospective randomized study was undertaken to evaluate the therapeutic effect of oral VPA as a neuroprotective agent in patients with advanced to almost end-stage glaucoma.

Methods

Advanced to end-stage glaucoma patients were recruited from the glaucoma services of our tertiary care center. Advanced glaucoma was defined as: A visual field limited <10° radius with size III stimulus on Humphrey visual-field analyzer and having a mean deviation worse than-24 dB. Clearance from our Institutional Ethics Committee was obtained. Informed consent was obtained from all patients, and the study was carried out as per the tenets of the Declaration of Helsinki.

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Patients with advanced glaucoma were randomized into intervention and control group using computer generated software (Sealed Envelope Ltd. 2016) for block randomization. Block randomization was used to ensure equal participants in each group. Inclusion criteria: Patients above the age of 30 years with advanced stage glaucoma in at least one eye and well-controlled IOP (<16 mmHg) in that eye with a Snellen visual acuity of at least 6/36. If both eyes of the patient were eligible the eye with the better visual field was included in the study. We excluded patients with any other disease that might have contributed to visual field loss at the time of initial visual-field test such as other optic neuropathies, stroke, brain tumors, retinal vein or artery occlusion, macular disease, cataract and proliferative diabetic retinopathy, patients allergic to VPA or to peanut oil, patients with deranged liver function test, hepatitis, alcoholic liver disease, and women likely to conceive.

Patients in the intervention group were given oral VPA 500 mg once a day for 3 months and followed up for 1 year. Controls not receiving VPA were followed up, to evaluate the change in visual function over 1 year and compared with patients who received VPA. Both the groups received routine IOP lowering therapy. Outcomes were monitored regarding Snellen Visual Acuity (converted to LogMAR for analysis), 10-2 SITA standard visual field and multifocal electroretinogram (ERG) (as per the guidelines of International Society of Clinical Electrophysiology of Vision using Monopack 3). We chose Multifocal ERG over pattern ERG to detect multiple local retinal responses as the latter does not provide the measure of pathological changes in localized retinal areas.[19-21] Assessment of these parameters was performed at baseline before starting therapy, at 3 months and 1 year of starting therapy (9 months of cessation of VPA, to look for a legacy effect of the drug). Liver function tests were measured before starting VPA and at 3rd month. Total plasma VPA concentrations were assessed at 3 months after completion of therapy using the liquid chromatographic technique coupled with tandem mass spectrometry (Q-Trap 4000, AB sciex).

Statistical analysis

The baseline characteristics between the groups were compared using independent *t*-test, Chi-square, or Mann–Whitney U test. For comparison of median visual acuity over time, we used the Wilcoxon Signed-rank test. $P < 0.05$ was considered statistically significant. The SPSS software (version 11.5, Chicago IL, USA) was used for statistical analysis.

Results

In this study, 68 patients with advanced stage glaucoma were recruited and randomized into Group 1 (receiving oral VPA) and Group 2 (controls). After attrition, 64 patients were included for analysis, 31 patients in VPA group, and 33 patients in control group who completed the 12 months follow-up. The baseline characteristics of the two groups are compared in Table 1.

Visual acuity

The median log of the minimum angle of resolution (logMAR) best-corrected visual acuity (BCVA) in the VPA group was 0.30 (range 0–0.78) at baseline, improving to 0.18 at 3 months ($P < 0.001$), and persisting at 12 months ($P = 0.0082$) with 0.18 (range 0–0.78) (Wilcoxon-signed rank test). On the other hand, the median logMAR visual acuity in control group was 0.18 (range 0–0.78) at baseline, 0.18 (at 3 months [$P = 0.56$] and 0.18 [range: 0–0.78] at 12 months [$P = 0.17$]). The improvement in VPA group was significant compared to the control group at 3 and 12 months ($P < 0.01$), Fig. 1. An improvement in one line (Snellen's visual acuity) was experienced in 11 out of 31 eyes in the VPA group compared to 1 out of 33 eyes among controls ($P = 0.003$) at 3 months. There was no patient who showed more than 1 line improvement. None of the eyes in the VPA had a drop in Snellen's acuity at 3 month while one eye in the control group had one line in drop in visual acuity ($P > 0.99$, Fisher's exact test).

Within the VPA group, those who showed a one-line gain in Snellen's visual acuity were compared with those who did not show a change. Table 2 shows the characteristics of patients who improved were not different from those who did not.

Visual field

In the VPA group, the mean deviation was −28.29 ± 3.12 at baseline, −28.308 ± 2.93 at 3 months ($P = 0.97$), and − 28.756 ± 28 at 12 months ($P = 0.087$). Among the controls, the visual field mean deviation was −26.96 ± 2.06 at baseline, −26.99 ± 2.08 at 3 months ($P = 0.86$), and −27.16 ± 2.73 at 12 months ($P = 0.52$). There was no statistically significant change noted in either groups or a difference seen between the groups.

Multifocal electroretinogram

We analyzed P1N2 amplitude and implicit time in five rings [Fig. 2a and b]. Ring 1 representing <2° field, ring 2 representing 2°–5° field, ring 3 representing 5°–10° field, ring

Table 1: Comparison of baseline characteristics between the two groups

	Valproic acid group (*n*=31)	Controls (*n*=33)	*P*
Age (years)	53.03±18.28	54.44±13.41	0.756[a]
Male:female	28:3	29:4	0.703[b]
Baseline visual acuity (logMAR)			
Median	0.3	0.18	0.21[c]
Range	0.78	0.78	
Baseline IOP (mmHg)	13.92±2.07	13.76±2.10	0.782[a]
Baseline visual fields (MD in db)	−28.29±3.12	−27.00±2.05	0.089[a]

Statistical test used: [a]Independent *t*-test, [b]Chi-square test, [c]Mann–Whitney test. IOP: Intraocular pressure, LogMAR: Logarithm of the minimum angle of resolution, MD: Mean deviation

Table 2: Comparison of those who showed objective improvement versus those who did not (in valproic acid group)

Objective improvement	Improvement (*n*=11)	No improvement (*n*=20)	*P*
Age (years)	58.3±13	48.07±20	0.14
Sex (male:female)	10:1	18:2	0.63
IOP (mmHg)	14.17±1.99	13.57±2.51	0.51
VF (MD in dB)	−28.58±3.31	−27.86±3.01	0.56

IOP: Intraocular pressure, MD: Mean deviation, VF: Visual field

4 representing 10°–15° field, and ring 5 representing >15° field. We did not find any significant difference in the multifocal ERG implicit time or amplitude between the two groups at any of the follow-up period.

Blood valproic acid concentration and adverse effects

The average blood VPA concentration measured at 3 months of therapy was 26 ± 8.9 μg/ml (range 8–55 μg/ml) [Fig. 3]. There was no correlation between the blood VPA concentration and the change in logMAR visual acuity seen among our patients (P = 0.12). No adverse effects that needed discontinuation of the drug were reported by any patient. Three patients had transient elevation of serum glutamic oxaloacetic transaminase/serum glutamate pyruvate transaminase enzymes that subsided after stopping therapy.

Discussion

Almost one-third of our glaucoma patients on VPA had improvement of 1 line of Snellen visual acuity. While in advanced stages of glaucoma, there is fluctuation of patients' vision not only during the day but also from one day to the other, one may not consider these results as substantial. At least, a 2 line visual acuity improvement is generally considered for a meaningful improvement in such cases of advanced glaucomas. However, the fact that this improvement did not happen in the control group gives credence to our observations that VPA might be associated with improvement in visual function in a subset of advanced glaucomas. Most studies except for a few on VPA in RP showed greater beneficial effect on visual field and less on visual acuity.[9-10,12,22,23] We did not perform a subjective assessment, but most patients felt their visual function to be subjectively better when on VPA therapy. This phenomenon has been explained in that, after degeneration has started, ganglion cells exhibit hyperactivity, firing spontaneously at rates many times greater than normal, and this hyperactivity is reduced by the GABAergic action of VPA; hence, patients feel that it was easier to see with reduced visual "noise" from spontaneous firing of ganglion cells.[10,24]

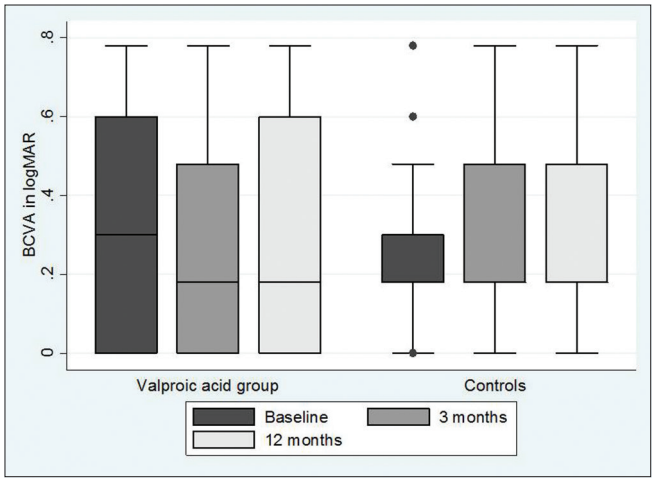

Figure 1: Log of the minimum angle of resolution best-corrected visual acuity over time in valproic acid group and controls

We found the improvement in visual acuity persisted even 9 months after stopping VPA therapy (legacy effect). In contrast in RP patients, both the visual acuity and visual field improvement were seen to reverse following discontinuation of therapy.[10]

We failed to show any improvement in visual field or ERG, however, probably because most eyes in our study had very advanced depression of visual field and we only analyzed the overall mean deviation. Similarly, for ERG, we failed to demonstrate a significant overall improvement because of end-stage loss. Probably, the beneficial effects could be more pronounced in early to moderate stages of glaucoma where VPA could be tried as an adjunct to IOP lowering therapy.

VPA has been shown to have neuroprotective effect in experimental studies. Lasseck *et al*. proposed that VPA (300 mg/kg) improves the retinal ganglion cell (RGC) survival in rats with optic nerve crush injury compared to controls.[14] The drug acts by decreasing the caspase 3 activity, induction of CREB, and activation of pERK1/2. Bierman *et al*. cultured purified RGCs in histone deacetylase (HDAC) inhibitors such as sodium butyrate, VPA, trichostatin A, and concluded that sodium butyrate and VPA increases RGC survival in culture.[25] They also added that mechanism other than histone deacetylation is responsible for increased RGC survival. It has been shown that HDAC activity is induced by

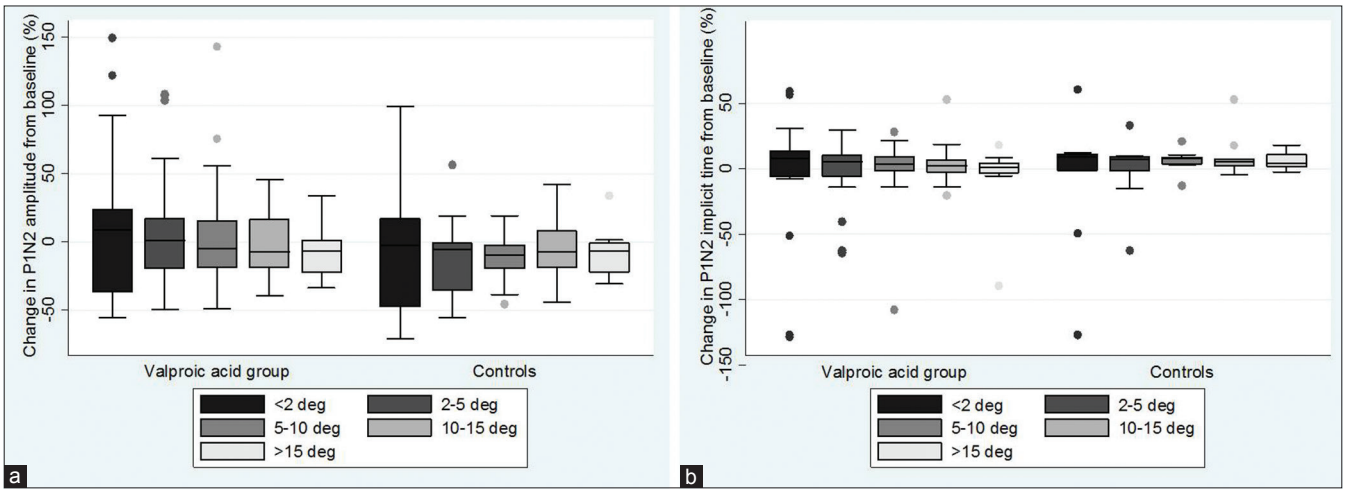

Figure 2: Percentage multifocal electroretinogram change in P1N2 amplitude (a) and implicit time (b) among valproic acid group and controls

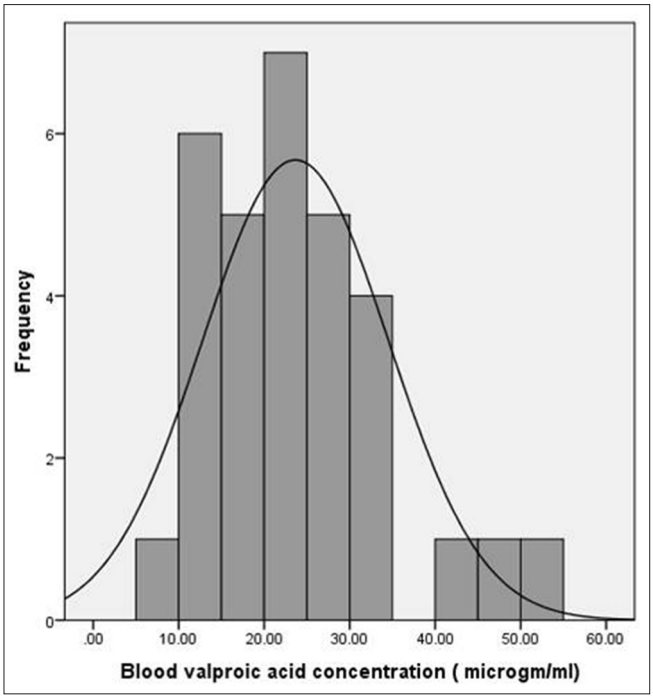

Figure 3: Histogram showing the blood valproic acid concentration measured 3 months after therapy

raised IOP that is detrimental to RGC and by inhibiting HDAC, VPA can be neuroprotective.[26]

In our study, we selected eyes with advanced to end-stage glaucoma that had a mean deviation worse than −24 dB. By the definition of Mills *et al*. for glaucoma staging, these would actually be severe glaucomas bordering on end-stage glaucoma.[27] We used this selection criterion, as these patients are the ideal group in need of neuroprotective therapy for improving their visual function. However, the neuroprotective effect may be more significant in the moderate stage of the disease rather than end-stage when there is almost complete loss of ganglions; hence, the need for a trial in less advanced stages of glaucoma.

In our study, the blood concentration of VPA in patients was found much lower than when it is used as an antiepileptic.[28] In fact, the average concentration in the blood achieved in studies using the same oral dose for RP was also higher compared to ours.[10] We could possibly explain this by ethnic differences in the pharmacokinetics of the drug or possibly poor compliance among our patients. Iraha *et al*. also did not find a correlation to the blood VPA concentration and BCVA change or change in the visual field noted in their patients with RP.[10] None of the patients in our study on VPA therapy experienced any adverse effects. While color vision, abnormalities are reported with VPA for epilepsy adverse effects on other visual functions have not been found.[29,30] In a study on visual side effects of VPA therapy among patients on antiepileptic therapy, Ozkul *et al*. found no adverse effect on visual acuity, visual field or ERG.[29] The patients in the study of Ozkul *et al*. were on a much higher dose of VPA for epilepsy and the aim was not to look for beneficial effects of VPA on visual function but to look for adverse effects.

One of the limitations of our pilot study was that being nonmasked; there could have been observer bias related to assessing visual outcomes among patients treated with VPA. We would thus recommend an observer masked randomized study for the future. In addition, patients taking VPA may have felt better with VPA having a placebo effect or even as a mood elevator thus giving a better subjective response while visual acuity assessment was performed. A placebo-controlled trial would further reduce this bias. We would also recommend a larger study with longer follow-up duration.

Conclusion

We can say that VPA therapy can be beneficial for improving visual function in a subset of advanced glaucoma patients. Even if this improvement is considered minimal, it cannot be ignored. More studies, especially in less severe stages of glaucoma, would help in further confirmation of the findings of our study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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