Testing Toxicity of Intravitreal Bevacizumab (Avastin) used for the Treatment of Choroidal Neovascularization Associated with Age-Related Macular Degeneration: A Full Field ERG Study

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Abstract

***Purpose*:** To evaluate the toxic retinal effects of intravitreal bevacizumab used for the treatment of exudative age-related macular degeneration (AMD) using Ganzfeld Electroretinography (G-ERG)

***Methods*:** In this prospective comparative interventional study, 23 patients with active choroidal neovascularization (CNV) associated with AMD were enrolled. Patients were received intravitreal injections of either 2.5 (12 patients) or 1.25 mg (11 patients) of intravitreal bevacizumab. Patients underwent complete ophthalmic examination including visual acuity testing, and G-ERG, at baseline, at one week, at one month, and at three months after intravitreal bevacizumab.

***Results*:** Best corrected visual acuity (BCVA) significantly increased from 1.34±0.59 (logMAR) in preinjection examination to 1.07±0.47 (logMAR) at one month (P=0.01), and 1.03±0.46 (logMAR) at three months (P=0.001). G-ERG did not show any significant change in the waveform parameters following intravitreal injection of bevacizumab. No significant difference was found between the two groups in the amount of change in visual acuity and G-ERG recordings of postinjection measurements.

***Conclusion*:** Intravitreal bevacizumab did not appear toxic to the retina based on G-ERG recordings.

***Keywords*:** Bevacizumab, Age-related Macular Degeneration, Choroidal Neovascularization, Electroretinography

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Introduction

Vascular endothelial growth factor (VEGF) plays a documented role in the formation of abnormal blood vessels. Some studies shown that development of choroidal neovascularization (CNV) as a result of age-related macular degeneration (AMD)[1] can be treated effectively by inhibition of VEGF by different agents.[2-4] Bevacizumab (Avastin, Genentech, Inc, South San Francisco, California, USA) is a humanized monoclonal antibody that inhibits all isoforms of VEGF, mainly designed and approved for intravenous administration in the treatment of metastatic colorectal cancer.[5] Recently, several clinical series using intravitreal bevacizumab for the treatment of CNV secondary to AMD have been reported.[6-9] These studies reported promising results demonstrating patients with significant improvement in visual acuity, and reduction in retinal thickness. Nevertheless, off-label treatment implicates the important disadvantage of missing safety studies and limited surveillance data.[10]

In recent years, limited reports have evaluated the possible toxicity of intravitreal bevacizumab. In animal studies using Electroretinography (ERG) testing, intravitreal bevacizumab did not appear toxic to the retina.[11-14] However, human ERG studies investigating the toxicity of intravitreal bevacizumab are less reported. In a short-term study, Maturi et al[15] reported relatively safe Ganzfeld-ERG (G-ERG) responses in five patients who were received 1.25 mg intravitreal bevacizumab for AMD. In another study, Ziemssen et al[16] reported no significant changes in the scotopic or photopic responses after intravitreal injection of 1.25 mg bevacizumab in 10 eyes with AMD.

The G-ERG response is a reflection of the sum total of the retinal electrical response measured at the corneal surface.[15] A global worsening of the G-ERG response may indicate retinal toxicity. Thus, G-ERG testing can provide important clues to the presence of diffuse retinal toxicity with any intervention.

To determine the possible retinal toxicity of intravitreal bevacizumab in humans, we evaluated the electrophysiologic changes in G-ERG recordings of subjects with CNV associated with AMD who were treated with 1.25 or 2.5 mg of intravitreal bevacizumab.

Methods

This study included patients with active CNV secondary to AMD. All angiographic subtypes were included. Exclusion criteria were the presence of ocular diseases other than AMD, which might affect visual acuity or G-ERG measurements such as hereditary retinal dystrophy, retinal vasculitis, diabetic retinopathy, recent or old retinal vein occlusion involving the fovea, presence of vitreous hemorrhage or significant cataract precluding retinal examination. Patients with previous intraocular surgery except uncomplicated cataract surgery performed more than three months before, previous treatment for CNV, history of external beam radiation to the skull region and inability to comply with the study protocol were also excluded. The study protocol was approved by the institutional review board of Rassoul-Akram hospital, and inform consent was obtained.

All patients at presentation underwent a complete ophthalmic examination, including measurement of best corrected visual acuity (BCVA) and intraocular pressure (IOP), fundus examination, and G-ERG. This examination protocol was repeated at one week, one month, and three months after the injection.

Patients were randomly assigned to receive intravitreal injections of 1.25 or 2.5 mg bevacizumab (0.05 or 0.1 ml of commercially available Avastin solution). The injections were performed according to the previous described protocol.[7]

The G-ERG recording procedures adhered to a recommended international standard for clinical electrophysiological measurements.[17] Pupils were fully dilated with 1% tropicamide and 2.5% phenylephrine. Silver/nylon fiber electrodes (DTL, Laird technologies, Sauquoit inc. Scranton, USA) were placed over the middle third of the lower eyelid of each eye. Stimulation was performed using a full field flash Ganzfeld stimulator (Roland consult, Electrophysiologic diagnostic systems, Wiesbaden, Germany). Dark-adapted ERG responses after a minimum of 30 minutes for dark adaptation including an isolated rod, standard flash (maximal) response, oscillatory potentials, and light adapted responses including a single white flash and 30 Hz flicker were recorded. Flash ERGs

(1–300 Hz), and 30 Hz flicker ERGs (1–300 Hz) were acquired in response to flashes of photopic luminance of 3.5 cd/m2 against a background light of 25 cd/m2. No color filter was used for scotopic and photopic recordings.

Data were entered using SPSS software (version 11.5, SPSS, Inc.). The mean of each parameter was compared between pre and postinjections. Analyses were performed using T-test, ANOVA test, Wilcoxon test and Chi square test. A P-value less than 0.05 was considered statistically significant.

Results

Twenty-three eyes of 23 consecutive patients (18 men and 5 women) with a mean age of 72±6.7 years were enrolled. Baseline BCVA was 1.34±0.59 (logMAR) which was increased to 1.1±0.44 (logMAR) at one week (P=0.1), 1.07±0.47 (logMAR) at one month (P=0.01), and 1.03±0.46 (logMAR) at three months (P=0.001, Figure 1).

There was no statistically significant difference between baseline and postinjection photopic and scotopic G-ERG measurements (Table 1).

Table 2 shows the patient characteristics in 1.25 and 2.5 mg groups. Age, sex, baseline BCVA and baseline G-ERG parameters were not statistically different between the two groups. There was no statistically significant difference between the two groups in the changes of BCVA in the postinjection examinations (Table 2). No significant difference was found between the two groups in G-ERG recordings of postinjection measurements. No serious ocular or systemic effects were found in the subjects.

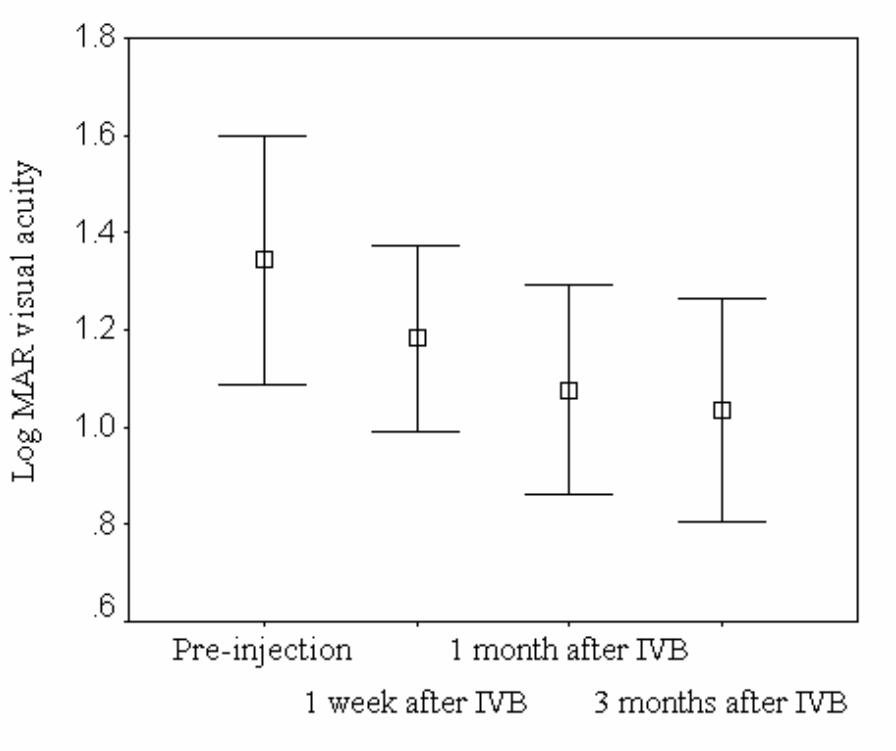

Figure 1. Best corrected visually acuity (BCVA) of 23 patients before and after intravitreal injection of bevacizumab for choroidal neovascularization (CNV)

Table 1. Photopic and scotopic electroretinographic parameters before and after intravitreal injection of bevacizumab

Parameter		Preinjection	One week after injection	One month after injection	Three months after injection
Scotopic 'b' wave implicit time (ms)	Total eyes	85.3±10.2	83.9±11.7 (P=0.5)	82.7±10.5 (P=0.3)	82.8±13.4 (P=0.5)
	1.25 mg group	82.9±11.3	81.3±13.4 (P=0.4)	79.5±11.8 (P=0.3)	83.3±9.3 (P=0.5)
	2.5 mg group	88.2±8.5	86.3±9.6 (P=0.4)	86.2±8.0 (P=0.5)	82.4±17.2 (P=0.3)
Scotopic 'b' wave amplitude (µV)	Total eyes	54.5±32.5	63.1±35.8 (P=0.5)	45.1±31.9 (P=0.5)	60.6±33.4 (P=0.6)
	1.25 mg group	60.67±30.5	66.8±30.8 (P=0.8)	42.7±30.2 (P=0.4)	66.1±29.1 (P=0.8)
	2.5 mg group	48.9±28.6	59.7±25.8 (P=0.4)	47.7±22.1 (P=0.8)	55.1±22.9 (P=0.4)
Combined maximal 'a' wave implicit time (ms)	Total eyes	23.5±2.1	24.6±2.1 (P=0.1)	23.5±1.5 (P=0.5)	98.7±38.7 (P=0.4)
	1.25 mg group	23.5±1.4	24.8±2.1 (P=0.07)	23.2±1.9 (P=0.2)	23.8±2.8 (P=0.1)
	2.5 mg group	23.5±3.0	24.5±2.5 (P=0.2)	23.8±1.4 (P=0.9)	23.5±3.5 (P=0.8)
Combined maximal 'a' wave amplitude (µV)	Total eyes	105.7±44.1	94.0±55.3 (P= 0.3)	101.5±41.0 (P=0.8)	98.7±38.7 (P=0.4)
	1.25 mg group	96.6±56.6	91.2±60.2 (P=0.7)	98.7±47.5 (P=0.8)	97.7±46.1 (P=0.8)
	2.5 mg group	114.3±33.7	96.5±52.1 (P=0.3)	104.5±34.6 (P=0.6)	99.6±32.7 (P=0.5)
Combined maximal 'b' wave implicit time (ms)	Total eyes	47.5±3.9	56.7±17.3 (P= 0.5)	47.1±3.5 (P=0.8)	46.8±2.1 (P=0.7)
	1.25 mg group	46.6±4.6	57.6±16.6 (P=0.1)	48.1±3.2 (P=0.4)	46.5±2.5 (P=0.3)
	2.5 mg group	48.3±3.2	55.9±18.6 (P=0.1)	46.1±3.8 (P=0.08)	47.2±1.8 (P=0.1)
Combined maximal 'b' wave amplitude (µV)	Total eyes	205.9±74.2	202.1± 98.0 (P=0.7)	214.2±84.6 (P=0.4)	223.0± 74.3 (P=0.3)
	1.25 mg group	207.7±93.7	204.2±116.6 (P=0.9)	211.5±87.4 (P=0.7)	229.7±82.4 (P=0.3)
	2.5 mg group	211.8±75.8	200.3±82.7 (P=0.6)	219.1±85.9 (P=0.4)	216.3±69.7 (P=0.6)
Photopic 'b' wave implicit time (ms)	Total eyes	33.3±2.4	34.5±1.7 (P=0.051)	37.2±1.9 (P=0.2)	32.5±1.1 (P=0.6)
	1.25 mg group	32.7±3.0	34.3±1.9 (P=0.1)	35.0±2.1 (P=0.052)	34.3±2.3 (P=0.1)
	2.5 mg group	34.0±1.4	40.4±2.1 (P=0.1)	34.0±1.2 (P=0.7)	30.6±6.4 (P=0.1)
Photopic 'b' wave amplitude (µV)	Total eyes	79.8±27.8	79.6±35.9 (P=0.4)	84.4±33.4 (P=0.5)	81.5±28.3 (P=0.7)
	1.25 mg group	74.9±26.8	73.7±39.3 (P=0.9)	79.8±25.8 (P=0.5)	82.2±34.0 (P=0.4)
	2.5 mg group	89.9±31.6	85.0±33.3 (P=0.4)	89.6±41.0 (P=0.6)	80.9±23.3 (P=0.8)
30 Hz flicker 'b' wave implicit time (ms)	Total eyes	32.3±3.6	32.7±2.1 (P=0.5)	32.7±3.1 (P=0.5)	31.9±1.9 (P=0.2)
	1.25 mg group	33.3±4.7	33.8±5.8 (P=0.8)	33.4±4.6 (P=0.8)	31.1±2.9 (P=0.2)
	2.5 mg group	31.9±2.3	32.3±1.7 (P=0.2)	32.1±1.6 (P=0.4)	32.4±1.5 (P=0.3)

ms: Millisecond, µV: Microvolt

Table 2. Patient demographics in 1.25 mg and 2.5 mg groups

Parameter	1.25 mg group	2.5 mg group	P-value
Age	71.4±8.9	72.5±4.2	0.1 †
Sex (Male/Female)	8/3	10/2	0.6 ‡
Preinjection BCVA (logMAR)	1.3±0.76	1.3±0.4	0.9 †
One week postinjection BCVA (logMAR)	1±0.56	1.2±0.28	0.2 †
One month postinjection BCVA (logMAR)	0.93±0.52	1.2±0.22	0.1 †
Three months postinjection BCVA (logMAR)	0.96±0.57	1.1±0.33	0.5 †

BCVA: Best corrected visual acuity, †: T-test, ‡: Chi square test

Discussion

This study showed that based on ERG results intravitreal, injection of bevacizumab in commonly used doses does not appear to be toxic to the retina. The end point ERG of the study eyes did not have a significant change.

Toxicity of bevacizumab has been tested in some animal studies. Lueke and coworkers[18] looked for increasing drug concentrations using a model of bovine retinas. They showed no effects of the bevacizumab at the concentration used in clinical routine. In solutions with more than 2.5 mg/ml bevacizumab, the solvent seemed to affect retinal function. Shahar et al[12] found bevacizumab to be nontoxic to the retina based on dark-adapted ERG and Visual evoked potential (VEP) response in 10 healthy rabbits up to four weeks after injection. Manzano et al[11] and Feiner et al[14] found no histologic and electrophysiologic alteration in rabbits, up to a concentration of 2.5 mg bevacizumab.

Few human studies reported the electrophysiologic effect of intravitreal bevacizumab.[15,16,19,20] Most of these studies focused on multifocal electroretinography (mf-ERG) to show the therapeutic effect of injections. However, some small studies reported on the G-ERG effects.[15,16] Maturi et al[15] evaluated nine patients with AMD who received 1.25 mg intravitreal bevacizumab for exudative AMD using mf-ERG or G-ERG. One month after injections, all five subjects who underwent G-ERG testing had no significant changes in electrophysiologic response, although some variation in amplitude and implicit time was noted at different testing times. Ziemssen and coworkers,[16] examined 10 patients with AMD by means of Electro-Oculography (EOG), ERG and color vision testing at baseline and four months following intravitreal injection of 1.25 mg bevacizumab. They reported no sign of clinically relevant retinal toxicity following the injections. Similarly, Shetty et al[20] evaluated the changes in mf-ERG and G-ERG, at one week and two months after intravitreal injection of bevacizumab for the treatment of macular edema secondary to retinal vein occlusion and diabetic retinopathy. They reported no change in waveform parameters following intravitreal injection of bevacizumab. Our study supports the results of previous studies, reporting no toxicity following intravitreal injection of bevacizumab.

We found some abnormalities in the baseline G-ERG recordings, which were repeated in each follow up recording, for example the b-wave amplitude was decreased in scotopic recordings. These changes may be attributed to the AMD-related abnormalities in the electroretinogram. It has been reported that the patients with various stages of AMD may have a global reduction of retinal function in flash ERG.[21] On the other hand, it may represent the normal ERG recordings specific

for our settings. Nevertheless, we compared pre and postinjection G-ERG recordings of the same patients, in the same settings and using the same methods, so, the results are still valid.

Despite its widespread use, the exact dose of bevacizumab for intravitreal injection providing the highest efficacy with relative safety is not determined. Its intravitreal injection for the treatment of CNV associated with AMD was first reported by Rosenfeld et al[6] whereby a 1.00 mg dose was used. These authors selected this dose based on phase III trials with ranibizumab used monthly intravitreal injection of 0.3 to 0.5 mg. Considering the fact that ranibizumab has a molecular weight approximately one third of bevacizumab, the dose of bevacizumab containing the same number of molecules would be 0.9 to 1.5 mg. They considered several other factors including the presence of two antigen binding site on bevacizumab molecule compared to a single site on ranibizumab molecule and that ranibizumab has been genetically engineered to increase its affinity for VEGF. Eventually they estimated that a dose of 1 to 1.25 mg seems to be a dose around which a dose response study can be designed. Based on this, most other clinicians have used 1.25 mg intravitreal injection for treatment of CNV associated with AMD.[7-9,22] Later some investigators used higher doses in the quest for higher efficacy.[23-25] To the best of our knowledge, this study is the only study which has compared safety of 1.25 mg and 2.5 mg doses using electrophysiologic recordings. In this study, the efficacy of 1.25 mg and 2.5 mg intravitreal injection of bevacizumab were the same regarding the visual acuity results. In general, based on our findings, it seems that 2.5 mg intravitreal bevacizumab has the same safety and efficacy as lower doses.

Conclusion

In the context with other studies where the effect of bevacizumab on the ERG had been tested in vitro and in vivo,[11-16] intravitreal injection of bevacizumab seems to be safe with regard to retinal function. Our study is the largest series of G-ERG testing ever reported, however, the sample size is still small, and the follow up duration is short to draw a definitive conclusion. In addition, the ultrastructural effects of bevacizumab on different retinal layers need to be further evaluated.

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