TESTING INTRAVITREAL TOXICITY OF BEVACIZUMAB (AVASTIN)

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Purpose: To evaluate the retinal toxicity of varying doses of bevacizumab when injected intravitreally in rabbits. Bevacizumab has been approved by the US Food and Drug Administration for the treatment of metastatic colorectal cancer.

Materials and Methods: Twelve New Zealand albino rabbits were used for this study and divided into four groups. Four concentrations of bevacizumab were prepared: 500 μg/0.1 mL, 1.0 mg/0.1 mL, 2.5 mg/0.1 mL, and 5.0 mg/0.2 mL. Each concentration was injected intravitreally in one eye of each of three rabbits; 0.1 mL volume of sterile balanced saline solution was injected into the contralateral eyes. Slit-lamp and funduscopic examinations were performed and the animals were observed for 2 weeks for signs of infection, inflammation, or toxicity. A baseline electroretinogram (ERG) was performed before the drug treatment and at day 14 before the animals were killed. The enucleated eyes were prepared for histologic evaluation of retinal toxicity.

Results: Histologic and ERG results in all groups showed no retinal toxicity. However, some inflammatory cells were found in the vitreous at the 5–mg dose.

Conclusions: Intravitreal bevacizumab did not appear toxic to the retina in albino rabbits at a concentration of 2.5 mg. Intravitreally injected bevacizumab should be evaluated for efficacy in choroidal neovascularization and macular edema.

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Angiogenesis is a highly complex, dynamic process regulated by a number of pro and antiangiogenic molecules. The vascular endothelial growth factor (VEGF) and its receptors play a vital role in normal and pathologic angiogenesis.[1] Activation of the VEGF-receptor pathway triggers a network of signaling processes that promote endothelial cell growth, migration, and survival from preexisting vasculature, differentiation, and mobilization of endothelial progenitor cells from the bone marrow into the peripheral circulation.[2–4] In addition, VEGF increases vessel permeability leading to deposition of proteins in the interstitium that facilitate the process of angiogenesis.[5]

There are many ocular diseases in which angiogenesis plays a major role; for example, proliferative diabetic retinopathy, age-related macular degeneration (ARMD), and retinopathy of prematurity. VEGF has been identified in neovascular membranes in both diabetic retinopathy and ARMD, and intraocular levels of the factor correlate with the severity of neovascularization in diabetic retinopathy.[6,7] Therapeutic antagonism of VEGF in animal models results in significant inhibition of both retinal and choroidal neovascularization (CNV) as well as a reduction in vascular permeability.[8,9]

One possible strategy for treating retinal neovascularization, CNV, and macular edema is to inhibit VEGF activity by competitively binding VEGF with a specific neutralizing anti-VEGF antibody.

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None of the authors has any proprietary interest in any technique or product described herein.

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Bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA) is a full-length humanized murine monoclonal antibody against the VEGF molecule. The amino acid sequence of this monoclonal antibody is 93% of human origin and 7% murine.[10] Bevacizumab is approved by the Food and Drug Administration for the treatment of metastatic colorectal cancer and is in Phase III trials for advanced breast cancer and advanced renal cancer.[11,12]

VEGF selectively stimulates endothelial cells by binding to two receptors, VEGFR-1 and VEGFR-2, which respond in typical fashion to ligand binding by activation of signal transduction cascades.[4] Bevacizumab can theoretically inhibit the activity of both receptors.[13]

In a recent study, patients with neovascular ARMD were treated systemically with bevacizumab; nine patients received either two or three infusions of bevacizumab at a dose of 5 mg/kg. Systemic bevacizumab was associated with a significant increase in visual acuity and decrease in central retinal thickness 1 week after therapy. These preliminary results are promising.[14] In addition to ocular side effects, there were some systemic disadvantages associated with systemic administration of bevacizumab; the most significant disadvantage was the possibility of life-threatening adverse events. There was an increased risk of potentially fatal thromboembolic events in patients with advanced metastatic colorectal cancer receiving concomitant chemotherapy and bevacizumab when compared to patients receiving chemotherapy alone. Other potential systemic side effects included hypertension, epistaxis, hemoptysis, proteinuria, wound healing complications, and gastrointestinal hemorrhage.[12,15–17]

The intravitreal administration of bevacizumab was evaluated in an effort to find a drug that could possibly decrease these adverse side effects. However, retinal toxicity is a primary concern when using intravitreal injections. The purpose of our study was to analyze the retinal toxicity of bevacizumab at various doses.

Materials and Methods

Twelve New Zealand albino rabbits weighing between 2 and 3 kg were used for this study. The animals were treated according to the Association for Research in Vision and Ophthalmology guidelines. Slit-lamp and indirect funduscopic examinations were performed on all eyes before the study began and on days 1, 7, and 14 after intravitreal injection. Any animals demonstrating corneal or lens opacity or retinal damage before the study were excluded. The animals were anesthetized before all procedures using approximately 1 mL of a mixture of ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (5 mg/kg). Topical anesthesia was applied using proparacaine (0.5%). The eyes were dilated by topical application of phenylephrine (2.5%) and tropicamide (0.5%).

Intravitreal Injections

All procedures were performed under sterile conditions using an operating microscope for visualization. An anterior chamber tap was performed with a 27-gauge needle, withdrawing 0.1 mL of aqueous fluid to reduce intraocular pressure and to minimize drug reflux after injection. Intravitreal injection was performed using a 30-gauge needle attached to a tuberculin syringe inserted bevel up approximately 2 mm posterior to the limbus. Various concentrations of bevacizumab were prepared: 500 μg/0.1 mL, 1.0 mg/0.1 mL, 2.5 mg/0.1 mL, and 5.0 mg/0.2 mL. Each concentration was injected in one eye of each of three rabbits, and 0.1 mL volume of sterile balanced saline solution was injected into the contralateral eye of each rabbit. Slit-lamp and funduscopic examinations were

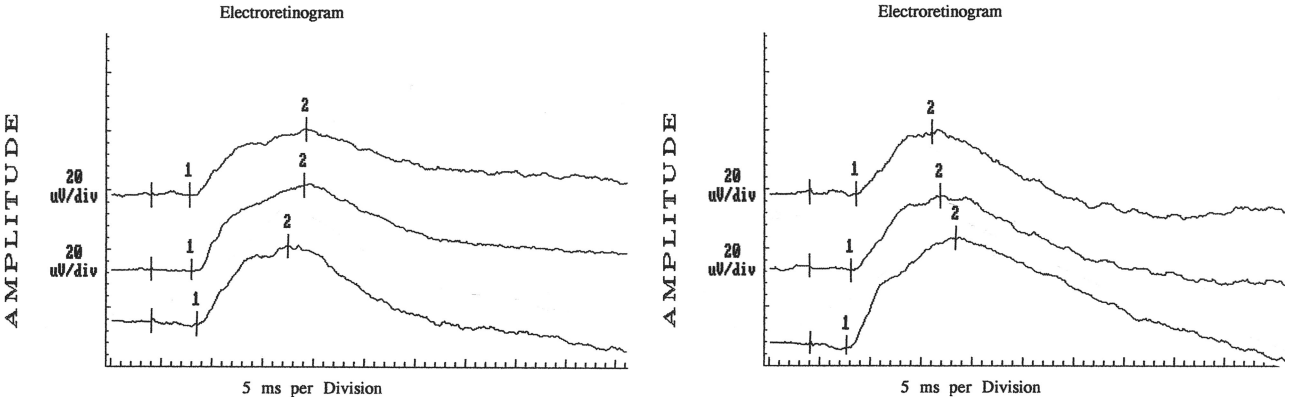

Fig. 1. Electroretinography step 1 of three eyes before (left) and after (right) injection of 500 μg of bevacizumab.

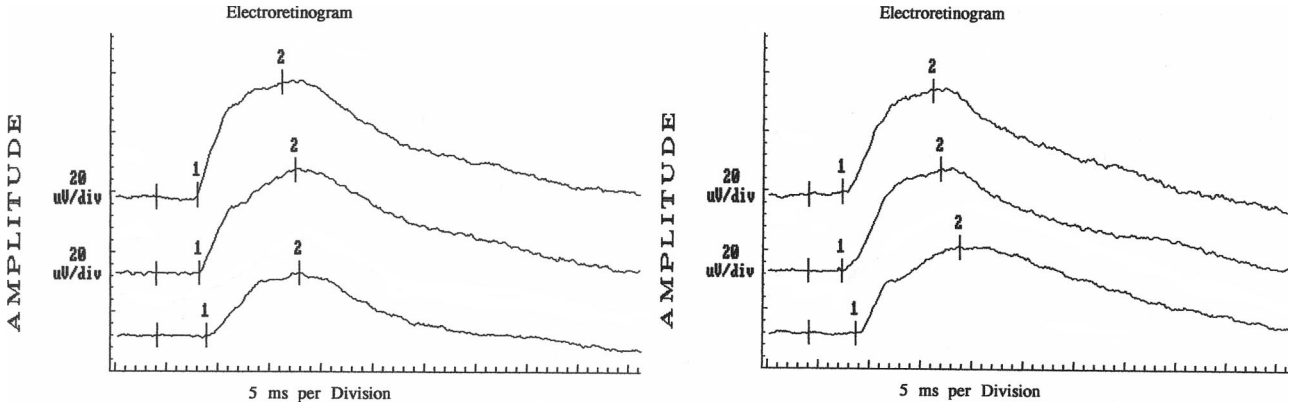

Fig. 2. Electroretinography step 1 of three eyes before (left) and after (right) injection of 1.0 mg of bevacizumab.

performed and the animals were observed for 2 weeks for signs of infection, inflammation, or toxicity.

Electrophysiologic Tests

Electroretinography (ERG) using the UTAS-2000 system (LKC Technologies, Gaithersburg, MD) was performed before the intravitreal injection and 14 days after injection. The rabbits were dark adapted for at least 30 minutes after pupillary dilation. Unipolar contact lenses (ERG jet electrodes) were put on both corneas with Goniosol (Ciba Vision, St. German, PR); the negative electrode was placed in the subcutaneous space of the forehead, and the ground electrode was clipped to the earlobe with some electric gel. The dark-adapted scotopic response (step 1, rod response), scotopic flash response (step 2, maximal response, cone and rod), and (after waiting for 3 minutes) light-adapted photopic response (step 4, cone response) were recorded. The average of five sweeps was determined for each step. The difference of a- and b-waves was calculated for each step. The baseline was compared to the response 2 weeks after injection. A decrease in the post injection response that was greater than 30% was considered significant.

Histologic Examination

All rabbits were killed with an intravenous injection of 100 mg/kg of sodium pentobarbital while under deep anesthesia. The eyes were enucleated and fixed in Karnovsky fixative for 48 hours and then processed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for light microscopy.

Results

Clinical Examination

No corneal opacity, cataract, vitreous hemorrhage, retinal detachment, or optic atrophy was observed in any of the eyes by funduscopic examination.

Electrophysiologic Test

ERG changes were considered significant if the follow-up differences in amplitude (a- and b-waves)

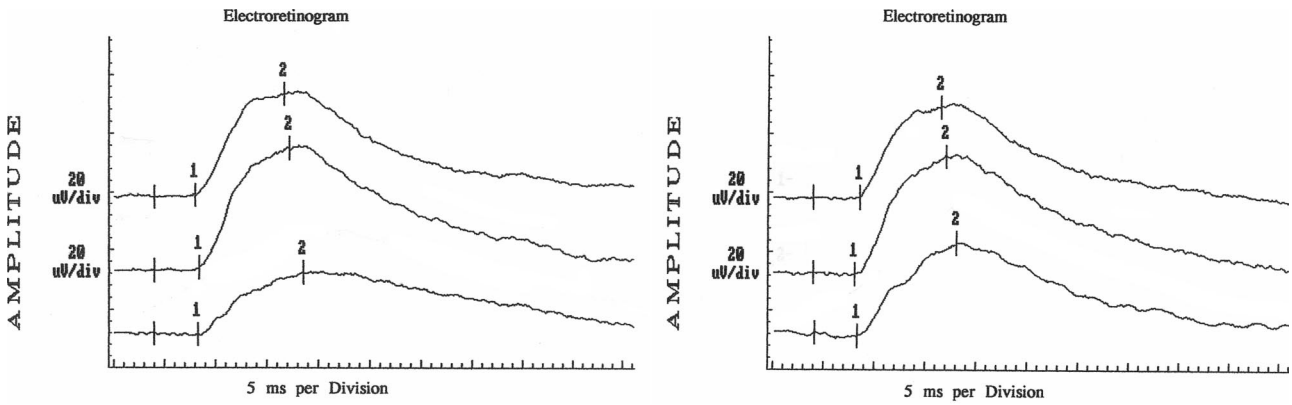

Fig. 3. Electroretinography step 1 of three eyes before (left) and after (right) injection of 2.5 mg of bevacizumab.

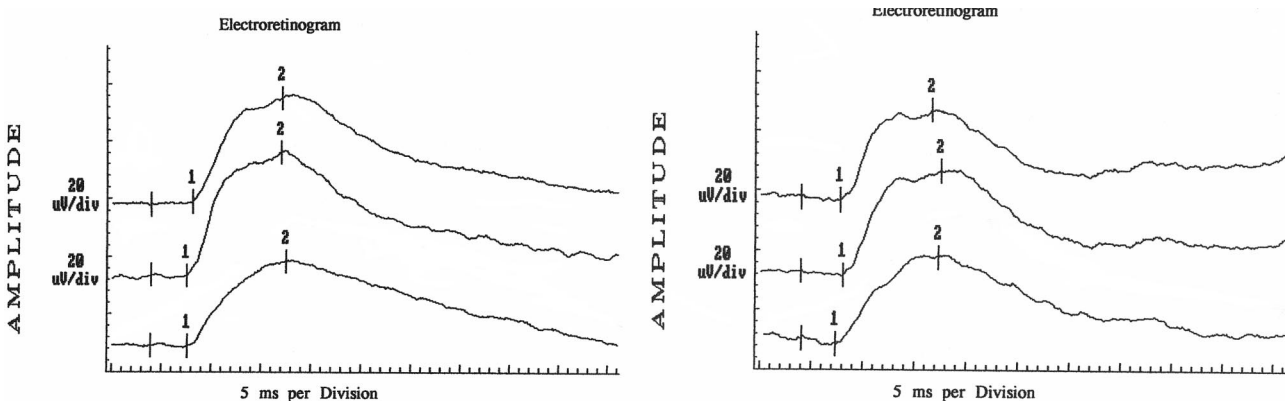

Fig. 4. Electroretinography step 1 of three eyes before (left) and after (right) injection of 5.0 mg of bevacizumab.

were decreased more than 30% from the preinjection values. This study did not show a significant decrease in amplitude in any group. Only one eye injected with the highest concentration (5 mg/ 0.2 mL) showed a decrease of 11% when compared to the baseline; one eye of the control group showed a decrease of 13% (Figs. 1–4, Tables 1–2).

Histologic Examination

Light microscopy was performed in all eyes. No retinal toxicity was found in any eyes. One eye in the 5–mg dose group had some inflammatory cells in the vitreous.

Discussion

Intravitreal injection is commonly used in ophthalmology for retinal and choroidal diseases and has been associated with a relatively low incidence of complications.[18] Triamcinolone acetonide is the most commonly used intravitreal drug to treat macular edema secondary to retinal vascular diseases. However, corticosteroids can lead to serious ocular complications such as cataract formation, elevation in intraocular pressure, and, potentially, retinal toxic effects.[19]

Pegaptanib sodium is another drug that has recently been used intravitreally to treat neovascular ARMD. Pegaptanib is an aptamer that targets the VEGF165 isoform. However, most patients treated with pegaptanib still lost some vision.[20] Ranibizumab (Lucentis), an antibody fragment of bevacizumab, is another inhibitor of VEGF designed specifically for ophthalmology and is currently in Phase III clinical trial for neovascular ARMD.[21]

Two recent case reports of intravitreal injection of bevacizumab have been published; the first was for exudative CNV resulting from ARMD and the other for macular edema caused by central vein occlusion.[22,23] In

Table 1. Difference in (a and b) amplitude (uV) of Step 1 at baseline, endpoint and the percentage of difference for all eyes.

Step 1	Baseline	Endpoint	Difference in %
500 μg	88.1	103.54	increase 14.91
	138.38	127.12	decrease 8.13
	138.35	195.17	increase 29.17
1 mg	106.51	147.43	increase 27.75
	189.81	171.29	decrease 9.75
	171.15	170.95	decrease 0.11
2.5 mg	170.98	160.94	decrease 5.87
	204.45	202.53	decrease 0.93
	103.63	161.92	increase 35.99
5.0 mg	180.82	160.26	decrease 11.37
	213.14	187.89	decrease 11.84
	149.52	150.05	increase 0.35
control	174.04	143.86	decrease 17.34
	90.76	104.21	increase 12.90
	102.06	106.29	increase 3.97

Table 2. Difference in (a and b) amplitude (uV) of Step 2 at baseline, endpoint and the percentage of difference for all eyes.

Step 1	Baseline	Endpoint	Difference in %
500 μg	170.1	195.35	increase 12.92
	202.17	178.15	decrease 11.88
	103.85	149.12	increase 30.35
1 mg	148.13	157.55	increase 5.97
	151.33	169.98	increase 10.97
	125.44	156.18	increase 19.68
2.5 mg	134.71	305.19	increase 55.86
	172.69	237.83	increase 27.38
	116.21	163.16	increase 28.77
5 mg	110.34	174.87	increase 36.90
	113.65	141.08	increase 19.44
	123.42	143.17	increase 13.79
control	192.68	149.01	decrease 22.66
	125.9	144.7	increase 12.99
	143.75	177.42	increase 18.97

both cases, a dramatic improvement was observed in the cross-sectional anatomy of the macula using optical coherence tomography (OCT). The improvement on OCT was maintained at the 4-week examination.

In our study, intravitreal bevacizumab did not show retinal toxicity even in the highest concentration although some inflammatory cells were found in the vitreous of one eye given the 5–mg dose. The end point ERG of almost all eyes of the study group did not have a significant decrease. However, this study does not rule out the possibility that longer-term follow-up in the albino rabbit or in human subjects might show untoward side effects.

Nevertheless, normal ERG results do not rule out functional and possible structural changes at the level of ganglion cells or the nerve fiber layer. The lack of changes by light microscopy does not exclude possible alterations on a submicroscopic level.

Bevacizumab at the concentrations studied did not appear to be toxic to the retina in albino rabbits. Doses (2.5 mg) of intravitreally injected bevacizumab higher than previously used may be beneficial for treatment of choroidal neovascularization and macular edema, but further studies are required to evaluate the long–term safety of this drug.

Key words: bevacizumab, choroidal neovascularization, intravitreal injection, macular edema, retinal toxicity, vascular endothelial growth factor.

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