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Research article

The Effect of Intravitreal Bevacizumab on Lateral Geniculate Body Electrical Activity in Rat's Eyes

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ABSTRACT

Objective: To evaluate the effects of intravitreal bevacizumab on Lateral Geniculate Body (LGB) electrical activity in rats eyes.

Methods: Twenty male Wister rats (280-320 g) were divided into two groups; the first group received 2 µl (0.05 mg) intravitreal bevacizumab and the second groupreceived2 µl intravitreal normal saline as the control group.The changes in the field electrical activity of LGB were comparatively assessed betweenthe two groups (pre-injection and post-injectionon the7th and 30th days post injection).

Results: The difference in electrical powers of LGB between the two groups was not statisticallysignificant ($p<0.05$).

Conclusion: Intravitreal bevacizumab injection in rat's eyes did not change the electrical activity of LGB.Further studies are needed to determine the possible changes in general brain activity.

Keywords: Bevacizumab, Intravitreal Bevacizumab, Rat, Lateral Geniculate Body

INTRODUCTION

Bevacizumab (Avastifl Geflentech Inc., San FranciscO, CA, USA) is acomplete, humanized monoclonal antibody directed against allisoforms of vascular endothelial growth factor (VEGF). Bevacizumabwas originally developed as a treatment for metastatic colorectalcancer (1);however, it has been successfully applied off-label for intravitreal treatment of VEGFmediated ocular diseases such as choroidalneovascular disorder, central retinal vein occlusion, proliferativediabetic retinopathy, and pseudophakic cystoid macular edema(2).

Recently, bevacizumab has been introduced as a new therapeuticstrategy for many other ocular disorders, such as recurrent pterygium,neovascularization caused by chemical burns, viral infections of thecornea and bleb survival after glaucoma surgery(3-7). Therefore,bevacizumab can be used to treat various ocular disorders, and ithas been applied by various administration routes: anterior chamberinjection, topical application or subconjunctival bevacizumab injection(3-7).Bevacizumab prevents VEGF from binding to its receptors(8). Bevacizumab binds to all isoforms of VEGF blocks and VEGF-inducedangiogenesis(9). However, there have been many concerns regarding potential ocularside effects of bevacizumab. Intravitreal bevacizumab enters thegeneral circulation, results in prolonged inhibition of VEGF and has a half-life of 1-2 weeks in primates. VEGF is critical for growth anddevelopment of vital organs such as kidneys, lungs and brain during thethird trimester(10). Determining systemic effects,pharmacokinetics, and dosage of anti-VEGF through controlled assessments, this agent, anti-VEGF, might be an opportunity fortreatment of severe ROP. As an alternative to laser, its effects are presently toopoorly

known(10). Very preterm infants at risk for severe ROP have subnormal functioning of many organ systems for the rest of their lives.

Anti-VEGF treatment may have the capacity to reduce their reserveseven further. These effects may not be obvious until decades aftertreatment. For clinical off-label use of a drug, basic research and animalexperiments are required to evaluate its safety and to reveal potentialadverse effects(10). Results of studies have suggested that VEGF is an important growthfactor during the development of the retina(11-13). The visualsystem in newborn infants is different from that in adults, in that it isstill rapidly developing. Whether anti-VEGF antibody usage affects thedevelopment of the visual system, especially the retina, is unclear. In addition, whether there is systemic exposure after the injection ofbevacizumab in newborns remains unknown (14). To answer these questions this study was designed to evaluate the effects of intravitrealbevacizumab on lateral geniculate body (LGB) electrical activityin rateyes.

Some authors have questions whether rats are suitable in general forevaluating the effects of agents directed against humanVEGF(15-17). LGB is the primary relay center for visual informationreceived from the retina of the eye.

The LGB receives information directly from the ascending retinalganglion cells via the optic tract and from the reticular activating system.Neurons of the LGB send their axons through the optic radiation, a direct pathway to the primary visual cortex(18).

MATERIALS AND METHODS

Twenty male Wistar rats(6 weeks, 280-320gr)were used in this study. Rats were purchased fromanimal laboratory center, JundishapurUniversity of medical sciences, Ahvaz, Iran. Animals were kept understandard laboratory conditions with a 12/12 hrs light-dark cycle andwere supplied with adequate food and water. Rats were anesthetized with an intraperitoneal injection of ketamine(50 mg/kg) and xylazine (10 mg/kg) and head of rats were fixed in the stereotaxic apparatus. The stereotaxic coordinates relative to the Bregmawere determined for the lateral GB. The electrode was implanted 4.5 mm posterior,4.2 mm lateral, and 6.2 mm ventral to the skull surface.Bipolar electrodes (stainless steel) were implanted into the LGB using micro driver and were secured to the skull withacrylic dental cement. After the recovery period of one week, the animals were randomly divided into two groups. First group received 2 µl of intravitreal (25 mg/ml) commercial bevacizumab solution and second group received normal saline. Injection was performed with a 30-gauge needle attached to a 10 µl Hamilton syringe through the sclera 1 mm posterior to the limbus, guided by a stereo microscope, with care taken to avoid lens and retinal injury,The person performing the injection was blinded to the kind of solution injected. The electrical power of LGB was recorded by power lab device (AD-instrument Co) and the data were analyzed with Labchart 7 software. The electrical power of LGB was compared in the frequency bands of Gamma, Beta, Alpha, Theta, and Delta waves between two groups before injection and after injection on 7th and 30th days. Data were presented as mean±standard error of mean (SEM) and were analyzed with post hoc test. For all statistical analyses the $P<0.05$ was set as statistical significant.

RESULTS

Twelve male Wistar rats (280-320 g at the time of surgery) were enrolled in this study. One rat from Avastin group was excluded because of detachment of electrode. There was no statistically significant difference between mean total wave of Avastin and saline group (n=6) before intervention ($p=0.931$) (Table 2).Moreover, after 7 and 30 days of injection,no significant difference was observedbetween two groups ($p=0.126$, $p=0.571$).

DISCUSSION

In the past few years, dramatic new therapies with anti-VEGF antibody have been developed as treatment options for patients having neovascular eye diseases, especially AMD (19, 20). The safety and efficacy of anti-VEGF agents in the treatment of these diseases has beenevaluated and the results indicate that the intravitreal administration of anti-VEGF agents has limitations(21, 22). In spite of its efficacy, bevacizumab sometimes causes complications

such as hypertension, gastrointestinal perforation, wound healing complications, arterial thromboembolic events, and bleeding, which are associated with mechanism-based adverse events. Diligentevaluation for these toxicities is important, because early intervention may decrease morbidity and mortality risk. Prompt recognition of an anti-angiogenic agent-relatedtoxicity may also mandate treatment cessation to avoid exacerbation of system the adverse event (23). Vascular endothelial growth factor(VEGF, VEGFA) is critical for blood vessel growth in the developing and adult nervous system of vertebrates. Several recent studies demonstratethat VEGF also promotes neurogenesis, neuronal patterning, neuroprotection and glial growth(24). This study was designed to investigate the effect of Avastin on the nervous system and showed that intravitreal bevacizumab had no effect on electrical activity of LGB in rat's eyes. In summary, bevacizumab did not appear to be toxic to the visual pathwayin rats. This study supports the safety of intravitreal anti-VEGF agent treatments of choroidal neovascularization and retinal neovascular diseases. Further studies are still required to evaluate the long-term safety of the drugs on general electrophysiology of brain.

Table 1. Characteristics and the values of the variables of the study

Group		All pre	All 7	All 30	Gamma pre	Gamma 7	Gamma 30	Beta pre	Beta 7	Beta 30	Alpha pre	Alpha 7	Alpha 30
Avastin	N	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	0.13460	0.08936	0.21120	0.00018	0.00088	0.00004	0.00088	0.00115	0.00126	0.00087	0.01960	0.01960
	SD	0.158053	0.173854	0.243345	0.000345	0.00152	0.000033	0.001294	0.001389	0.001416	0.001381	0.043266	0.043266
saline	N	6	6	3	6	6	6	6	3	6	6	3	3
	Mean	0.23750	0.48933	0.08366	0.00199	0.00054	0.00072	0.00075	0.00034	0.00046	0.00202	0.00136	0.00136
	St. deviation	0.438755	0.505511	0.069716	0.00470	0.000808	0.001180	0.001355	0.000408	0.000512	0.003130	0.001939	0.001939
Total	N	11	11	8	11	11	11	11	8	11	11	8	8
	Mean	0.1907	0.30752	0.16337	0.0011	0.0006	0.00041	0.00072	0.00084	0.00083	0.00150	0.01276	0.01276
	St. deviation	0.33035	0.42835	0.19895	0.00346	0.00112	0.00090	0.00126	0.001151	0.001051	0.002454	0.034056	0.034056

Table 1.Cont.

Group		theta pre	theta 7	theta 30	delta pre	delta 7	delta 30
saline	N	5	5	5	5	5	5
	Mean	0.01526	0.01050	0.08674	1.89600	0.25400	2.168
	St. deviation	0.025312	0.019892	0.096015	1.88893	0.32633	2.752
Total	N	6	6	3	6	6	3
	Mean	0.00616	0.03786	0.0226	3.33925	5.1007	1.460
	St. deviation	0.009012	0.074209	0.028360	7.450639	7.3781	1.795
saline	N	11	11	8	11	11	8
	Mean	0.01030	0.02543	0.06271	2.68322	2.8976	1.902
	St. deviation	0.017874	0.055820	0.081224	5.454478	5.8023	2.320

Table 2. Test Statistics of the measured variables between the two groups[b]

Group	All pre	All 7	All 30	Gamma pre	Gamma 7	Gamma 30	beta pre	beta 7	beta 30	alpha pre	alpha 7	alpha 30	theta pre	theta 7	theta 30	delta pre	delta 7	delta 30
Mann-Whitney U	14.0	6.0	5.50	11.0	6.0	5.500	15.000	11.500	4.50	10.500	12.500	7.000	14.000	13.000	5.000	10.500	14.000	6.500
Wilcoxon W	35.0	21.0	11.50	26.0	21.0	20.500	36.000	26.500	10.500	31.500	27.500	22.000	35.000	28.000	11.000	31.500	29.000	12.500
Z	-0.183	-1.643	-0.60	-0.732	-1.68	-0.604	0.000	-0.640	-0.900	-0.823	-0.457	-0.149	-0.183	-0.365	-0.745	-0.823	-0.183	-0.300
Asymp. Sig. (2-tailed)	0.855	0.100	0.549	0.464	0.09	0.546	1.000	0.522	0.368	0.410	0.647	0.881	0.855	0.715	0.456	0.410	0.855	0.764
Exact Sig. [2*(1-tailed Sig.)]	0.931[a]	0.126_a	0.571_a	0.537[a]	0.126_a	0.571_a	1.000_a	0.537_a	0.393_a	0.429_a	0.662_a	0.1.000[a]	0.931_a	0.792_a	0.571_a	0.429_a	0.931_a	0.786_a

a. Not corrected for ties.

b. Grouping Variable: group

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