ORIGINAL ARTICLE

The Adjunctive Use of Anti-vascular Endothelial Growth Factor Agents in the Management of Iris Neovascularisation in Malaysia

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SUMMARY

This retrospective study investigated the role of anti-vascular endothelial growth factor agents (VEGF), ranibizumab, bevacizumab and pegaptanib sodium in patients with iris neovascularisation (INV), in which 9 eyes received intraocular injections for various ischaemic ocular conditions.

Ocular sequelae included recurrence of rubeosis (n=2) and hyphaema (n=2). Systemic complication included one case of cerebrovascular accident. INV regressed in all cases from day one. INV recurrence occurred in 2 cases. The mean intraocular pressure of the study eyes decreased from 25.3 mmHg to 18.3 mmHg at one month. Five eyes are medication free. Visual acuity improved in 5 eyes. Four eyes achieved a Snellen visual acuity of 6/24 or better. We conclude that the use of intraocular anti-VEGF agents are safe and effective for inducing the regression of INV. Patients with multiple systemic risk factors should be counseled on stroke risk.

KEY WORDS:
Anti-vascular growth factor agents, Ranibizumab, Bevacizumab, Pegaptanib sodium, Iris neovascularisation

INTRODUCTION

The presence of rubeosis iridis or iris neovascularisation (INV) is associated with the development of open-angle and subsequently angle closure glaucoma. Vascular endothelial growth factor (VEGF) is implicated in the development of INV and intraocular levels of VEGF increase in ischaemic situations.

Ranibizumab (Lucentis™, Genentech Inc, San Francisco, United States of America) is a humanized antigen-binding fragment designed to inhibit all isoforms and active degradation products of vascular endothelial growth factor A (VEGF-A)[1].

Ranibizumab [2-4] and other anti-VEGF agents such as bevacizumab (Avastin™, Genentech Inc, San Francisco) and pegaptanib sodium (Macugen™)[5] have been shown to reduce neovascularisation in age-related macular degeneration (AMD). Bevacizumab is a full length recombinant humanized antibody against VEGF-A[6]. It has been shown to induce a rapid regression of retinal neovascularisation in proliferative diabetic retinopathy (PDR). Pegaptanib is an aptamer specific for the VEGF-A 165 isoform [5].

The aim of our study was to investigate the safety, efficiency and utility of giving an intraocular injection of various anti-VEGF agents to induce regression of INV from various etiologies.

MATERIALS AND METHODS

A retrospective, interventional, non-comparative study was performed at the Universiti Kebangsaan Malaysia Medical Centre (UKMMC) Ophthalmology clinic of the medical records of 8 patients who received 9 injections of various anti-VEGF agents (ranibizumab, bevacizumab or pegaptanib sodium) for INV from March 2008 until July 2009 either at UKMMC (n=6) or at International Specialist Eye Centre (ISEC) (n=2) . UKMMC is a major tertiary referral centre in Malaysia's capital city. ISEC is a private Ophthalmology centre that receives mainly specialist Ophthalmology referrals.

Intravitreal anti-VEGF is considered for all patients with INV who are scheduled for surgery who have raised intraocular pressure. Another indication for anti-VEGF injection is a patient who declines surgical intervention or wishes to defer surgery. It is also offered to patients who have no view of their retina or persistent INV despite panretinal laser photocoagulation (PRP) therapy for proliferative retinopathy. Gonioscopic examination is performed to note whether the drainage angle is closed or open. Patients with a prior history of cerebrovascular accident (CVA) are excluded from receiving anti-VEGF injections.

Informed consent was obtained from each patient prior to intraocular injection of the anti-VEGF agents. The risk of cataract, infection, retinal detachment and the off-label use of the agents were explained to the patients. Each injection was performed in a clean treatment room in an outpatient setting. Topical anaesthesia was achieved with proparacaine hydrochloride (Alcaine™) and subconjunctival anaesthesia with 2% lignocaine (Xylocaine™). 5% povidone iodine lid scrub and douche was performed prior to sterile draping. All

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patients who received ranibizumab or bevacizumab had shared it with another patient who had purchased the vial for treatment of AMD. Only two injections were performed per vial of ranibizumab. The original container of ranibizumab or bevacizumab had been placed in a refrigerator whose sole use was for storage of sterile medications, at a temperature of 4˚C[7]. Each bottle cap was swabbed with 100% alcohol prior to aspiration into a new 30G insulin needle attached to a 1ml syringe.

Each intravitreal injection was given 3.5 to 4 mm from the limbus, directing the needle into the center of the vitreous cavity. Anti-VEGF agents are administered in the following dosages: 0.3mg of pegaptanib, 0.25-0.5mg of ranibizumab or 1.25mg of bevacizumab. 5% povidone iodine was then re-instilled. The intraocular pressure was then checked. Post injection, each patient received guttae ciprofloxacin 2 hourly for one week. Patients were given instructions on symptoms and signs of infection and were reviewed the following day and prior to any surgical procedure if planned.

The intracameral injection was performed under aseptic conditions in the operating theatre. A corneal incision was made at 2 o'clock in the left eye with a 15˚ blade, allowing egress of aqueous fluid prior to injection at 10 o'clock with an insulin syringe and 30G needle by the right-handed surgeon. There is no back-flush of injected agent using this method.

The ophthalmic notes were reviewed for demographical data (age, gender and ethnicity), injection related data, indications for surgery and operation record; visual acuity and examination findings at one week, one month, 3 months, 6 months and at last review. Duration to clearance of any haemorrhage including hyphaema and any complication of the intravitreal injection was noted as was recurrence of the INV.

RESULTS

Table I summarises the patient demographics, the anti-VEGF agents used and the ocular status pre and post anti-VEGF agent injection following any surgical interventions. Patient age ranged from 44 to 71 years of age with a mean of 57.4 years. There were 4 males and 4 females. Four patients were of Malay ethnicity, 1 was Chinese and 3 were of Indian parentage. Five were left eyes.

The indications for injection were INV secondary to proliferative diabetic retinopathy (PDR) (n=6), ischaemic central retinal vein occlusion (n=2), rhegmatogenous retinal detachment with lasered PDR (n=1; Patient H) and anterior proliferative vitreoretinopathy in an oil-filled, non-diabetic eye (n=1; Patient A). One eye had persistent vitreous haemorrhage post-pars planar vitrectomy (PPV) in which there was pre-operative INV (patient D) and one eye had rapid acceleration of INV after complicated cataract surgery (patient B). A major risk factor for INV was diabetes mellitus (n=8).

Five eyes of five patients received an intravitreal injection of 0.05 ml (0.5 mg) (n=5) or 0.025 ml (0.25 mg) of ranibizumab. One eye received intracameral injection of 0.05 ml (0.5 mg) ranibizumab. Two eyes of one patient received 0.05 ml (1.25mg) of intravitreal bevacizumab. One eye received 0.9 ml (0.3 mg) of intravitreal pegaptanib sodium. (Table I)

Panretinal photocoagulation (PRP) had been performed previously in 8 eyes (patients B-H). Patient A was not a known diabetic. Laser photocoagulation was therefore given intraoperatively during the vitrectomy to the ischaemic anterior retinal remnant. In addition to anti-VEGF, patients B-H also received top up PRP where possible to areas of attached retina only.

Intraocular pressure (IOP) at baseline ranged from 7 mmHg to 45 mmHg (mean 25.8 mmHg). Following injection, IOP ranged from 6 to 33 mmHg at one week (mean 21.3 mmHg) with two eyes defaulting. At post-injection 1 month, the mean IOP was 17.1 mmHg (4-32 mmHg; no eyes defaulting). Five eyes were medication free after injection while the others required further medical therapy including various doses of oral acetazolamide. Follow-up duration ranged from 3 months to 24 months with all eyes having 3 or more months of follow-up.

Of 7 eyes in which gonioscopy was performed, open angles were noted in 5 eyes including presence of silicone oil globules in patient B who had anterior chamber intraocular lens placement and patient C who had neovascularisation in the angles (NVA). Two eyes with closed angles had NVA.

All cases showed regression of INV as early as day 1 (Patient A) or day 2 (Patient G). Complete regression of INV occurred up to 2 months post-injection. Two cases had recurrence of INV at 6 weeks and 4 months post-injection (Patient A and H). Patient A wished to defer his second injection until after a religious festival and patient H declined any further intraocular injection or operation due to a fear of recurrent stroke. He did however consent to orbital floor triamcinolone injection, which alleviated some of his discomfort but could not salvage the vision.

All eyes that were operated had minimal to moderate intraoperative bleeding. There was no documented case of progression of tractional detachment during the study period although fibrosis of neovascularisation was consistently observed to various degrees in patients with fundal view.

Pre-operative visual acuity (VA) ranged from 6/36 to hand motions with the majority (n=7) with pre-operative vision of 6/60 or worse. VA had either remained unchanged (n=2), improved (n = 5) or worsened (n=2) with best corrected VA at last review ranging from 2/60 or better (n=5) to no perception of light (n=1). Four eyes achieved 6/24 or better at last review.

There were no complications locally from the intravitreal injection. One patient who received the injection for annular retinal detachment post PPV who wanted to defer surgical intervention, developed a left hemiparesis approximately 24 hours after intravitreal injection of Lucentis. This diabetic individual had concurrent risk factors of hypertension, hyperlipidaemia and triple vessel cardiac disease.

Table I: Shows the demographic details and interventions performed in the study population

Eye		Age (yrs)	Gender	Ethnicity	Indication(s): Rubeosis secondary to:	Anti-VEGF used	Intraocular pressure (IOP) (mmHg)			Gonioscopy findings	Rubeosis regression onset (days post injection)	Operation performed (if any)	Intra-operative bleeding	Snellen visual acuity (VA)		Comp-lications
Patient	Eye number						Baseline	1 month	Last review (duration of follow-up/ months)					Pre-injection	At final review	
A	1L	66	M	1	1	IC Lucentis 0.5mg	7	4	4 (3)	3	1 (day 7 post injection)	IOL explantation/ROSO/EL	1	CF	CF	Recurrence of rubeosis at 6 weeks
B	2L	54	F	3	2	IV Lucentis 0.3mg	26	15	20 (11)	1 (oil globules superiorly)	4	20G PPV/MP/EL/SO (day 6 post injection)	1	3/60	6/60, N24	Hyphaema
C	3R	63	M	3	3	IV Lucentis 0.5mg	28	20	11 (20)	1 (INV noted)	7	20G vitreous washout/EL/SF6 (6 weeks post last injection)	1	HM	6/24, N10	Nil
D	4R	44	F	2	4	IV Macugen 0.3ml	40	14	11 (8)	2 (NVA)	5	Trabeculectomy with 5 fluorouracil (day 6 post-injection)	Not documented	6/60	NPL (after subsequent vitrectomy surgery)	Hyphaema post trabeculectomy
E	5L	71	M	1	2	IV Lucentis dose not stated	45	32	18 (10)	1	7	Trabeculectomy with MMC (2 months post injection)	0	6/36	6/12	Nil
F	6L	56	F	1	4	IV Avastin 1.25mg	20	20	15 (20)	1	7	Left PEA/IOL (5 months post injection)	0	6/36	6/18	Nil
F	7R	56	F	1	4	IV Avastin 1.25mg	20	20	16 (20)	1	7	Nil	Not relevant	6/60	6/24	Nil
G	8L	54	F	1	5	IV Lucentis 0.5mg	33	17	12 (24)	1	2	Left PEA/IOL (6 weeks post injection)	0	HM	HM	Nil
H	9R	53	M	3	6	IV Lucentis 0.5mg	13	12	20 (9)	2 (NVA)	7	No - patient declined	Not relevant	2/60	PL	Left hemiparesis day 1 post-injection. Hyphaema & recurrence at 4 months.

Abbreviations:
Eye: R-Right, L-Left;
Gender: M-male, F-female;
Ethnicity: 1-Malay, 2- Chinese, 3- Indian;
Indications (rubeosis secondary to): 1- Non-diabetic anterior proliferative vitreo-retinopathy, 2- Proliferative diabetic retinopathy (PDR) (accelerated) post complicated cataract surgery, 3- PDR (lasered-incomplete) (LI) with vitreous haemorrhage, 4- PDR (LI) with cataract
5- Ischaemic central retinal vein occlusion, 6- PDR (lasered) (complete) with rhegmatogenous retinal detachment;
Anti-VEGF used: IC- intracameral, IV- intravitreal;
Gonioscopy findings: 1- open, 2- closed, 3- not done, INV- iris neovascularisation, NVA- new vessels in the angle;
Operations performed: PPV- Pars planar vitrectomy, PEA/IOL-Phaco-emulsification + intraocular Lens implant (IOL), MMC- mitomycin C, MP-membrane peeling, EL-endolaser,
Cryo-cryotherapy, SF6- sulphur hexafluoride gas, SO-silicone oil, ROSO-removal of SO;
Intraoperative bleeding: 0-nil,1-mild, 2-moderate, 3-severe;
Snellen VA: 6/ , CF- counting fingers, HM – hand motions, PL-light perception, NLP-no light perception, N –near vision chart

DISCUSSION

Diabetic retinopathy (DR) is the leading cause of blindness among people of working age worldwide and leads to significant loss of quality of life. The standard treatment for proliferative diabetic retinopathy is panretinal photocoagulation (PRP), which ablates large areas of retina in order to reduce the hypoxic stimulus of the remaining retina. Anti-VEGF agents increase vessel permeability by increasing the phosphorylation of tight junction proteins including retinal vasculature permeability in in vivo models. Studies have demonstrated correlation of VEGF levels in the retina, vitreous and aqueous of human eyes with the severity of proliferative diseases [8-10].

The effectiveness of bevacizumab, a sister compound to ranibizumab, is demonstrated in case reports to cause regression of INV [11-13]. INV also occurs in eyes with history of complex retinal detachments that are injected with SO due to persistent peripheral annular detachments [14-15] VEGF is implicated in the development of INV and is released by residual ischaemic peripheral retina.

In patient A, ranibizumab was injected intracamerally as the retinal ischaemia was deemed to be limited to the anterior segment. The concentration of ranibizumab and its diffusibility in silicone oil was also uncertain. Bevacizumab is non toxic to the corneal endothelium when injected intracamerally [16].

INV regression was rapid in these cases, typically occurring in the first week in cases of ranibizumab injection with maximum effect seen up to 1 month later in those injected with bevacizumab. There is limited information in the literature on this. In the case of a patient with INV following central retinal artery occlusion, regression was seen in the first week after intracameral injection of bevacizumab with improvement up to 6 months after laser photocoagulation [17].

Other series support our finding that regression after anti-VEGF injection takes place in the first week [12] but may take up to 3 weeks [11]. Recurrence of INV occurs up to 6 months later but may stabilize after repeat injections without IOP elevation[12]. In our cases, recurrence occurred as early as 6 weeks after the injection, but in view of the half life of the agents, a review at no more than 4 weeks later will be judicious [11].

In deciding on the anti-VEGF agent used, we considered availability and cost. Ranibizumab was our first choice. However, it costs on average 6 times more than bevacizumab. Hence, patients were injected with ranibizumab only if the drug was available by virtue of purchase by a patient with AMD. If this was not available, then the patient had to obtain an injection of bevacizumab at a private institution as mentioned earlier. Studies of bevacizumab and other drugs show a low rate of microbial contamination with multi-aliquot usage [7] and its anti-VEGF activity is stable for six months [18]. The pegaptanib sodium was a sample from the company.

The Tuebingen Bevacizumab Study group concluded that bevacizumab, pegaptanib and ranibizumab all significantly suppress choroidal endothelial cell proliferation but none was superior with respect to endothelial cell growth inhibition[19]. Hence, all the agents may be used but more studies need to be done to determine which the best in each clinical situation is. The incidence of endophthalmitis in intravitreal injections of anti-VEGF agents is low at approximately 0.029% [20-21]. There were no local complications from the injection such as uveitis or endophthalmitis in this study.

Wong *et al* found that persons with early-stage AMD, followed for ten years, had a higher cumulative incidence of stroke than those without the disease (4.08% vs. 2.14%) [22]. The annual prevalence rate for myocardial infarction in patients with past history of arterial thromboembolic events is 7.4% and 35.1% for inpatient stroke. The PACORES group found a 0.5% incidence of stroke in patients receiving bevacizumab [23]. However, the Macugen study group found an undetectable level of systemic pegaptanib absorption after 0.3 mg injections. Pegaptanib appears to have a good safety profile as there is no increase in deaths, in events associated with systemic VEGF inhibition such as hypertension, thromboembolic events, serious haemorrhagic events up to 3 years after the injections [24-25]. This has potential implications for diabetics already at risk for thromboembolic events due to concurrent hypertension and hyperlipidaemia.

The limitations of the study include its retrospective nature and small numbers. However, it adds to the wealth of information on the effectiveness of various anti-VEGF agents in INV and their rapid onset of activity. All the agents used in the study were able to induce temporary regression of rubeosis and increase safety margins where intraoperative bleeding was a concern.

Future improvements in studies of this topic will include prospective randomised studies that incorporate standardised documentation of the rubeosis including investigations with iris fluorescein angiography.

CONCLUSION

In conclusion, anti-VEGF agents have a role in reducing INV from various etiologies. However, patients receiving anti-VEGF agents must be counseled on the risk of cerebrovascular events.

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