Alterations in intraocular cytokine levels following intravitreal ranibizumab

Farzin Forooghian, MD, FRCSC,* Peter J. Kertes, MD, FRCSC,† Kenneth T. Eng, MD, FRCSC,† David A. Albiani, MD, FRCSC,* Andrew W. Kirker, MD, FRCSC,* Andrew B. Merkur, MD, FRCSC,* Nader Fallah, PhD,‡ Sijia Cao, MD,* Jing Cui, MD,* Chris Or, BSc,* Joanne A. Matsubara, PhD*

ABSTRACT • RÉSUMÉ

Objective: Our previous work has shown that, after intravitreal bevacizumab (IVB) administration, decreases in the levels of vascular endothelial growth factor (VEGF)-A and placental growth factor (PlGF), along with increases in the levels of interleukin (IL)-8 and transforming growth factor (TGF)-β_2, can be observed. It is not yet known if similar changes occur after intravitreal ranibizumab (IVR). The purpose of this study was to examine intraocular cytokine changes after IVR.

Design: Prospective clinical study.

Participants: Subjects with proliferative diabetic retinopathy requiring pars plana vitrectomy (PPV) were recruited.

Methods: Participants received IVR as pre-treatment before PPV. Aqueous humour levels of IL-8, VEGF-A, PlGF, and TGF-β_2 were measured at time of pre-treatment and PPV. Results were analyzed using univariate statistical models.

Results: A total of 14 participants were recruited. After IVR administration, we observed a decrease in the levels of VEGF-A and PlGF, and an increase in the levels of IL-8 and TGF-β_2. These results were statistically significant only for VEGF-A ($p = 0.0001$) and IL-8 ($p = 0.0002$).

Conclusions: The changes in cytokine levels after IVR mirror the changes seen after IVB. Further studies are warranted in order to determine if there are any differences between IVB and IVR in this regard.

Objectif : Nos travaux antérieurs ont montré que, suite à l'administration de bevacizumab intravitréen (BIV), on constate une diminution des niveaux de FCEA-A et du facteur de croissance placentaire (PlGF), et une augmentation des niveaux d'IL-8 et de TGF-β2. Nous ne savons pas encore si des changements similaires se produisent suite à l'administration de ranibizumab intravitréen (RIV). Le but de l'étude était d'examiner les changements au niveau des cytokines intraoculaires après l'administration de RIV.

Nature : Étude clinique prospective.

Participants : Nous avons recruté des sujets atteints de rétinopathie diabétique proliférante (RDP) nécessitant une vitrectomie par la pars plana (VPP).

Méthodes : Les participants ont reçu du RIV comme prétraitement avant de subir une VPP. Nous avons mesuré le niveau d'humeur aqueuse de l'IL-8, du FCEA-A, du PlGF et du TGF-β2 au moment du prétraitement et de la VPP. Nous avons analysé les résultats à l'aide de modèles statistiques univariés.

Résultats : Au total, 14 participants ont été recrutés. Après l'administration du RIV, nous avons observé une diminution des niveaux de FCEA-A et de PlGF, et une augmentation des taux d'IL-8 et de TGF-β2. Nous n'avons constaté des résultats statistiquement significatifs que pour le FCEA-A ($p = 0{,}0001$) et l'IL-8 ($p = 0{,}0002$).

Conclusion : Les changements au niveau des cytokines après l'administration de RIV sont semblables aux changements observés après l'administration de BIV. D'autres études sont nécessaires afin de déterminer s'il y a des différences entre le BIV et le RIV à cet égard.

Intravitreal bevacizumab (IVB) and intravitreal ranibizumab (IVR) are used widely for the treatment of a variety of neovascular ocular diseases, including age-related macular degeneration (AMD), diabetic macular edema (DME), and retinal venous occlusion. Prospective randomized clinical trials have shown that IVB and IVR are equivalent with respect to their effect on visual acuity in AMD,[1] and their effect on central subfield optical coherence tomography thickness in DME.[2] The therapeutic effect of IVB and IVR is based on their ability to bind and neutralize vascular endothelial growth factor (VEGF), a powerful angiogenic mediator that plays a pathological role in various neovascular ocular diseases.[3] Studies of intraocular cytokines have revealed that, in addition to VEGF, various inflammatory cytokines also appear to be elevated in patients with AMD,[4,5] DME,[6] and retinal venous occlusion.[7,8]

We have previously shown that, after a mean of 10 days after IVB in patients with proliferative diabetic retinopathy (PDR), there is an increase in cytokine levels (interleukin [IL]-8 and transforming growth factor [TGF]-β_2) detected in the aqueous humour.[9] This rise in inflammatory mediators may occur via compensatory mechanisms in response to decreased VEGF levels, and this rise in the levels of these inflammatory mediators may be responsible for some of the complications that can occur after IVB. Further studies have confirmed these findings, showing

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that intraocular levels of several inflammatory cytokines (IL-6, IL-8, and TGF-β_2) are elevated 1–7 days after IVB.[10] Studies that have examined intraocular cytokine levels 4 weeks after IVB failed to find any effect,[11–13] suggesting that the elevation of inflammatory mediators after IVB is acute and transient. With respect to IVR, studies of intraocular cytokines have shown that platelet derived growth factor (PDGF) levels decrease after IVR.[14] Thus, both IVB and IVR appear to have an effect on various mediators of inflammation and angiogenesis.

Aflibercept is a novel anti-VEGF compound that also binds placental growth factor (PlGF), a member of the VEGF family that plays an important role in pathological angiogenesis.[15] In vitro studies have shown that, although aflibercept is able to effectively bind PlGF, both bevacizumab and ranibizumab show no ability to bind PlGF.[16] However, our previous study in patients with PDR showed a (statistically insignificant) decrease in PlGF levels after IVB.[9] Examination of changes in PlGF in human eyes after IVR would thus be interesting to examine.

To our knowledge, this is the first study to examine changes in PlGF, IL-8, and TGF-β_2 after IVR.

Methods

The study design of this study was similar to that of our previously published work that examined intraocular cytokine levels after IVB.[9] Eligibility criteria for this prospective pilot study included the presence of PDR with nonclearing vitreous hemorrhage (VH) and/or tractional retinal detachment requiring pars plana vitrectomy (PPV). Exclusion criteria included VH secondary to ocular disease other than diabetes. Approximately 1 week before the planned surgery, the patients were injected with IVR (0.50 mg, 0.05 mL). Study drug was stored in refrigerated glass vials and drawn into plastic syringes immediately before use. Immediately before the intravitreal injection, an anterior chamber paracentesis was performed and 50–150 μL of aqueous humour was withdrawn and stored at −80°C. At the scheduled time of surgery, another anterior chamber paracentesis was performed immediately before commencement of PPV and another 50–150 μL of aqueous humour was obtained and stored at −80°C. To prevent dilution of aqueous humour, the infusion cannula was left clamped after placement. After this, the anterior chamber paracentesis was performed, the infusion cannula was unclamped, and PPV was started. Anterior chamber paracentesis was performed under sterile conditions with a 30-gauge needle attached to an insulin syringe. PPV was performed by 1 of 5 different surgeons (D.A.A., A.W.K., and A.B.M. in Vancouver; P.J.K. and K.T.E. in Toronto) using standard 3-port 23-gauge or 25-gauge techniques. Demographic information, including age, sex, and $HbA1_C$ values (collected within 30 days of the vitrectomy date), was obtained from subject charts.

IL-8, PlGF, and VEGF-A were measured by customized Bio-Plex Pro Human Cancer Biomarker Panel 2 assay, and TGF-β_2 was measured by customized Bio-Plex Pro TGF-β assays, according to the manufacturer's instructions (Bio-Rad Laboratories, Mississauga, Ont.). Whenever possible, cytokines were assayed in triplicate. The assays use xMAP technology (Luminex, Austin, Tex.), which permits the quantification of multiple cytokines in a single well with 50 μL of diluted or undiluted sample. Briefly, the samples were diluted into 1:4, and for TGF-β_2 assay, the samples were activated by 1N HCl and then neutralized by 1.2 N NaOH/0.5 mol/L HEPES before dilution. Fifty microliters of cytokine/growth factor standards and diluted samples were incubated with the premixed anticytokine/growth factor–conjugated beads in 96-well filter plates at room temperature, with agitation (1100 rpm for 30 seconds, and then 300 rpm for 2 hours). After incubation, plates were washed 3 times via vacuum filtration with 100 μL of Bio-Plex wash buffer per well using the Bio-Plex Pro wash station. Plates were then incubated with 25 μL of diluted biotinylated detection antibody for 30 minutes at room temperature with agitation. After another 3 washes, 25 μL of streptavidin–phycoerythrin were added in each well, and the plates were incubated for 10 minutes at room temperature with agitation. After another 3 washes, the beads were resuspended in 125 μL of Bio-Plex assay buffer and vortexed for 30 seconds at 1100 rpm. Standards and samples were analyzed using the Bio-Plex 200 Suspension Array System, and subsequent raw median fluorescent intensity data were captured and analyzed using Bio-Plex Manager software 4.1 (Bio-Rad Laboratories). The detection limits for our assays were as follows: TGF-β_2 = 15–30 080 pg/mL, VEGF-A = 6–56 237 pg/mL, IL-8 = 2–26 403 pg/mL, and PlGF = 1–9590 pg/mL.

Statistics were performed using SPSS software (version 20; IBM Corp, Armonk, NY). All data were tested for normality using histogram graphical analysis and Kolmogorov–Smirnov numerical analysis. When the data conformed to normality, the *t* test was used for 1-sample testing of the mean. When the data did not conform to normality, the Wilcoxon signed-rank test (for 1-sample testing of the mean) was used. Given the pilot nature of this study, a formal sample size calculation was not performed and sample size was based on feasibility.

Table 1—Univariate analyses of cytokine level changes after intravitreal ranibizumab

	Mean Change (pg/mL)	p^*
Change in VEGF-A level	−354‡	0.0001
Change in PlGF level	−2.66‡‡	0.47
Change in IL-8 level	83.3‡	0.0002
Change in TGF-β_2 level	2831‡	0.38

*p-Value represents significance of change in cytokine level compared to zero. ‡One-sample Wilcoxon signed-rank test. ‡‡One-sample *t* test.

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