Vitreous levels of interleukine-8 and monocyte chemoattractant protein-1 in macular oedema with branch retinal vein occlusion

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Abstract

Purpose To investigate whether interleukine-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1) are related with macular oedema in patients with branch retinal vein occlusions (BRVOs).

Design Retrospective case-control study.

Participants Nineteen patients who had macular oedema due to BRVO and nine patients with non-ischaemic ocular diseases (control group).

Methods Macular oedema was examined by optical coherence tomography. Both venous blood and vitreous samples were obtained at the time of vitreoretinal surgery. IL-8 and MCP-1 levels in vitreous fluid and plasma were determined with enzyme-linked immunosorbent assay kits. Variables were compared with the Mann-Whitney *U*-test, Wilcoxon's signed-ranked test, and the χ^2 -test, when appropriate. To examine correlations, Spearman's rank-order correlation coefficients were calculated. Statistical significance was set at $P < 0.05$.

Results The vitreous fluid levels of IL-8 (median: 63.5 pg/ml) and MCP-1 (median: 1522.4 pg/ml) were significantly higher in the patients with BRVO than in the control group (median: 5.1 and 746.5 pg/ml respectively; $P < 0.001$ and < 0.001 respectively). Vitreous IL-8 and MCP-1 were significantly correlated in patients with BRVO ($P = 0.009$).

Conclusions Both IL-8 and MCP-1 were elevated in the vitreous fluid of patients with BRVO and macular oedema. Both chemokines may contribute to the pathogenesis of macular oedema in patients with BRVO.

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Introduction

Macular oedema often complicates branch retinal vein occlusion (BRVO) and causes a reduction in visual acuity.¹ Increased vascular permeability due to conformational changes in the tight junctions of retinal vascular endothelial cells is thought to be a fundamental event in the pathogenesis of macular oedema.² Some cytokines, such as vascular endothelial growth factor (VEGF) and interleukine-6 (IL-6), provoke these changes,^{2–4} and their vitreous levels are elevated in patients with BRVO and central retinal vein occlusion (CRVO).^{5,6}

Chemokines are chemoattractant cytokines involved in the recruitment of inflammatory cells. IL-8 is the most important C-X-C chemokine and monocyte chemoattractant protein-1 (MCP-1) the most important C-C chemokine. Experimental studies have shown that both IL-8 and MCP-1 increase blood-brain barrier permeability, and that MCP-1 causes alteration of tight junctions in brain endothelial cells.^{7–9} Moreover, both IL-8 and MCP-1 are expressed by human retinal endothelial cells and retinal pigment epithelial cells (RPE).¹⁰ In this study, we measured the concentrations of IL-8 and MCP-1 in vitreous fluid of patients with macular oedema secondary to BRVO to investigate the relationship between the

development of macular oedema and the vitreous levels of these chemokines.

Materials and methods

Subjects

The study included non-diabetic patients with BRVO and secondary macular oedema involving the fovea, who had been enrolled in a study to assess the role of adventitia sheath dissection in the management of this condition. Exclusion criteria for this study were duration of BRVO more than 4 weeks and presence of ischaemic areas on fundus fluorescein angiography (FFA) imaging. If retinal haemorrhages precluded proper assessment of ischaemia but cotton wool spots were observed patients were also excluded. In addition, patients were excluded if they had a history of retinal photocoagulation or intraocular treatment with any drug, steroid use, ocular surgery during the past 3 months, or uveitis or vitreal haemorrhage. Nineteen patients with BRVO were included. Vitreous fluid samples were also obtained from nine patients with non-ischaemic ocular diseases to serve as controls. The same exclusion criteria were applied. The control group included six patients with macular epiretinal membrane and three patients with a macular hole. Baseline characteristics of the patients are shown in Table 1. Characteristics of BRVOs are summarized in Table 2. Informed consent was obtained from each participant following an explanation of the purpose and potential adverse effects of the procedures. This study was performed in accordance with the Declaration of Helsinki Principles and was approved by the ethics committee of our hospital.

Ophthalmologic examinations

Preoperative examinations included determination of visual acuity using Snellen charts, a complete biomicroscopic evaluation, measurement of intraocular pressure with a Goldmann tonometer, and fundus

examination using a quadraspheric fundus contact lens (Volk Optical Inc, Mentor, Ohio, USA). Macular oedema was confirmed by FFA performed with a Topcon TRC-50IX fundus camera, and using optical coherence tomography (OCT) (Zeiss-Humphrey Ophthalmic Systems, Dublin, CA, USA), which was also used to measure retinal thickness at the central fovea. This latter parameter was defined as the distance between the inner limiting membrane and the retinal pigment epithelium, including serous detachments.

Idiopathic epiretinal membrane and macular hole were diagnosed on clinical criteria and using OCT.

Vitreous samples

Undiluted vitreous samples (1 ml) were obtained at the start of vitrectomy by aspiration into a 1 ml syringe attached to the vitreous cutter, subsequently transferred to a sterile tube, and then frozen at -80°C until analysis. For plasma determinations, blood samples were collected at the same time as the vitrectomy and centrifuged at $1600 \times g$ for 10 min at 4°C . Aliquots of plasma were obtained and stored at -80°C until assayed.

Measurement of IL-8 and MCP-1

Enzyme-linked immunosorbent assay to measure IL-8 and MCP-1 in vitreous and plasma samples was performed with commercial kits (QuantiGlo Human IL-8 and Quantikine Human CCL2/MCP-1; R&D Systems, Minneapolis, MN, USA). According to the manufacturer the minimum detectable concentrations of IL-8 and MCP-1 were 0.28 pg/ml (inter-assay coefficient of variation (CV) 6.0–11.5%) and 5.0 pg/ml (inter-assay CV 4.6–6.7%) respectively.

Statistical analysis

Data are presented as the mean $\pm$ standard deviation, or the median and range. Unpaired continuous variables

Table 1 Baseline characteristics of patients and controls

	BRVO (N = 19)	Controls (N = 9)
Gender		
Female	11	5
Male	8	4
Age	65 ± 6.7^a	67 ± 7.1^a
Glaucoma	3	0
Hypertension	9	4
Pseudophakia	2	4
Macular thickness (μm)	619.94 ± 189.87^a	

Abbreviation: BRVO, branch retinal vein occlusion.

^aMean $\pm$ standard deviation.

Table 2 Characteristics of BRVO

Anatomic type	n
Superotemporal	11
Inferotemporal	8
FFA findings (macula)	
Enlargement of FAZ	0
Macular haemorrhages	12
Macular leakage	19
Central macular thickness (μm)	619.94 ± 189.97^a

Abbreviations: n, number of cases; FFA, fluorescein angiography; FAZ, foveal avascular zone.

^aMean $\pm$ standard deviation.

were compared with the Mann–Whitney *U*-test. Wilcoxon's signed-ranked test was used to compare paired continuous variables, and the χ^2 -test to compare discrete variables. To examine correlations, we calculated Spearman's rank-order correlation coefficients. Statistical significance was set at $P < 0.05$. All statistical analyses were performed with SPSS, version 13.0 (SPSS Inc, Chicago, IL, USA).

Results

The concentration of IL-8 in vitreous fluid was significantly higher in patients with BRVO than in the control patients (63.5 pg/ml (25–240.1) *vs* 5.1 pg/ml (2.3–18.3); $P < 0.001$). The vitreous concentration of IL-8 was 8.5-fold higher than the plasma concentration in BRVO patients (63.5 pg/ml (25–240.1) *vs* 7.5 (1.3–23.8); $P < 0.001$), whereas there were no differences in the control patients (5.1 pg/ml (2.3–18.3) *vs* 7.4 (4.9–29.4); $P = 0.8$) (Figure 1). The vitreous fluid concentration of MCP-1 was also significantly higher in BRVO patients than in the controls (1522.4 pg/ml (462.7–3445.3) *vs* 746.5 (479.7–980.3); $P < 0.001$). In BRVO patients, the vitreous concentration of MCP-1 was fivefold higher than the plasma concentration (1522.4 pg/ml (462.7–3445.3) *vs* 312 pg/ml (134.5–694); $P < 0.001$) and in control patients it was twofold higher than the plasma concentration (746.5 pg/ml (479.7–980.3) *vs* 369.6 (269.5–436.6);

$P = 0.002$) (Figure 2). There was a statistically significant correlation between vitreous concentrations of IL-8 and MCP-1 in patients with BRVO ($\rho = 0.579$, $P = 0.009$; Figure 3).

No correlation was observed between vitreous and plasma concentrations of IL-8 ($\rho = 0.151$, $P = 0.538$) and MCP-1 ($\rho = 0.070$, $P = 0.775$) in patients with BRVO.

There was no significant correlation between vitreous concentrations of either factor or central macular thickness (IL-8, $\rho = -0.036$, $P = 0.880$; MCP-1, $\rho = -0.04$, $P = 0.861$).

Conclusion

Branch retinal vein occlusion is the second most common cause of retinal vascular disease after diabetic retinopathy.¹¹ Interruption of venous flow in these eyes almost always occurs at an arteriovenous intersection, where a retinal artery crosses a retinal vein. Thrombus formation or focal endothelial swelling occurs at the crossing site and results in elevated venous pressure, which leads to macular oedema, ischaemia, and haemorrhage. Standard care for macular oedema consists of laser photocoagulation in a grid pattern throughout the leaking area. This treatment is based on the results from the Branch Vein Occlusion Study, which showed that macular grid laser improves visual acuity in selected patients. In patients with persistent BRVO for more than

Figure 1 Vitreous interleukine-8 levels in patients with branch retinal vein occlusion compared with controls.

Figure 2 Vitreous monocyte chemoattractant protein-1 levels in patients with branch retinal vein occlusion compared with controls.

Figure 3 Relationship between vitreous levels of interleukine-8 and monocyte chemoattractant protein-1 in patients with branch retinal vein occlusion ($\rho = 0.579$, $P = 0.009$).

3 months, visual acuity was less than 20/40 and oedema affected the fovea.¹¹ Nonetheless, although central vision improves after laser treatment, scotomas and reduced retinal function have been reported at follow-up.^{12–14} Hence, new therapeutic options may be warranted to avoid this complication.

In recent years, research has focused on the role of cytokines in the pathogenesis of macular oedema. VEGF and IL-6 cause conformational changes in retinal endothelial cells that increase their permeability.^{2–4} Vitreous levels of VEGF and IL-6 have been found to be elevated in patients with BRVO and CRVO;^{5,6} hence, both cytokines may contribute to oedema formation in these patients. Intravitreal anti-VEGF treatment reduces macular oedema and increases visual acuity, supporting the concept that this peptide is an important contributor to the pathogenesis of oedema.^{15–17} Intravitreal triamcinolone also achieves a reduction in macular oedema, and this fact points to inflammation as an important event occurring after BRVO and CRVO.^{18–21}

The chemokine family of cytokines is mainly considered to be pro-inflammatory; they are inducible in inflammatory conditions and function primarily as chemoattractants and activators of specific leucocytes at the sites of inflammation. The most important chemokine is IL-8, which has been recognized as a potent chemoattractant and activator of neutrophils and T lymphocytes, but not monocytes, and MCP-1, which exhibits chemoattractant potential for monocytes and lymphocytes, but not for neutrophils. Tight junctions of endothelial cells from different tissues are reported to be regulated by both IL-8 and MCP-1. In one study using primary cultures of mouse brain endothelial cells, MCP-1 induced re-organization of actin cytoskeleton and re-distribution of tight junction proteins, ZO-1, ZO-2, occludin, and claudin-5. These morphological changes were associated with an increase in permeability.²² In another study, addition of IL-8 to monolayers of the human dermal microvascular endothelial cell line HMEC-1 caused changes in tight junction structure; the changes were inhibited by IL-8 antibodies.²³

The role of IL-8 and MCP-1 in eye disease has also been investigated. *In vitro* expression and production of IL-8 and MCP-1 by human retinal endothelial cells and HRPE after stimulation by pro-inflammatory cytokines have been reported.¹⁰ MCP-1 significantly reduced ZO-1 protein expression in a mouse model of experimental autoimmune uveoretinitis.²⁴ Recently, Funk *et al*²⁵ reported elevated aqueous humour levels of IL-8 and MCP-1 in patients with CRVO, but not in those with BRVO. In this study we investigated whether IL-8 or MCP-1 are related with macular oedema in patients with BRVO by measuring the vitreous fluid levels of these

chemokines. Analysis of vitreous fluid is a better method than aqueous humour analysis to investigate the role of peptides in retinal disease, because of its proximity to retinal tissue. Moreover, it may contain cells that secrete these factors. To investigate the source of MCP-1 and IL-8, we also measured the plasma levels of these chemokines. Vitreous fluid IL-8 and MCP-1 levels were elevated in comparison to plasma levels in patients with BRVO, and there was no statistical correlation between vitreous fluid and plasma levels of either IL-8 or MCP-1. These results suggest that the elevated vitreous concentration of these chemokines is caused by intraocular hyperproduction, a conclusion supported by studies reporting IL-8 and MCP-1 production by human retinal endothelial cells.¹⁰ In the control group, comprised of patients with macular hole and macular epiretinal membrane, vitreous MCP-1 was higher than plasma MCP-1, suggesting that there is intraocular production of this chemokine.

We found that vitreous levels of IL-8 and MCP-1 were significantly higher in patients with BRVO than in the control subjects. In the study by Funk *et al*,²⁵ the levels of both chemokines in aqueous humour were elevated in patients with CRVO, but not BRVO, compared with control subjects. As was stated by the authors, extension of the area of thrombosis may explain the higher concentrations of chemokines in CRVO. Nonetheless, we found elevated vitreous concentrations also in BRVO. As has been mentioned earlier, vitreous fluid analysis is a better specimen for investigating retinal physiology than aqueous humour analysis and this may explain the differences with respect to study by Funk *et al*. Our findings suggest that IL-8 and MCP-1, and hence inflammation, contribute to the development of macular oedema in BRVO. An inflammatory response has been reported after systemic venous thrombotic events. Roumen-Klappe *et al*²⁶ found high plasma levels of IL-8 and IL-6 in patients with deep venous thrombosis. In an experimental baboon model, level of IL-6 and IL-8 peaked on day 2 after stasis-induced deep venous thrombosis and was associated with a venous inflammatory response consisting of early neutrophil infiltration into the vein wall, followed by extravasation of monocytes, macrophages, and lymphocytes.²⁷ Interestingly, Yoshida *et al*²⁸ reported that thrombin, a peptide whose main actions are related with clot formation (ie, converting fibrinogen to fibrin and activating platelet adhesion, aggregation, and secretion), induces secretion and mRNA expression of IL-8 and MCP-1 by HRPE cells. The inflammatory response elicited after a thrombotic event is needed for resolution of the thrombus.²⁹ We speculate that this physiological response may contribute to disruption

of the blood–retina barrier and formation of oedema due to the action on tight junctions mentioned earlier.

It should be mentioned that VEGF, probably the best known peptide related with permeability and angiogenesis, induces endothelial cell production of IL-8 and other cytokines, such as IL-6 and growth-related oncogen- α .³⁰

We believe that thrombosis may be the stimulating agent for the increased levels of both chemokines. Moreover, previous reports have shown that retinal ischaemia stimulates IL-8 and MCP-1 production, and both chemokines are involved in retinal neovascularization.^{31–34} Although ischaemia as seen using FFA was one of the exclusion criteria, we cannot absolutely exclude that this was present in our patients. So retinal ischaemia may have also contributed to hyperproduction of IL-8 and MCP-1.

We did not find a statistical correlation between macular thickness and vitreous IL-8 and MCP-1 levels. We believe that these chemokines contribute to oedema formation, but that many other peptides may also be involved, and some of them (eg, VEGF) may have a greater and more determinant role in regulating vascular permeability. In fact, Noma *et al*⁵ reported a statistical correlation between vitreous fluid levels of VEGF and IL-6 and foveal thickness in patients with BRVO.

Our study has some limitations. First, chemokine levels may have been elevated before the development of retinal vein thrombosis and not afterwards, and acted as risk factors for the development of BRVO. Vitreous samples in patients at risk of developing BRVO should be analysed to exclude this point, but such a study would be ethically unacceptable. Second, we have not corrected the concentration of the peptides under study for the total vitreal protein levels because of a small volume of the sample. However, plasma concentrations of both chemokines were strikingly lower than those obtained in the vitreous (~8.5 time less for IL-8 and ~5 time less for MCP-1). Therefore it seems clear that the elevated concentrations found in the vitreous fluid are not derived from blood. Last, further studies should be carried out to elucidate the role of IL-8 and MCP-1 in the regulation of tight junctions of retinal endothelial cells. Exposure of these cells to chemokines and study of the conformational changes occurring in the components of the tight junctions or transendothelial permeability could clarify this point.

In summary, vitreous levels of MCP-1 and IL-8 are elevated in patients with BRVO. Our study results suggest that intraocular synthesis is responsible for this increase. Inflammation associated with thrombotic events might be the stimulating agent for hypersecretion of these chemokines. Retinal ischaemia may also be related to this hypersecretion. IL-8 and MCP-1 may

contribute to increased vascular permeability and macular oedema in patients with BRVO.

Summary

What was known before

- Cytokines and macular oedema in retinal venous occlusive disease: vitreous levels of Interleukine-6 and vascular endothelial growth factor are increased in patients with macular oedema secondary to branch retinal vein occlusion, which suggests a possible role for these peptides in the pathogenesis of this condition.
- Interleukine-8 and monocyte chemoattractant protein-1 have also been involved in the pathogenesis of retinal venous occlusive disease after finding elevated levels in aqueous humour taps. Vitreous levels have not been measured before.

What this study adds

- We have determined vitreous levels of interleukine-8 and monocyte chemoattractant protein-1 in patients with branch retinal vein occlusion
- We have found elevated vitreous levels of both cytokines in these patients compared with controls, which suggests that they may have a role in the pathogenesis of macular oedema in branch retinal vein occlusion.

Conflict of interest

The authors declare no conflict of interest.

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