Aqueous flare and cells in eyes with retinal vein occlusion – correlation with retinal fluorescein angiographic findings

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Abstract
The laser flare cell meter (LFCM) was used to evaluate alterations of the blood-aqueous barrier in eyes with retinal vein occlusion (RVO). In 42 eyes of 42 patients with RVO (30 branch vein occlusions, 12 central vein occlusions) aqueous flare and aqueous cells were determined and retinal fluorescein angiography was performed. Flare and cell values were compared with 59 normal age and sex-matched control eyes and correlated with fluorescein angiographic findings. Both aqueous flare (12-321 (SD 6-717) photon counts/ms) and aqueous cells (mean 3-37 cells/0-075 x 10^6/l) were significantly increased in eyes with RVO in comparison with the normal control group (flare 4-288 (SD 1-144) photon counts/ms, cells 0-54 cells/0-075 x 10^6/l, p<0-0001 and p<0-005). Flare values in eyes with central RVO (19-517 (SD 7-762) photon counts/ms) were significantly higher than in eyes with branch RVO (9-443 (SD 3-307) photon counts/ms, p<0-0001). Significant correlations were found between flare values and area of RVO (r=0-70, p<0-0001) and between flare values and area of retinal non-perfusion (r=0-76, p<0-0001). There were also significant correlations between flare values and number of cotton wool spots (r=0-54, p<0-0002) and between cell counts and degree of cystoid macular oedema (r=0-46, p<0-006).

Our findings show that aqueous protein concentration, as indicated by flare, and aqueous cells are increased in RVO and that changes of aqueous flare correlate with fluorescein angiographic findings that reflect the severity of RVO. Measurements with LFCM may be useful non-invasively to quantify changes of the ocular barrier functions in RVO.

Methods and patients
Aqueous flare and aqueous cell were measured with the LFCM (FC-1000, Kowa Co, Ltd, Tokyo, Japan). The apparatus and techniques have been described previously.1 2 The sensitivity and reproducibility of the method have been confirmed by a number of groups in a series of studies.1 12–16

For this study, flare values were indicated as photon counts per millisecond (photon counts/ms). A total of 69 eyes of 69 patients with RVO were examined. Twenty seven patients were excluded because they had already undergone argon laser treatment. Thus, 42 patients (30 branch retinal vein occlusion (BVRO), 12 central retinal vein occlusion (CRVO)) were included in this study within 2 months of onset of their symptoms (22 men, 20 women, mean age 58·3 (SD 12-6) years, age range 25–84 years). Measurements with the LFCM and fluorescein angiography were performed within 1 week. As a control group, 59 randomly selected age and sex-matched normal eyes of 59 subjects (31 men, 28 women, mean age 58·6 (SD 11·6) years, age range 25–84 years) were examined.

In both groups, exclusion criteria were presence of diabetes, pseudoexfoliation, and previous ocular surgery. Also excluded were patients with iris neovascularisation visible at slit-lamp examination. All patients with RVO included in this study had at least one fluorescein angiogram available for review. All of the fluorescein angiograms were reviewed by the authors. In each angiogram, the area of venous outflow obstruction and the area of retinal non-perfusion were estimated in disc areas. In addition, the number of cotton wool spots were counted and degree of retinal haemorrhages and cystoid macular oedema (CMO) were determined semiquantitatively (grade 0–3).

Flare values and cell counts were measured 30 minutes after pupillary dilatation with 0·5% tropicamide and 5% phenylephrine hydrochloride by a total of three examiners. From each eye five measurements were taken and averaged, and care was taken to exclude all measurements spoiled by artefacts. The data were processed using personal computers and statistically analysed with the non-parametric Mann-Whitney test and regression analysis.

Results
Both flare values and cell counts of 42 eyes with RVO (flare 12-321 (SD 6-717) photon counts/ms, cell 3-37, range 0–19·0 cells/0-075 x 10^6/l) were significantly higher than in normal control eyes (flare 4·288 (SD 1·144) photon counts/ms,
cell 0.54, range 0.3-6 cells/0.075×10^6, p<0.0001 and p<0.005).

Flare values in eyes with CRVO were 19.517 (SD 7.762) photon counts/ms, and in eyes with BRVO were 9.443 (SD 3.307) photon counts/ms. Statistically significant differences in flare were found between eyes with CRVO and normal control eyes (p<0.0001), and between eyes with BRVO and normal eyes (p<0.0001). Flare values were significantly higher in eyes with CRVO than in BRVO (p<0.0001) (Table 1).

Cell counts in all groups showed considerable variation. In eyes with CRVO, cell counts were 3.87 cells/0.075×10^6 (range 0.15-6). The group of eyes with BRVO had cell counts of 3.16 cells/0.075×10^6 (range 0.19-0). Statistically significant cell count differences were found between CRVO eyes and normal eyes (p<0.005), and between BRVO eyes and normal eyes (p<0.005). No significant differences were found between eyes with CRVO and eyes with BRVO (p>0.5) (Table 2).

Regression analysis showed correlation between flare and the area of RVO (r=0.70, p<0.0001) (Fig 1) and between flare and the area of retinal non-perfusion (r=0.76, p<0.0001) (Fig 2). Flare values also showed some correlation to number of cotton wool spots (r=0.54, p<0.0002). No significant correlations were observed between flare and extent of retinal haemorrhages, and between flare and degree of CMO (p<0.5) (Table 3). There appeared to be some correlation between cell counts in eyes with RVO and degree of CMO (r=0.46, p<0.006) whereas no such correlation was seen between cell counts and any of the rest of the above mentioned angiographic parameters (Table 3).

Discussion

The results of our study clearly indicate that aqueous protein concentration, as determined by aqueous flare, is increased in eyes with RVO. This is in accordance with previous studies. Zirm, who used anterior chamber tap and biochemical analysis, found increased albumin concentration in the aqueous of eyes with CRVO, and observed flare in the aqueous humour in cynomolgus monkeys following experimental RVO. Increased protein concentration in the anterior chamber indicates an impairment of the blood-ocular barriers. Accordingly, studies using vitreous fluorophotometry demonstrated significant changes of the blood-retinal barrier permeability to fluorescein in patients with CRVO, and these changes correlated with retinal fluorescein angiographic findings. Our results also showed correlations between flare values and area of RVO, between flare values and area of retinal non-perfusion, and between flare values and the number of cotton wool spots. Miyake et al. measured aqueous flare with the LFCM in eyes with RVO and found significantly increased values. Virdi et al. observed flare in the aqueous humour in cynomolgus monkeys following experimental RVO. Increased protein concentration in the anterior chamber indicates an impairment of the blood-ocular barriers. Accordingly, studies using vitreous fluorophotometry demonstrated significant changes of the blood-retinal barrier permeability to fluorescein in patients with CRVO, and these changes correlated with retinal fluorescein angiographic findings. Our results also showed correlations between flare values and area of RVO, between flare values and area of retinal non-perfusion, and between flare values and the number of cotton wool spots. Miyake et al. measured aqueous flare with the LFCM in eyes with RVO and found significant differences in flare values between eyes with CRVO and normal control eyes (p<0.0001), and between eyes with BRVO and normal eyes (p<0.0001). Flare values were significantly higher in eyes with CRVO than in BRVO (p<0.0001) (Table 1).
least higher values of aqueous flare and aqueous fluorescein than in normal control eyes.

Alterations of the blood-aqueous barrier similar to the retinal changes may occur in the anterior segment of the eye with RVO. Using iris fluorescein angiography, iridal neovascularisation can sometimes be detected very early after RVO before it is detectable on slit-lamp examination.16 17 Virdi et al.18 found increased fluorescein in the aqueous and iris vascular leakage on fluorescein angiography in eyes with major branch or CRVO without any evident iris abnor-
mality or rubeces. Fluorescein leakage from iris vessels was also found in monkeys with experimental RVO before the development of iris neovascularisation, and there was a correlation between the retinal leakage and the development of ocular neovascularisation.19 Therefore, leakage of protein through altered iris vessels is a likely cause of increased flare values in eyes with RVO. The alterations of iris vessels in RVO are believed to be caused by growth factors that are released by the ischemic retina into the intra-
ocular fluids to the iris.20 21 Another possible mechanism that could be responsible for increased aqueous protein may be passive diffusion of protein from the retina via vitreous cavity and posterior chamber into the anterior chamber, as there is no dense barrier that prevents diffusion from the vitreous into the aqueous.22-24 This possible mechanism may correspond with the observations by Amuzi et al.25 in eyes with diabetic retinopathy, who found that aqueous flare is increased distinctly after vitreous haemorrhage. However, Miyake et al.,26 who used anterior chamber and vitreous fluorophotometry in eyes with RVO, observed that fluorescein concentrations were only increased in the anterior chamber and the posterior vitreous, whereas values in the middle vitreous were low or normal. Therefore they concluded that increased aqueous flare in RVO mainly reflects blood-aqueous barrier disruption.

Although the mechanisms by which aqueous flare is increased in eyes with RVO are not fully understood, our results suggest that the LFCM is a useful tool for quantification of changes in the aqueous in eyes with RVO.

Our measurements also showed increased cell counts in eyes with RVO. These 'cells' may represent leucocytes or even melanin granules. Interestingly, the finding of a correlation of cell counts with degree of CMO might point to pathogenetic mechanisms in the development of CMO such as release of prostaglandins that lead to increased vascular permeability and diapedesis of leucocytes. For clinical evaluation of RVO with the LFCM, flare values seem to be more useful than cell counts as cell counts show high variations both in normal eyes and in eyes with RVO.

Measurements of the aqueous flare with the LFCM can be useful to quantify the extent and severity of the alterations of the blood-ocular barriers in eyes with RVO, as we and others27 28 found a correlation between flare values and both the area of RVO and the area of non-perfused retina. However, as the correlation between flare and area of retinal non-perfusion is stronger, retinal non-perfusion might possibly be the factor that affects the blood-aqueous barrier more severely. But still, eyes with CRVO without significant retinal non-perfusion may show high flare values (Fig 2), thus indicating that RVO without non-perfusion also alters the blood-aqueous barrier. Aqueous flare may also be of prognostic significance in the development of iris neovascularisation, because the development of iris neovascularisation with RVO correlates with the extent of retinal non-perfusion29 and with the degree of retinal vascular leakage.30 Further studies are needed to follow up aqueous changes in eyes with RVO and to correlate these changes with electrophysiological finding, visual outcome, and the development of complications such as iridal neovascularisations and neovascular glaucoma, and to assess the effects of argon laser photoocoagulation on aqueous flare and aqueous cells.


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