SCIENTIFIC REPORT

Diabetic cataract removal: postoperative progression of maculopathy—growth factor and clinical analysis

J I Patel, P G Hykin, I A Cree

Br J Ophthalmol 2006;**90**:697–701. doi: 10.1136/bjo.2005.087403

Background: Diabetic cataract extraction can be frequently complicated by macular oedema, progression of retinopathy, or development of iris neovascularisation. The pathogenesis of these complications may be the result of changes in the concentration of angiogenic and anti-angiogenic cytokines in the immediate postoperative period. The study aims to prospectively analyse this.

Methods: Uneventful phacoemulsification with intraocular lens implant was performed in seven eyes of six patients with diabetic retinopathy ranging from severe non-proliferative to quiescent proliferative. Patients were reviewed 1 day, 1 week, 1 month, and 3 months after surgery with fundus fluorescein angiography (FFA) and aqueous sampling. Each sample was analysed for VEGF, HGF, IL-1 β (pg/ml), and PEDF (μ g/ml) by sandwich ELISA.

Results: Clinically significant macular oedema (CSMO) occurred in one patient although increased macular hyperfluorescence occurred in three patients on FFA at 1 month. VEGF 165 concentration increased 1 day after surgery from a median baseline of 68 pg/ml (range 22–87 pg/ml) to 723 pg/ml (range 336–2071) at day 1. By 1 month it had decreased to 179 (range 66–811 pg/ml). HGF concentrations steadily increased over the month while IL-1 β and PEDF concentrations demonstrated an acute rise on day 1 after surgery and then IL-1 β returned to baseline concentrations while PEDF decreased to below baseline.

Conclusion: These results confirm altered concentrations of angiogenic and antiangiogenic growth factors after cataract surgery, which may induce subclinical and clinical worsening of diabetic maculopathy.

Diabetic cataract extraction can be complicated by macular oedema, progression of retinopathy, or development of iris neovascularisation.¹ The incidence of such complications is greater with more advanced retinopathy. Results of cataract surgery in such patients compared to those without diabetes are less favourable with a final post operative visual acuity of 6/60 or worse in 28%–50% of patients with diabetic retinopathy.²

The pathogenesis of such developments after surgery may be related to alterations in the angiogenic growth factors profile at the vitreoretinal interface. VEGF 165 (vascular endothelial growth factor) and HGF (hepatocyte growth factor) are both important angiogenic growth factors. Raised concentrations of both have been described in the vitreous of patients with proliferative diabetic retinopathy and hence thought to mediate neovascularisation.^{3–4} Both have also been shown to mediate vasopermeability. Inflammatory cytokines—for example, interleukin 1 β (IL-1 β), may also contribute to the breakdown of the blood-retinal barrier leading to the development of macular oedema.⁵

Furthermore, a change in the concentration of pigment epithelial derived growth factor (PEDF), an important intraocular anti-angiogenic agent, may allow for the unhindered action of VEGF and HGF.

However, no study to date has examined the changes in these cytokines after cataract surgery in diabetic patients or correlated them with post-surgery degree of retinopathy and maculopathy.

As diffusion gradients for cytokines may exist from the vitreoretinal interface to the anterior chamber (as reflected in the development of rubeosis), relative differences in the aqueous concentrations of these growth factors may reflect their interactions after cataract surgery. Such interactions may determine the pathophysiology of worsening retinopathy after cataract surgery.

METHODS

Clinical assessment at baseline, 1 day, 1 week, and 1 month after cataract surgery included best corrected visual acuity, clinical slit lamp evaluation with non-contact fundus lens for clinically significant macular oedema (CSMO), and intravenous fluorescein angiography (FFA). Slit lamp evaluation was performed by JP and FFA were assessed by PH.

Patients with severe non-proliferative (severe NPDR), quiescent non-proliferative PDR, or low risk proliferative diabetic retinopathy (PDR) who underwent routine phacoemulsification and intraocular lens implant were recruited to this study. Those patients with coexistent retinal disease or those who had a complicated cataract procedure—for example, vitreous loss, were excluded. Patients were recruited in accordance with the Declaration of Helsinki and with the approval of the ethics committee of Moorfields Eye Hospital.

Patients underwent standard scleral pocket phacoemulsification with implantation of a 7 mm PMMA lens. Aqueous samples were taken at baseline, 1 day, 1 week, and 1 month.

Analysis for VEGF-165, HGF, IL-1 β , and PEDF in the aqueous samples was correlated with simultaneous clinical findings. Where sufficient aqueous was obtained, a detailed growth factor analysis for each study patient was performed.

ELISA VEGF-165, HGF, IL-1 β

A volume of 30 μ l of the primary capture antibody specific to the growth factor was added to wells in a 384 well plate and incubated overnight at room temperature (20–25°C). The wells were blocked with 50 μ l of phosphate buffered saline (PBS) 0.1% bovine serum albumin (BSA) for 1 hour. Then,

Abbreviations: BSA, bovine serum albumin; CSMO, clinically significant macular oedema; DM, diabetes mellitus; FFA, fundus fluorescein angiography; HGF, hepatocyte growth factor; HRP, horseradish peroxidase; IL-1 β , interleukin 1 β ; NAD, no abnormality detected; NPDR, non-proliferative diabetic retinopathy; PBS, phosphate buffered saline; PDR, proliferative diabetic retinopathy; PEDF, pigment epithelial derived growth factor; PRP, panretinal photocoagulation; TBS, TRIS-buffered saline; VEGF, vascular endothelial growth factor

Table 1 Profile of antibodies used for ELISA for VEGF, HGF, IL-1 β

R & D antibodies	Primary antibody concentration (ng/ml)	Secondary capture concentration (ng/ml)	Inter-assay CoV	Intra-assay CoV	Sensitivity
VEGF-A (isoform 165)	Polyclonal 125 ng/ml	Polyclonal, biotinylated 100 ng/ml	10%	3%	1 pg/ml
HGF	Monoclonal, 2000 ng/ml	Biotinylated, 250 ng/ml	14%	1%	5 pg/ml
IL-1 β	Polyclonal, 2000 ng/ml	Biotinylated, Polyclonal, 200 ng/ml	17%	18%	1 pg/ml

30 μ l of aqueous was added (1:4 dilution with PBS, pH 7.4) and incubated for 2 hours. Thereafter, 30 μ l of the human biotinylated affinity purified detection or secondary antibody followed by the addition of 30 μ l of Neutravidin horseradish peroxidase conjugated (Pierce, IL, USA) at 0.125 μ g/ml for 30 minutes. Finally, 30 μ l of LumiGLO Reagent and Peroxide (from New England Biolabs, USA) was added for 5 minutes and the plate read in a luminometer. Between the addition of each antibody and reactive agent the wells were washed with PBS, the last wash being with TRIS-buffered saline (TBS 50 mM, pH 8). All sample readings were quantified from a standard curve produced using recombinant human cytokine (from R & D).

The nature and the concentrations of each pair of antibodies for each growth factor and their respective inter-assay and intra-assay coefficient of variation (CoV) and concentration sensitivity are shown in table 1. All antibody pairs and standard growth factor peptides were obtained from R and D Systems, Minneapolis, MN, USA. Inter-assay CoV was determined by evaluating the concentration of the cytokine in a similar control sample on three consecutive ELISAs, while the intra-assay CoV was similarly determined in a similar control sample but which was evaluated three times in the same assay.

Luminescence immunoassay for PEDF

A volume of 30 μ l of 10 μ g/ml PEDF were incubated using a 384 well plate overnight at 4°C. Then PEDF standards or samples were incubated with PEDF antibody at 1:1000 for 1 hour at room temperature. The wells were blocked with 50 μ l of PBS, BSA 0.5% for 1 hour at room temperature. The wells were washed with 100 μ l PBS six times. A volume of 30 μ l of samples and standards in duplicate were added to the wells for 2 hours at room temperature. The wells were washed as before. Then 30 μ l of secondary antibody horseradish peroxidase (HRP) goat anti-rabbit antibody at 1:10 000 for 1 hour at room temperature was added to each well and subsequently the plate washed with PBS. Lastly, 30 μ l of LumiGLO Reagent and Peroxide (from New England Biolabs, USA) was added for 5 minutes and the plate read in a luminometer.

RESULTS

Patient data

Six patients (seven eyes) were recruited with the following characteristics. The average age was 62 years (53–71 years) with a mean duration of diabetes mellitus (DM) of 12 years (4–25 years). All were type 2 DM with an average HbA_{1c}: 8% (5.8–9.9%).

At baseline no eye had preoperative CSMO, two had severe NPDR, two had low risk PDR, and three had quiescent PDR. Previous macular laser had been applied to five eyes. Previous panretinal photocoagulation (PRP) had been applied in six eyes.

All patients underwent routine uncomplicated phacoemulsification with insertion of a posterior chamber intraocular lens.

Fluorescein angiographic assessment at 1 month post-surgery revealed that in three eyes there was only an increased macular hyperfluorescence compared to preoperative intravenous fluorescein angiography. At 1 month, one eye demonstrated CSMO. No increase in hyperfluorescence was noted in the three remaining eyes at 1 month. No eyes progressed in relation to their retinopathy status. The one eye with CSMO was treated with extra macular laser at 1 week (see table 2)

Biochemical data

Owing to the small sample volumes and the fact that not all the patients consented to all samples, the cytokine levels given represent the actual number of samples available for each patient at each time point

Overall aqueous cytokine profile after cataract

VEGF-165 demonstrated an increase 1 day after surgery from a median baseline of 68 pg/ml (range 22–86 pg/ml) to 723 pg/ml (range 336–2071). At 1 week it had decreased to 113 pg/ml (range 2–203 pg/ml). At 1 month it increased to 179 pg/ml (range 66–811 pg/ml) (see fig 1 and table 2)

HGF increased in concentration from baseline of 119 pg/ml (range 80–130 pg/ml) to a 1 month concentration of 1214 pg/ml (range 228–2975 pg/ml) (see fig 1 and table 2).

IL-1 β increased acutely 1 day after surgery (median 18.5 pg/ml (range 2–31.75 pg/ml), with a median baseline of 2.3 pg/ml and range 0.6–5.17 pg/ml). It then decreased back to baseline concentrations over the proceeding month (see fig 1 and table 2).

PEDF concentrations increased in the first postoperative day but then declined to a concentration below baseline at 1 month after surgery (baseline 0.82 μ g/ml, range 0.7–1.73 μ g/ml), 1 month median 0.65 μ g/ml (range 0.55–0.7 μ g/ml) (see fig 1 and table 2).

Statistical analysis could not be performed because a complete set of aqueous samples was not taken at each time point owing to compliance despite consent at the start of the trial. Hence these figures represent a trend.

Aqueous profile for each individual outcome

A growth factor descriptive analysis of the three macular related clinical outcomes (no abnormality/change detected compared to baseline, NAD, only angiographic hyperfluorescence and CSMO) showed that in both hyperfluorescence and CSMO there was an acute increase in IL-1 β concentration. In hyperfluorescence the concentration then returned to baseline by 30 days after surgery. Owing to lack of aqueous, a similar complete 1 month profile could not be made for CSMO. The VEGF-165 concentration demonstrated an acute increase but decreased towards baseline by 1 month after surgery. HGF concentration increased above baseline for both hyperfluorescence and CSMO, but in CSMO it was greater

Table 2 Cytokine aqueous concentrations after cataract surgery and corresponding 1 month retinopathy and maculopathy for each study eye

	Baseline	Day 1	1 Week	1 Month	1 Month clinical status
Eye 1	QPDR				QPDR
VEGF	83.6	2071.5	113	465.4	Hyperfluorescence
HGF	101	665	1105	227	
IL-1 β	2.6	22.6	3.7	2.8	
PEDF	0.97	0.77	0.6	0.65	
Eye 2	Low risk PDR				Low risk PDR
VEGF	53	1920	41	179	Hyperfluorescence
HGF	119	953	1105	1214	
IL-1 β	0.65	8.02	10.9	2.0	
PEDF	0.72	1.53	0.57	0.55	
Eye 3	Severe NPDR				No change
VEGF	22.4	380	NA	NA	No change
HGF	131	620	NA	NA	
IL-1 β	1.26	2.2	NA	NA	
PEDF	1.73	1.21	NA	NA	
Eye 4	QPDR				QPDR
VEGF	44.5	722.7	NA	NA	Hyperfluorescence
HGF	81	529.5	NA	NA	
IL-1 β	5.18	18.6	NA	NA	
PEDF	0.50	1.7	NA	NA	
Eye 5	Severe NPDR				Severe NPDR
VEGF	86.6	336	2.6	65.8	CSMO
HGF	131	2042.7	NA	2975	
IL-1 β	2.32	31.7	NA	NA	
PEDF	0.82	3.18	2.63	0.71	
Eye 6	Low risk PDR				Low risk PDR
VEGF	NA	1418	203	811	No change
HGF	NA	NA	NA	NA	
IL-1 β	NA	NA	NA	NA	
PEDF	NA	NA	NA	NA	
Eye 7	QPDR				QPDR
VEGF	114	517	192	119	No change
HGF	NA	NA	NA	NA	
IL-1 β	NA	NA	NA	NA	
PEDF	NA	NA	NA	NA	

Macular hyperfluorescence on fundus fluorescein angiography, No change compared to baseline, CSMO, clinically significant macular oedema. VEGF, HGF, and IL-1 β in pg/ml, PEDF in μ g/ml. NA, no information available. At baseline there was no macular hyperfluorescence or CSMO; QPDR, quiescent proliferative retinopathy; Low risk PDR, low risk proliferative retinopathy; Severe NPDR, severe non-proliferative retinopathy.

Figure 1 The aqueous cytokine profile seen in the month following cataract surgery (n = the number of aqueous samples for each cytokine at each time point).

Figure 2 The aqueous cytokine profile for individual clinical outcomes. A few of the outcomes for each cytokine at each time point may not be available owing to lack of compliance by patients for aqueous sampling. NAD, no abnormality detected; hyperfluorescence, macular hyperfluorescence on FFA; CSMO, clinically significant macular oedema.

and continued to increase over the 1 month after surgery. PEDF concentrations on the whole decreased after surgery to a concentration lower than baseline over the postoperative month. These results represent trends as statistical analysis was not possible (see fig 2 and table 2).

DISCUSSION

This is the first prospective study examining the relation of the baseline and postoperative aqueous growth factors in patients with diabetes mellitus undergoing routine phacoemulsification and correlating these concentrations with the development of postoperative macular changes either clinically or on fundus fluorescein angiography. Central macular angiographic hyperfluorescence developed at 1 month after surgery in three of the seven eyes, and one patient progressed to CSMO although visual acuity was unaffected.

VEGF-165 and HGF are both angiogenic and in both clinical outcomes of angiographic macular hyperfluorescence and in CSMO, their concentrations increased in the postoperative period, suggesting that the increase in these factors (both of which can damage the blood retinal barrier^{6,7}) are able to induce the clinical and angiographic changes seen 1 month after surgery.

The inflammatory marker IL-1β was also raised after surgery as would be expected after a surgical procedure. IL-1β can also damage the tight junctions of retinal vascular endothelial cells in an experimental model of uveitis.⁸ It can also stimulate VEGF⁹ and HGF release by both fibroblasts and endothelial cells in culture.^{10,11} Therefore, the increase in IL-1β in the acute stage may also contribute to the increase in VEGF and HGF. This triad of growth factors is then in a position to damage the integrity of the blood-retinal barrier.

In conjunction with these changes, there was a reciprocal decrease in the anti-angiogenic PEDF concentration. This decrease in the protective function of PEDF, together with the increase in VEGF-165 and HGF concentration, may explain the observed postoperative macular changes.

The main limitation of this study was the low recruitment of patients and also despite the patients being informed of the study protocol, some enrolled patients declined to complete the full postoperative aqueous sampling schedule. As a result, despite having a complete set of clinical data on each patient at each time point, aqueous sampling was not as complete and therefore the growth factor data presented suggest only trends.

This is a prospective examination of the possible molecular explanation of postoperative macular hyperfluorescence and development of CSMO. It suggests that these macular changes are preceded by increases in angiogenic and inflammatory cytokines with a decrease in the antiangiogenic cytokine PEDF.

Authors' affiliations

J I Patel, I A Cree, Institute of Ophthalmology, London, UK
J I Patel, P G Hykin, Moorfields Eye Hospital, London, UK

Correspondence to: Mr J I Patel, Department of Pathology, Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK; jigs37@hotmail.com

Accepted for publication 23 February 2006

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