The –174G/C interleukin-6 promoter polymorphism influences the development of macular oedema following uncomplicated phacoemulsification surgery

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Abstract

Aim To determine whether the functional –174 G/C interleukin-6 gene polymorphism is a risk factor for the development of cystoid macular oedema (CMO) following routine uncomplicated phacoemulsification surgery in patients with no established risk factors.

Methods A total of 40 patients who underwent routine phacoemulsification surgery as part of a randomised controlled trial comparing the use of postoperative steroid drops against a single sub-tenon injection of triamcinolone were genotyped for the IL-6 –174G/C polymorphism. All patients underwent fluorescein angiography at 30 days and anterior chamber flare measurements pre-operatively and at day 1, 7, and 30.

Results Angiographic CMO developed in 14 patients of the 40 studied. 9 out of the 14 patients carried the GG genotype (Fisher's exact test $P = 0.05$, Hazard ratio for GG genotype; 4.05 (1.02–16.00)). There was no difference in flare measurements between the GG and Non-GG (GC/CC) group. The two groups were otherwise well matched in terms of age, sex, phacoemulsification energy used intraoperatively, and proportion of patients receiving postoperative triamcinolone or steroid drops.

Conclusion The –174G/C interleukin-6 promoter gene variant appears to modulate the

response to phacoemulsification surgery and to influence the development of postoperative CMO. These data suggest a genetic predisposition to this complication.

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Introduction

Despite considerable advances in the field of cataract surgery, cystoid macular oedema (CMO) continues to be an important cause of suboptimal postoperative vision.¹ CMO following cataract surgery was initially reported by Irvine in 1953 and is known as the Irvine–Gass syndrome.² Approximately 20% of the patients who undergo uncomplicated phacoemulsification develop angiographically proven CMO.³ The exact mechanisms underlying the development of CMO following cataract surgery are unclear. It has been suggested that inflammatory mediators, in particular prostaglandin E₂, released in response to surgical trauma may play an important role in the development of CMO,⁴ through the breakdown of the blood–retinal barrier. The inflammatory response to cataract

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surgery is, however, complex and is likely to involve multiple factors.

Interleukin-6 (IL-6) is a pleiotropic cytokine with proinflammatory characteristics and is thought to be one of the main mediators of anterior uveitis.⁵ Malecaze *et al*⁶ showed that aqueous level of IL-6 increased dramatically in patients following extracapsular cataract surgery. Intra-vitreous injection of IL-6 has been reported to induce ocular inflammation, accompanied by a breakdown of the blood ocular barrier.⁷ Recent studies have examined the role of interleukin-6 in the pathogenesis of diabetic macular oedema.

Elevated levels of IL-6 were found in the vitreous of patients with diabetic macular oedema.⁸ Another study found elevated levels of IL-6 in the aqueous humour in patients with diabetic macular oedema,⁹ with increasing levels of IL-6 correlating with the severity of macular oedema. Funatsu *et al*¹⁰ found that the preoperative aqueous concentration of IL-6 was a predictor of subsequent macular oedema following phacoemulsification in patients with nonproliferative diabetic retinopathy. IL-6 may thus be an important factor in the pathogenesis of CMO in non-diabetics following uncomplicated cataract surgery.

A common G/C polymorphism located within the IL-6 gene promoter at position -174 has been reported to influence IL-6 expression, with the G allele being associated with higher expression levels in response to stimulation.¹¹ We thus sought to identify whether carriage of the (GG) IL-6 genotype increased the risk of CMO following routine cataract surgery.

Materials and methods

Patient population

From April 2001 to March 2003, a randomised prospective controlled trial was carried out at the Queen's Medical Centre, Nottingham, to ascertain the efficacy of a single per-operative triamcinolone injection as compared to a standard routine postoperative regime of steroid drops. The methodology and results of this study are detailed elsewhere.¹² During this period, 54 eyes of 54 Caucasian patients undergoing elective uncomplicated phacoemulsification surgery at the Queen's Medical Centre, Nottingham, were enrolled.

Inclusion criteria were patients with uncomplicated, age-related cataracts listed for phacoemulsification surgery. Exclusion criteria include patients with diabetes, patients on oral or topical anti-inflammatory agents (steroids and non-steroidals), family history of glaucoma, a history of steroid responsiveness, eyes with uveitis, glaucoma, age-related macular degeneration, corneal disease, and a history of CMO if the fellow eye had had

previous cataract surgery. Ethical committee approval was sought and gained for the genetic aspect of the study.

Outcome measures

Anterior chamber (AC) flare

AC inflammation was assessed both preoperatively and at each follow-up visit by one of the investigators (AN). This was performed by measuring AC flare on the Kowa-500 laser flare metre. All measurements were made with undilated pupils before the instillation of any local anaesthetic drops or dilating drops. The mean of five readings was taken.

Oral fluorescein angiography (OFA)

OFA was performed at day 30 postoperatively as described elsewhere.¹² The angiograms were read by a masked observer for the presence or absence of CMO and graded into four grades depending upon the severity of fluorescein leakage.¹³

Selection of patients for genetic testing

The randomised controlled trial described above showed no difference in postoperative inflammation measures or angiographic CMO rates in patients treated with one month of drops compared with a single per-operative injection of triamcinolone acetate. Owing to this lack of difference, we felt that this was a suitable population to study. Of the 54 patients enrolled into the original trial, three patients had died, therefore 51 patients were contacted by post. However, of these, only 40 proved contactable for the purposes of this study. Patients had either changed address or simply did not respond despite multiple telephone calls. For all those contacted successfully, 10 ml of blood was collected from each patient into a EDTA-containing bottle and frozen at -20°C until screened for the IL-6 polymorphism.

IL-6 genotyping

DNA was extracted from the peripheral blood leucocytes and the IL-6 -174 G/C polymorphisms were identified as described elsewhere.¹⁴ Briefly; the *Sfa*NI (restriction enzyme) polymorphism is owing to a replacement of C by G at position 174, and primers were designed to amplify the promoter region of IL-6 gene. The primers used in the PCR were: 5'-TGACTTCAGCTTTACTCTTT GT-3' and 5'-CTGATTGGAAACCTTATTAAG-3'. PCR products were digested with the *Sfa*NI restriction enzyme and electrophoresed on a 2% agarose gel stained with ethidium bromide. The identified genotypes were named according to the presence or absence of the

enzyme restriction site. The genotyping was carried out at another centre by PC who was blinded to the results of the clinical data.

Statistical analysis

The unpaired *t*-test and Fisher's exact test was used to determine associations between the -174 G/C IL-6 polymorphism and various demographic and outcome measures. All statistical analyses were carried out on SPSS version 13.0.

Results

Patient's characteristics according to genotype are depicted in Table 1. Patient characteristics were similar in the GG and non-GG genotype groups in terms of basic demographics, proportion of patients receiving the triamcinolone injection, and the amount of energy used during phacoemulsification. There was no association between anterior chamber flare at any time point and genotype.

Angiographic CMO developed in 14 patients of the 40 studied. Nine out of the 14 patients carried the GG genotype. Table 2 shows the association between genotype and angiographic CMO.

Discussion

Cystoid macular oedema is a relatively common (1–2%)¹⁵ postoperative cause of poor vision after routine uncomplicated phacoemulsification surgery. Given that 300 000 such procedures were performed in the UK last year, this amounts to 3000–6000 patients developing significant CMO. Little is known about the precise mechanisms underlying the development of CMO especially in patients with no recognizable risk factors undergoing routine surgery.

In our investigation, the -174 G/C polymorphism of the promoter of the IL-6 gene (a potential genetic

modulator of the inflammatory reaction to surgery) was associated with postoperative angiographic cystoid macular oedema. Given the data linking diabetic macular oedema to elevated interleukin-6 levels in the human eye,^{8,9} it is entirely conceivable that this important multi-functional cytokine may have an important role to play in the development of CMO in non-diabetics. CMO is a result of leakage from perifoveal capillaries, and IL-6 may mediate this through increasing the expression of VEGF,¹⁶ a potent factor in the modulation of vascular permeability. IL-6 itself has also been shown to increase endothelial permeability directly by affecting the shape of endothelial cells.¹⁷ Our investigation also shows that in order for to have a significantly increased susceptibility to developing macular oedema following cataract surgery, a patient must carry two copies of the G allele. Patients with the GG genotype would produce increased levels of IL-6 in response to equivalent surgical trauma compared to patients who were GC/CC at the locus. Increased levels of IL-6 could cause CMO through some of the mechanisms described above.

Interestingly, we found no correlation between IL-6 genotype and AC flare, despite evidence that IL-6 can induce a breakdown of the blood–aqueous barrier.¹⁸ This might be explained by the modulating effect of postoperative steroids on anterior segment inflammation. It has also been shown previously that eyes developing angiographic CMO after phacoemulsification surgery do not necessarily have higher levels of aqueous flare.³

The main limitation of this study is the small sample size, hence the association between the GG genotype and

Table 2 Incidence of postoperative angiographic CMO according to IL-6 promoter polymorphism

Genotype	With CMO	Without CMO	Odds ratio (95% CI)
GG (n = 17)	9 (53%) ^a	8 (47%)	4.05 (1.02–16.00)
Non-GG (n = 23)	5 (22%)	18 (78%)	Reference

^aFisher's exact test: *P* = 0.05.

Table 1 Characteristics of the patients according to IL-6 promoter polymorphism

	GG (n = 17)	GC/CC (n = 23)	P-value
Age	71 (±8)	73 (±9)	0.34
Males	12	15	1.00
No. in group receiving triamcinolone injection	8	14	0.52
Preoperative VA Log MAR	0.32 (±0.16)	0.43 (±0.34)	0.22
Preoperative Flare (photons/ms)	8.7 (±3.6)	8 (±3.6)	0.53
Absolute Phaco power (seconds at 100%)	0.14 (±0.20)	0.10 (±0.09)	0.48
Month 3 flare (photons/ms)	8.8 (±2.6)	10.3 (±3.5)	0.15
Month 3 Log MAR VA	0.001 (±0.08)	-0.016 (±0.06)	0.45

Statistical analysis revealed lack of difference between the GG and GC/CC groups for all the variables tabulated above. Figures provided are means (±) standard deviation (*t*-test for continuous variables and Fisher's exact test for comparing proportions).

CMO is only just significant. However, this particular group of patients was selected because they had no risk factors whatsoever for CMO, having been carefully studied previously. It is also possible that the result may have been biased by the absence of 11 patients we were unable to contact. The study could be further enhanced by measuring the aqueous levels of IL-6 postoperatively to provide a comparison with the genetic analysis; however, this would only be possible in a prospective experiment. Despite the limitations, this study provides some evidence that a functional polymorphism in the IL-6 gene may contribute to CMO following routine phacoemulsification. Clearly, this area needs further studies to confirm the above association and to study other genes that may be relevant.

Ethical approval was granted by the LREC (Nottingham).

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