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Expression of 12 cytokines in aqueous humour of uveal melanoma before and after combined Ruthenium-106 brachytherapy and transpupillary thermotherapy

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ABSTRACT.

Purpose: **To determine the aqueous humour levels of 12 cytokines in eyes with uveal melanoma and whether their expression changes after combined Ru-106 brachytherapy and transpupillary thermotherapy (TTT).**

Methods: **Aqueous humour samples were collected from 20 patients with previously untreated uveal melanoma undergoing combined Ru-106 brachytherapy and TTT, both at the time of plaque placement and removal. Using multiplex biochip array technology, 12 different cytokines were measured, including interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), tumour necrosis factor (TNF)-α, interferon (IFN)-γ, epidermal growth factor (EGF) and monocyte chemoattractant protein (MCP)-1. Aqueous humour from 20 patients undergoing cataract surgery was used as control.**

Results: **IL-6, IL-8, IFN-γ and MCP-1 were highly expressed in uveal melanoma, whereas IL-2, IL-10 and TNF- α were low in expression. There was a positive correlation between tumour height and IL-8 level ($p = 0.020$). Vascular endothelial growth factor tends to be highly expressed in melanoma-containing eyes ($p = 0.056$). Levels of IL-6, IL-8 and IL-1β increased after the mean 117 $\pm$ 38 hrs of brachytherapy and adjunctive TTT with a tumour apex dose of 61 $\pm$ 28 Gy and a scleral contact dose of 786 $\pm$ 226 Gy. Increase in levels of IL-6 ($p = 0.003$) and IL-8 ($p = 0.046$) positively correlated with scleral contact dose.**

Conclusions: **Cytokines such as IL-6, IL-8, IFN-γ and MCP-1 may be implicated in the progression of uveal melanoma. Ocular irradiation from a Ru-106 plaque promoted an increase in the levels of IL-6, IL-8 and IL-1β, modulation of which could be useful in managing radiation-related side effects.**

Key words: angiogenesis – brachytherapy – cytokine – inflammation – uveal melanoma

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Introduction

Uveal melanoma is the most common primary intraocular tumour in adults. Up to 50% of patients may develop systemic metastasis with a fatal outcome (Kujala et al. 2003). Enucleation had been the standard treatment, but eye-conserving plaque radiotherapy has become a popular alternative. Brachytherapy using isotopes such as Ruthenium (Ru)-106 and Iodine (I)-125 has achieved local control rates above 90% at 5 years (Char et al. 2002; Damato et al. 2005) and survival rates comparable to those of enucleation (COMS report No. 28. 2006). Despite current developments in diagnosis and treatment, the survival rate of uveal melanoma has not significantly changed for the last several decades because of limited knowledge of its molecular biology and lack of active treatments for metastatic diseases. Although specific cytogenetic abnormalities, especially monosomy 3, have been closely associated with metastatic disease and shed some light on this subject, the mechanisms of uveal melanoma progression and metastasis remain poorly understood (Prescher et al. 1996; Damato et al. 2007; Lee et al. 2011).

Angiogenesis plays a critical role in the development and progression of

all malignant tumours (Hanahan & Weinberg 2000). As the eye has no lymphatic drainage, the metastasis of uveal melanoma is primarily hematogenous; therefore, angiogenesis may be even more important in the biology of this tumour. Inflammation, together with angiogenesis, contributes to an important aspect of cancer immunity. Studying the expression of angiogenic and inflammatory factors in uveal melanoma can provide valuable information about tumour biology and is important for development of new treatments.

During brachytherapy for uveal melanoma, radioactive plaques that are sutured to sclera overlying the tumour emit β- (Ru-106) or γ-irradiation (I-125) that kills cancerous cells. As an adjunctive treatment to brachytherapy, especially in large tumours, transpupillary thermotherapy (TTT) using an infrared laser can be used (Oosterhuis et al. 1995; Shields et al. 2002; Bartlema et al. 2003). Despite the eye-conserving advantage over enucleation, various vision-affecting side effects from radiation have been described after brachytherapy, including cataract, radiation retinopathy, optic neuropathy, neovascular glaucoma, retinal detachment, keratitis, vitreous haemorrhage and uveitis (Shields et al. 2002; Puusaari et al. 2004). Study of changes in intraocular environment following brachytherapy can be useful for understanding of its therapeutic effects and prevention or management of radiation-related complications.

Using a multiplex biochip array system, we determined the baseline and posttreatment expression of 12 immune cytokines in aqueous humours from melanoma-containing eyes that received brachytherapy using Ru-106 plaque and adjunctive TTT as a primary therapy.

Methods and Materials

This study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Yonsei University Medical Center, Seoul, Korea. All patients signed a written informed consent after an explanation of the nature and possible consequences of this study.

Patients diagnosed with uveal melanoma at the Ophthalmology Department of Yonsei University Medical Center between 1 January 2009 and 31 December 2010 were included in the study. Exclusion criteria included (i) a history of any treatment for uveal melanoma, (ii) a history of any intraocular surgery in the affected eye, (iii) concomitant ocular disorders in the affected eye that could confound interpretation of cytokine levels, such as diabetic retinopathy, and (iv) primary treatment other than combined brachytherapy using a Ru-106 plaque (BEBIG, Berlin, Germany) and TTT. Diagnosis of uveal melanoma was established by a comprehensive ophthalmic examination, ultrasonography, fluorescein angiography and magnetic resonance imaging. Largest basal diameter (LBD) and tumour thickness were measured with B-scan ultrasonography (Hi-Scan; Optikon 2000, Rome, Italy).

Undiluted aqueous humour samples (100 μl) were obtained through anterior chamber paracentesis using a 30 gauge needle from 20 eyes of uveal melanoma patients just before they underwent TTT and Ru-106 brachytherapy in the operating room. After removal, aqueous humour was not replaced by any material. All cases with brachytherapy received adjunctive TTT of the tumour apex using an indirect ophthalmoscope in the operating room before placing the Ru-106 plaque. Exposure time was 1 min per application, and laser energy was adjusted stepwise until the tumour surface turned slightly gray at the end of a 1-min exposure. In five eyes, rectus muscles (two inferior, two lateral and one medial) were removed at the time of plaque implantation and repositioned at the original insertion site when the plaque was removed. In one eye, an inferior oblique muscle was detached and left disinserted. Undiluted aqueous humour samples (100 μl) were re-collected just before the plaque was removed under local anaesthesia.

Undiluted aqueous humours (100 μl) were also obtained as control samples from 20 patients without ocular pathology other than cataracts, immediately before their cataract surgery using a 30 gauge needle and replaced with viscoelastic materials. Aqueous humour samples were transferred to sterile tubes and stored at −76°C until the assay. The aqueous levels of 12 cytokines were simultaneously determined using a commercially available multiplex biochip array system (EVIDENCE Investigator Biochip Array Technology; Randox Laboratories Ltd., Crumlin, UK) (Molloy et al. 2005). Analyzed cytokines included interleukin (IL)-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), tumour necrosis factor (TNF)-α, interferon (IFN)-γ, epidermal growth factor (EGF) and monocyte chemoattractant protein (MCP)-1. Samples were immediately thawed before analysis, and undiluted samples were directly applied to each reaction well in the biochip, a solid substrate with multiple specific cytokine antibodies attached at predefined sites on the surface. After each cytokine was bound to its specific antibody, appropriate enzyme-labelled secondary antibodies were added and cytokine levels were quantified by chemiluminescence using a charge-coupled device camera and imaging system. The analyte concentration was calculated from the calibration curve. If the concentrations of the raw data were less than the minimal detectable levels or more than the maximal levels, they were set to zero or the maximal level of each calibration curve.

The Mann–Whitney U test and Pearson's chi-squared test were performed to compare the differences in demographics and expression of cytokines between the uveal melanoma group and control group. The Wilcoxon signed rank test was performed to compare the cytokine levels before brachytherapy with adjunctive TTT and after plaque removal. Spearman's rho was performed to assess whether there were correlations between cytokine levels. Linear regression was applied to see whether clinical characteristics correlated with each cytokine levels before treatment and to determine which factors affected the differences in cytokine levels following the treatment. All data were analyzed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

During the study period, 34 patients were diagnosed with uveal melanoma

in our institute. Of these patients, four had received previous surgeries or radiation therapy for uveal melanoma, and one had concurrent diabetic retinopathy. Nine patients underwent primary enucleation. All of these 14 patients were excluded from the study. Remaining 20 patients underwent combined Ru-106 Brachytherapy and TTT. Subsequently, aqueous humour samples were obtained from 20 uveal melanoma patients before and after plaque irradiation. The mean and standard deviation of tumour apex dose and scleral contact dose was 61 ± 28 and 786 ± 226 Gy, respectively. Plaque removal occurred at a mean of 117 ± 38 hrs.

Demographics of patients are provided in Table 1. The mean age of patients was 48 years (range, 25–69), and 14 (70%) were men. There was a significant difference in age between the uveal melanoma group and the control group (48 and 58, respectively). However, a linear regression test showed that there was no correlation between age and expression of each cytokine level (data not shown). The mean visual acuity was 20/80 (range, HM-20/20). The mean LBD of the tumour was 12.3 mm (range, 7.6–17.3), and the mean height was 6.6 mm (range, 3.7–9.8). No tumour involved ciliary body. Serous retinal detachment involving more than 1 quadrant of retina was present in nine eyes (45%).

Aqueous humour levels of 12 cytokines

No case showed complication after anterior chamber paracentesis. The cytokine levels in the aqueous humour samples are provided in Table 2. The following cytokines were not detected in the aqueous humour of melanoma-containing eyes and were set to zero: IL-2 in 9 (45%) patients; IL-4 in 1 (5%) patient, IL-10 in 9 (45%) patients; TNF-α in 13 (65%) patients; IL-1α in 5 (25%) patients; IL-1β in 8 (40%) patients; and EGF in 6 (30%) patients. The aqueous humour level of MCP-1 in uveal melanoma was set to 900 in 14 (70%) patients, because this was the maximal level of the standard curve (900 pg/ml). In the control group, following cytokines were not detectable and were set to zero: IL-2 in 1 (5%), IL-10 in 3 (15%), INF-γ in 4 (20%), TNF-α in 7 (35%), IL-1α in 2 (10%), IL-1β in 3 (15%) and EGF in 5 (25%).

Table 1. Demographics of choroidal melanoma group and control group.

Feature	Melanoma group	Control group	p-value
No. of eyes	20	20	
Male/female (no. of patients)	14/6	9/11	0.110
Right/left (no. of eyes)	8/12	9/11	0.749
Mean age in years (±SD)	48 (±14)	58 (±10)	0.024
Associated systemic findings			0.343
None	13 (65%)	15 (75%)	
Hypertension	5 (25%)	5 (25%)	
Gastrointestinal stromal tumour	1 (5%)	0 (0%)	
Ovarian benign tumour	1 (5%)	0 (0%)	
Mean snellen visual acuity (range)	20/80 (HM-20/20)	20/50 (20/400–20/32)	0.758
Mean tumour size in mm (±SD)			
Largest basal diameter	12.3 (±2.7)	–	
Height	6.9 (±1.9)	–	
Anterior margin of tumour (no. of eyes)			
Pre-ora serrata	0 (0%)	–	
Post-ora serrata	20 (100%)	–	
Posterior margin of tumour (no. of eyes)			
1–2 DD from fovea	2 (10%)	–	
<1 DD from fovea	5 (25%)	–	
1–2 DD from optic disk	3 (15%)	–	
<1 DD from optic disk	2 (10%)	–	
>2 DD from fovea or optic disk	8 (40%)	–	
Associated serous retinal detachment (no. of eyes)			
<1quadrant	11 (55%)	–	
1–2 quadrants	7 (35%)	–	
>2 quadrants	2 (10%)	–	

DD = disk diameter; HM = detecting hand movement; SD = standard deviation.

The levels of IL-2, IL-10 and TNF-α significantly decreased, whereas IL-6, IL-8, IFN-γ and MCP-1 were increased in the uveal melanoma group, compared with the control group. The VEGF level tended to be higher in the uveal melanoma group, but this increase was not statistically significant ($p = 0.056$).

Some cytokines correlated with each other in their expression (Table 3). Positive correlation was seen between VEGF and IL-6 ($p = 0.034$), VEGF and IL-8 ($p = 0.030$), IL-4 and IFN-γ ($p < 0.001$), IL-4 and IL-1α ($p = 0.019$), and IL-2 and EGF ($p = 0.035$).

Comparison study between clinical characteristics (age, LBD, humour height, anterior and posterior tumour margin, and degree of associated retinal detachment) and cytokine concentrations showed a positive correlation between tumour height and IL-8 ($p = 0.020$) and degree of retinal detachment and VEGF ($p = 0.018$).

Short-term changes in aqueous humour cytokine levels of after brachytherapy

We aimed to assess the short-term effect of Ru-106 brachytherapy and adjunctive TTT on the tumour environment by comparing cytokine levels before and after brachytherapy. Levels of IL-6, IL-8 and IL-1β significantly increased during therapy (Table 2).

Positive correlations were seen between scleral contact dose and increase in levels of IL-6 (Fig. 1) and IL-8 (Fig. 2), which were calculated by subtraction of the baseline value from the final value. Comparison study between clinical characteristics and increase in levels of IL-6, IL-8 and IL-1 β showed a positive correlation between tumour height and increase in IL-6 ($p = 0.041$) and IL-8 ($p = 0.033$).

Discussion

We used the multiplex biochip array system to simultaneously analyze the aqueous levels of 12 different cytokines in patients with uveal melanoma. The aqueous levels of seven cytokines significantly differed between the melanoma-containing eyes and control eyes; IL-2, IL-10 and TNF-α were low, while IL-6, MCP-1,

Table 2. Aqueous humour cytokine levels in the control group and uveal melanoma group before and after Ru-106 brachytherapy and transpupillary thermotherapy (mean ± standard deviation).

	Control group	Uveal melanoma before treatment	Uveal melanoma after treatment	p-value*	p-value†
EGF(pg/ml)	0.9 ± 0.8	1.0 ± 0.8	1.0 ± 0.9	0.820	0.698
IFN-γ(pg/ml)	2.2 ± 2.2	6.9 ± 6.7	6.2 ± 5.2	0.001	0.681
IL-1α(pg/ml)	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.3	0.565	0.856
IL-1β(pg/ml)	0.6 ± 0.3	0.5 ± 0.5	1.0 ± 0.8	0.314	0.008
IL-2(pg/ml)	4.1 ± 2.2	2.3 ± 2.5	1.8 ± 2.2	0.018	0.300
IL-4(pg/ml)	4.3 ± 2.1	3.3 ± 1.7	3.9 ± 2.0	0.183	0.103
IL-6(pg/ml)	5.7 ± 6.1	108.4 ± 168.4	321.3 ± 280.9	< 0.001	< 0.001
IL-8(pg/ml)	8.5 ± 8.0	25.1 ± 18.4	57.9 ± 54.7	< 0.001	0.001
IL-10(pg/ml)	0.7 ± 0.6	0.4 ± 0.4	0.5 ± 0.4	0.026	0.115
MCP-1(pg/ml)	434.0 ± 198.0	862.6 ± 63.8	864.8 ± 84.8	< 0.001	0.575
TNF-α(pg/ml)	1.5 ± 2.3	0.3 ± 0.5	0.5 ± 0.6	0.005	0.139
VEGF(pg/ml)	45.6 ± 35.7	109.5 ± 117.6	175.1 ± 198.1	0.056	0.100

EGF = epidermal growth factor; IFN = interferon; IL = interleukin; MCP = monocyte chemoattractant protein; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor.
* Mann–Whitney *U* test between control and melanoma group before therapy.
† Wilcoxon signed rank test between before and after treatment for melanoma.

Table 3. p-values* of correlation between aqueous humour cytokine levels in uveal melanoma patients.

	EGF	IFN-γ	IL-1α	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	MCP-1	TNF-α
VEGF	0.934	0.369	0.797	0.863	0.709	0.371	0.034†	0.030†	0.494	0.432	0.739
TNF-α	0.764	0.169	0.563	0.418	0.372	0.100	0.187	0.482	0.303	0.648	
MCP-1	0.524	0.371	0.431	0.979	0.561	0.753	0.057	0.071	0.105		
IL-10	0.095	0.926	0.063	0.337	0.129	0.619	0.496	0.820			
IL-8	0.975	0.052	0.466	0.581	0.645	0.422	0.107				
IL-6	0.223	0.494	0.335	0.207	0.864	0.313					
IL-4	0.363	< 0.001†	0.019†	0.186	0.091						
IL-2	0.035†	0.330	0.800	0.416							
IL-1β	0.879	0.084	0.697								
IL-1α	0.174	0.113									
IFN-γ	0.720										

EGF = epidermal growth factor; IFN = interferon; IL = interleukin; MCP = monocyte chemoattractant protein; TNF = tumour necrosis factor; VEGF = vascular endothelial growth factor.
* Spearman's correlation.
† Positive correlation with p-values < 0.05.

IL-8 and IFN-γ were high in melanoma-containing eyes.

Uveal melanoma progression has been attributed to a variety of immune evasion strategies, including down-regulation of IL-2 (Yang et al. 2008). IL-2 acts as a growth factor and activator of T cells, resulting in increased number of cytotoxic T cells that can kill tumour cells. Investigators have used systemic IL-2 in the treatment of various tumours with varying success, including metastatic cutaneous melanoma, but did not show promising results for uveal melanoma (Atkins et al. 1999).

IL-10 is a Th2-specific cytokine and generally acts as an anti-inflammatory cytokine. IL-10 was shown to be expressed in several uveal melanoma cell lines, which may be important for evading the immune surveillance (Ijland et al. 1999). In case of an intraocular lymphoma, elevated ratios of vitreous IL-10 to IL-6 were suggested to be diagnostic (Whitcup et al. 1997). In our cases, aqueous IL-10 was not increased in melanoma-containing eyes and expression of IL-6 was significantly higher than that of IL-10. A recent study also showed that on average, IL-6 was higher than IL-10 in uveal melanoma(Ly et al. 2010).

TNF-α is a proinflammatory cytokine that can inhibit tumorigenesis. Regional application of high-dose TNF-α has shown some successes with cutaneous melanoma (Lejeune et al. 1998). However, its role in melanoma appears to be complicated; it has been shown that TNF-α can actually upregulate melanoma invasion and migration *in vitro* (Katerinaki et al. 2003).

IL-6 is a pro-inflammatory cytokine typically produced by T lymphocytes and macrophages. Various malignant tumour cells have also been reported to produce IL-6 including cutaneous and uveal melanomas (Colombo et al. 1992; Cools-Lartigue et al. 2004). IL-6 can stimulate tumour cell growth as an autocrine growth factor or through the inhibition of apoptosis (Kawano et al. 1988). Another possible source of IL-6 is tumour-associated macrophages (TAMs) in uveal melanoma (Elgert et al. 1998). In fact, IL-6 was suggested as an important cytokine in

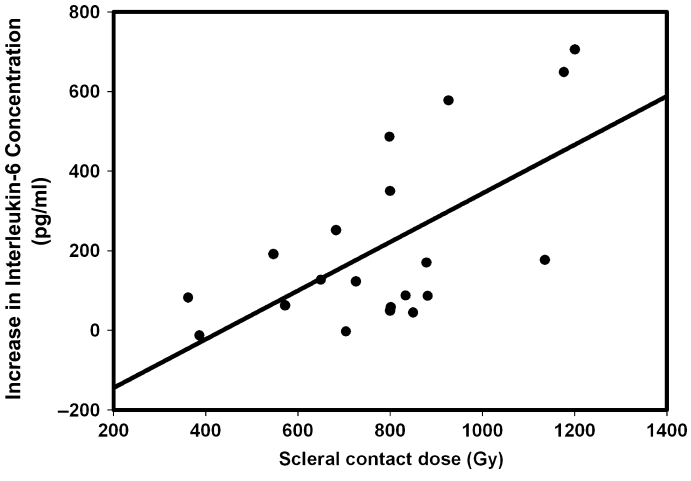

Fig. 1. A graph showing a positive correlation between scleral contact dose of Ru-106 brachytherapy and increase in interleukin-6 concentration during therapy ($R^2 = 0.392$; p = 0.003).

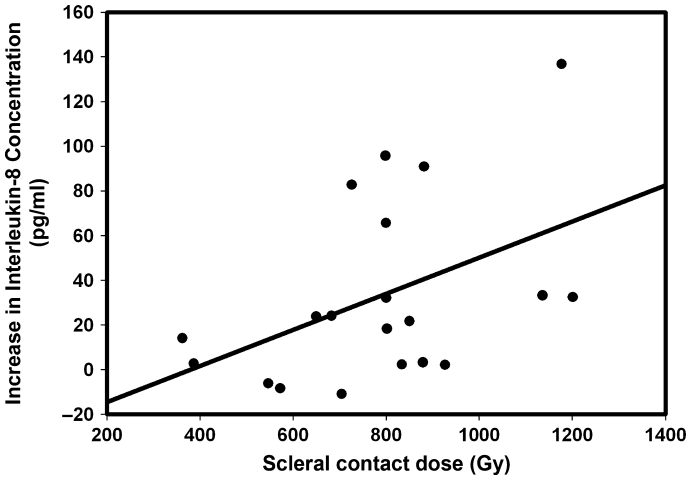

Fig. 2. A graph showing a positive correlation between scleral contact dose of Ru-106 brachytherapy and increase in interleukin-8 concentration during therapy ($R^2 = 0.203$; p = 0.046).

the cross-talk between uveal melanoma cells and TAMs (Cools-Lartigue et al. 2004). High density of TAMs was reported to correlate with decreased survival (Makitie et al. 2001). A recent study showed that brachytherapy increased the number of macrophages within the sclera under the tumour, which also correlated with increasing numbers of TAMs (Toivonen & Kivela 2010).

Tumour-associated macrophages are recruited from circulating monocytes into tissues in response to chemoattractants such as MCP-1. An elevated aqueous humour level of MCP-1 was shown to be correlated with TAM in uveal melanoma in a recent study (Ly et al. 2010). In other malignancies, such as breast cancer, MCP-1 correlated with TAMs and disease progression (Fujimoto et al. 2009). MCP-1 can also promote angiogenesis by itself and inhibition of MCP-1 resulted in reduced angiogenesis and growth of cutaneous melanoma in a mouse model (Koga et al. 2008). Further studies are necessary to understand the role of MCP-1 in uveal melanoma progression.

Originally described as a chemotactic factor for leukocytes, IL-8 belongs to the superfamily of CXC chemokines and is highly angiogenic. It has been associated with tumorigenesis and even metastasis in numerous tumours including cutaneous melanoma (Bar-Eli 1999). IL-8 can directly promote angiogenesis (Li et al. 2003) or can facilitate angiogenesis by stimulating VEGF expression (Martin et al. 2009). We found that IL-8 was highly expressed in larger tumours with greater height (p = 0.020), which may suggest its role in tumorigenesis of uveal melanoma.

Leukocytes from late-stage uveal melanoma patients secrete increased amounts of INF-γ, so it was proposed as a negative prognostic marker (Likhvantseva et al. 1999). Many tumours, including cutaneous melanoma, become resistant to INF-γ, and this may also be the case for uveal melanoma (Hallermalm et al. 2008).

Many studies have dealt with VEGF, a potent angiogenic factor, to look for its implications in uveal melanoma. Vascular endothelial growth factor has been reported as highly expressed in the aqueous humour and/or vitreous humour of uveal melanoma patients (Missotten et al. 2006; Ly et al. 2010). However, VEGF mRNA and protein was not significantly expressed in uveal melanoma, unlike retinoblastoma in one study (Kvanta et al. 1996). There appears to be no correlation between VEGF expression and metastasis in uveal melanoma (Sheidow et al. 2000; Ly et al. 2010). Although aqueous VEGF level was elevated in our cases, this was not statistically significant. Increased VEGF level may be partially attributed to the associated serous retinal detachment, as detached retina can also be the source of VEGF (Missotten et al. 2006). Aqueous VEGF level correlated with the degree of retinal detachment in this study. It may be noteworthy that no tumour involved ciliary body in our cohort; melanomas involving ciliary body appear to express higher level of VEGF than tumours without ciliary body involvement (Missotten et al. 2006), which may partially explain relatively lower VEGF level in this study. Whether VEGF expression is implicated in uveal melanoma requires further study.

We found that expressions of IL-6, IL-8 and IL-1β were acutely elevated in uveal melanoma after brachytherapy. Radiation kills tumour cells basically in two ways: indirect and direct. The indirect effect is believed to be a more common mechanism and results from the generation of free radicals in the cells via ionization by photons. Free radicals, in turn, damage DNA or some other vital components of the cell, causing cell death. The direct

effect is a result of photons themselves interacting directly with the cells causing cell death. If tumour cells die following irradiation, cytokines that are presumably produced or induced by tumour cells would also decrease after therapy. However, this was not demonstrated in this study; no cytokine whose baseline level was elevated decreased after treatment. Maybe it was too early to reflect tumour control, as we obtained aqueous humour after 117 hrs of irradiation on average. Acute increase in pro-inflammatory cytokine expression, therefore, appears to reflect radiation-induced inflammation. Neurons are generally resistant to radiation because they do not divide on a regular basis. Rapidly dividing cells, especially vascular endothelial cells, are probably the source of elevated IL-6 and IL-8 after radiation (Meeren et al. 1997); though, it may also be possible that these cytokines were expressed from tumour cells following radiation.

We found that an increase in IL-6 and IL-8 expressions was positively correlated with an increase in scleral contact dose and tumour height. Unlike brachytherapy performed in other medical fields, which irradiate the tumour from all sides, episcleral plaque therapy delivers radiation from only one side. This leads to a steep dose gradient in which the dose to the sclera and the tumour are dependent on the tumour height. Therefore, tumour of greater height will receive much higher sclera dose than tumours of lesser height to achieve therapeutic apex dose. Increased sclera dose and tumour size have been associated with increased risks for radiation retinopathy, one of the most common vision-threatening complication following radiation (Puusaari et al. 2004). After plaque brachytherapy, 42% and 8% of patients developed non-proliferative and proliferative retinopathy, respectively, at 5 years follow-up in one study (Shields et al. 2002). Clinical features of radiation retinopathy are similar to those of diabetic retinopathy, and vitreous humour levels of IL-6 and IL-8 have been shown to be elevated in diabetic retinopathy, especially in proliferative types (Canataroglu et al. 2005). Other ocular irradiation complications such as cataracts, neovascular glaucoma and uveitis are also related to inflammation and angiogenesis, and cytokines such as IL-6, IL-8 and IL-1β may have a role in mediating these radiation-related side effects. However, elevation of these cytokines in our study reflects a short-term change after plaque radiotherapy and may not be significant for these side effects, as they usually occur in a delayed manner. Long-term follow-up would be necessary to elucidate the implication of these changes.

Limitations of this study include small sample size and a combination therapy including a single session of TTT, which could have affected the concentration of aqueous humour cytokines independent of plaque therapy. Tumours, transpupillary thermotherapy, however, was only applied to the apex of the tumour as an adjunctive treatment and thus may have not contributed as significantly to the changes in cytokine profiles as the plaque radiotherapy.

In conclusion, this study demonstrated that certain pro-inflammatory and pro-angiogenic cytokines are highly expressed in the aqueous humour of uveal melanoma. IL-8, in particular, was highly expressed in tumours with greater heights and thus possibly be associated with tumorigenesis in uveal melanoma as in other malignancies. Combined brachytherapy using Ru-106 and TTT resulted in acute elevation of aqueous humour levels of pro-inflammatory cytokines, such as IL-6, IL-8 and IL-1β. Increases in IL-6 and IL-8 levels positively correlated with scleral contact radiation doses. Modulation of these cytokines may be useful in managing radiation-related side effects. This study is limited by the small number of patients, and its findings need confirmation in a larger study. Further studies are warranted to investigate the role of various cytokines, especially IL-6, IL-8, IFN-γ and MCP-1, in the progression of uveal melanoma.

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