

**Radiation Biology of CNV of AMD**

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## **Age-related macular degeneration**

Age Related Macular Degeneration (AMD) is a disorder of older people where there is progressive deterioration of central vision due to degenerative changes within the macula of the human eye. The wet form of this disease is present in only a tenth of the individuals with AMD although it accounts for over 90% of those who suffer severe central visual loss (Ferris, 1983). In the sub group with wet AMD, the degenerative process results in the breakdown of the barrier between the choroid and the retina in the macular region and this is ushered by the growth of new vessels originating from the choriocapillaris (Gass, 1967). These new vessels invade the sub-pigment epithelial and sub retinal spaces and are accompanied by inflammatory cells, detached retinal pigment epithelial cells and fibroblasts. The new vessels are incompetent and leak fluid and blood which are inimical to the health of the RPE and the photoreceptors. Fibrosis within the CNV leads to distortion of the RPE and photoreceptor architecture and is incompatible with normal central visual function.

Thermal laser photocoagulation has been used to destroy the CNV and has been the mainstay of treatment for wet AMD. However randomized controlled clinical trials have shown that laser is only effective when delivered to carefully selected cases (where the new vessels can be clearly delineated and where they lie outside the foveal avascular zone) in an optimised manner (Macular photocoagulation study group 1986a, 1986b, 1991, 1994), however these criteria are fulfilled in fewer than 15% of all cases of wet AMD. Furthermore even after the most optimal and effective ablation of CNV, recurrence is common and thus visual outlook is uniformly poor (Yanuzzi 1995). Although AMD does not result in total blindness, the loss of critical central visual function, severely impacts on the quality of life of our rapidly aging population. Thus the search for new and more effective treatment modalities has been ongoing.

## **Angiogenesis**

The use of ionizing radiation for the treatment of CNV of AMD has captured the interests of ophthalmologists, radiation oncologists and radiobiologists primarily because eight decades of radiation oncology has demonstrated its profound anti-angiogenic effect. Angiogenesis is the term used to describe the growth of new blood capillaries. For angiogenesis to occur there must be degradation of the basement membrane of the pre-existing capillaries, migration of the component cells (endothelial cells and pericytes), followed by proliferation of these cells to form solid tubes. Canalisation of solid tubes results in the formation of the lumen, new basement membrane synthesis and the commencement of perfusion within these vessels. Angiogenesis may be physiological and normal for example in foetal tissues or pathological as in a corneal pannus or choroidal neovascularisation.

### **Radiosensitivity of the vascular endothelium**

Vascular endothelial cells appear to be inherently more radiosensitive than other mesenchymal cell types such as vascular smooth muscle and fibroblasts (Johnson et al 1982). The  $D_{01}$  values (the dose that reduces cell survival by  $1/e$ ) for cultured endothelial cells range from 0.94 to 1.65 Gy. These values fall within the lower half of experimentally obtained  $D_{01}$  values for normal cultured human cells (Rhee et al 1986, Fuks et al 1992, Hei et al 1987, Martin and Fisher 1984). The reasons for the increased radiosensitivity of endothelial cells are not fully understood. However, in general, cell radiosensitivity increases with active proliferation, proportion of cells at the sensitive phase of the cell cycle, reduction in cytoplasmic organelles, and increased chromosome volume. Endothelial cells in culture exhibit many of these attributes, depending upon culture conditions. Following irradiation protein synthesis and gene expression may be changed and some of the reported changes include altered enzymic activity (Gerritsen et al 1993, Verheij et al 1999), increased permeability (Phillips et al 1966), and modification of the levels of expression of adhesion molecules and

attachment filaments (Hirsch et al 1983). In vivo and in vitro studies of radiosensitivity of endothelial cells yield remarkably similar  $D_0$  values. Some of the earliest quantitative histological in vivo studies of radiosensitivity of irradiated vascular endothelium demonstrate a  $D_0$  of 1.0 Gy which is similar to that obtained by in vitro studies (Rheinhold 1974). However irradiation in vivo may cause viable endothelial cells to lose contact with their basement membrane and become dislodged into the vascular lumen. Tritiated thymidine uptake following irradiation indicates that endothelial tissue begins new DNA synthesis earlier and to a greater extent than the surrounding tissues (Hirst et al 1980). This has been interpreted as a healing response to replace cells lost through radiation damage. Although some cells may have been viable at the time of shedding, this effect is considered to contribute to the radiosensitivity of the tissue. Because irradiation causes upregulation of growth factors and cytokines in endothelial cells (Witte et al 1989, Wilson et al 1993), inflammatory cells are recruited to the site of the damaged vascular tissue. These cells secrete additional cytokines, which interfere with endothelial function, destabilize endothelial metabolism and weaken vessel wall structure (Aubin et al 1983). How the various pathological changes contribute to endothelial injury and impact upon cell survival and replication and ultimately radiosensitivity are as yet unclear.

Many synergistic biological mechanisms have been identified which also contribute to the overall pathological vascular tissue response to radiation exposure. Firstly radiation causes swelling of endothelial cytoplasm resulting in mechanical obstruction to flow particularly within small vessels such as capillaries (Adamson et al 1983). The resulting relatively anoxic conditions weaken the downstream cells. Secondly radiation acts to alter blood flow within vessels through modified release of vasoactive substances leading to thrombosis and vessel closure and endothelial activation and expression of injury (Donlon, 1988). Thirdly radiation results in the expression of adhesion molecules and recruitment of inflammatory cells with increased release of cytokines with additional tissue damage (Hirsch, et al 1983.). Thus the overall picture

following exposure to radiation is one of endothelial damage within a procoagulant vasospastic environment.

Within the vasculature itself there appears to be differential radiosensitivity in the components of large and small vessels (Dimitrievich et al 1984). Many studies have shown that new capillary growth is inhibited by extremely low doses of ionising radiation. Takahashi et al found that new capillaries are more sensitive to radiation than larger vessels or fibroblasts. (Takahashi 1930) One mechanism accounting for the increased radiosensitivity of capillaries is the greater proportion of cycling endothelial cells when compared with more established larger vessels (Gillete et al 1975). Later studies revealed that the radiosensitivity of capillaries was due to a specific subpopulation of the endothelial cells experiencing a short cell cycle time (Hirst et al 1980). Other reasons may include altered expression of growth factors in basement membranes in different vessel types. For example basic fibroblastic growth factor (bFGF) an important angiogenic survival factor is present in the basement membranes of blood vessels, but capillaries exhibit diminished immunoreactivity when compared with larger vessels (Cordon-Cardo et al. 1990). Exogenously added bFGF reduced the occurrence of in situ terminal transferase labeling in irradiated lung microvascular endothelial cells, indicating a reduction of radiation induced apoptosis (Fuks et al 1994).

### **Radiation retinopathy**

The radiosensitivity of the retina is principally due to the damage sustained by the retinal microvasculature and there is a substantial body of literature on the pathogenesis of the clinical condition known as radiation retinopathy (Gunduz K et al 1999, Takahashi K et al 1998, Kinyoun et al 1996). A significant proportion of our knowledge on the response of the retina to ionising radiation is based on clinical observations in vivo and histopathological studies of enucleated eyes. Usually enucleation has followed failed radiotherapy for intraocular neoplasms or when the eye has been damaged either directly or gratuitously through inclusion in the radiation field during treatment of tumours of adjacent

tissues. However, when the total dose to the eye is less than 40Gy and when the fraction size has been kept below 1.8Gy, radiation retinopathy is relatively uncommon (Harris and Levene 1973, Gunduz et al 1999, Parsons et al 1994). Experimental studies have shown that proliferating fibrovascular intraocular membranes in the animal model of a posterior perforating ocular injury can be inhibited by the application of a radioactive plaque to the site of perforation (Chakravarthy et al 1989a, Chakravarthy et al 1989b). In these studies, the formation of granulation tissue between the opposing margins of an ocular wound was inhibited and delayed. It was particularly noteworthy that the inflammatory component was attenuated and that endothelial buds were sparse within the granulation tissue of irradiated wounds. Continuous irradiation of the wound using the radioactive plaque resulted in a total dose of radiation of between 6 Gy and 16Gy. Doses in excess of 9.5Gy were more effective in containing granulation tissue formation with maximal inhibition at the highest dose of 16Gy. The investigators also noted that the adjacent retinal neuropile, normal choroidal vasculature and retinal pigment epithelium which were unaffected by the perforating injury appeared histologically intact, suggesting that in the short term at least these tissues were unaffected by radiation exposure in doses below 16Gy. The cellular composition of the granulation tissue in the wound healing response is not dissimilar to that seen in choroidal neovascularisation (Grossniklaus et al 1992). These observations led Chakravarthy et al to suggest that low dose ionising radiation may be useful in the induction of regression of CNV of AMD (Chakravarthy et al 1993).

Clinical studies have shown that CNV of AMD grow rapidly implying that the endothelial cells of the new vessels are mitotically more active than those of the established vasculature. (Klein et al.1989). As it is conventional wisdom that cycling cells exhibit increased radiation sensitivity, this attribute may be used to create a therapeutic advantage in terms of sparing healthy tissue.

### **Radiotherapy in CNV of AMD**

Based on the above rationale, a number of phase I and phase II clinical trials, investigating the potential for low dose radiation therapy for CNV of AMD using total doses between 10 and 15 Gy have been performed (Chakravarthy et al 1993, Hart et al 1996, Berson et al, 1996, Brady et al, 1997, Spaide et al 1998, Stalmans et al 1998). While the majority of these studies suggested that radiotherapy was associated with less scarring and a better visual outcome than could be expected if no treatment were given, two studies have suggested that visual outcome in terms of natural history may be worse in radiotherapy treated patients (Spaide et al 1998, Stalmans et al 1998). However all the above studies lack prospective concurrently recruited controls and thus cannot be viewed as conclusive evidence for or against treatment.

All of the above mentioned phase 1 radiotherapy studies for CNV of AMD have used conventional fractionated teletherapy regimes with fraction sizes kept below 3Gy and total doses below 15 Gy. However a few have used nonstandard large fraction teletherapy regimes or brachytherapy (Bergink et al, 1995, Yonemoto et al 1996, Jaakkola et al 1998, Finger et al, 1999). In cancer therapy, it is normal to use a large number of small fractions of radiation, as time-dose fractionation exploits the differences between the dose responses of the cancerous and normal tissues. Cells within normal tissues are able to repair DNA damage and proliferate during the intervals between radiotherapy. However, when the target is non-neoplastic vascular malformations or blood vessels of benign tumours, significantly improved involution is seen following smaller total doses but larger fraction sizes of radiation (Lundsford et al 1991, Steinder et al 1992). Intuitively the vessels comprising the choroidal neovascular membrane are more likely to resemble those of the normal vasculature than blood vessels of cancerous tissue. Thus it may be argued that larger fraction sizes may be more appropriate for the purposes of inducing vascular injury in non-neoplastic tissue. Nonetheless a multicentre pooled analysis of phase I data on radiotherapy for CNV of AMD did not reveal any additional benefit through the use of larger fraction sizes (Chakravarthy and

Mackenzie 2000). More recent data from two small randomized controlled trials however do suggest a visual benefit following radiotherapy, and in both these trials fraction sizes in excess of 4Gy were used (Bergink et al 1998, Char et al, 1999).

Substantial controversy exists regarding the initiation, maintenance and regression of CNV of AMD. Natural history studies suggest that there is an initial period of expansion and leakage from the CNV and this is followed by involution, closure of the

vessels and scar formation (Stevens et al 1997). The anti-angiogenic properties of ionising radiation suggest that it is likely to be a useful tool in inducing and hastening neovascular regression while minimising scar formation. Ultimately however radiotherapy for CNV of AMD can only be used as a therapeutic measure if clinical effectiveness can be proved in terms of maintained visual function. The many ongoing, randomized, controlled clinical trials should answer this question in the near future.

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