IGA Research Grants

Project: The Role of Thrombospondin 1 and 2 in open angle glaucoma

Grant Recipient: Mr Anshoo Choudhary, Consultant Ophthalmologist and Honorary Clinical Lecturer, University of Liverpool

It is known that, in primary open angle glaucoma (POAG), the increased intraocular pressure (IOP) is due to increased resistance in the drainage mechanism of the eye – the trabecular meshwork (TM). This increase in resistance appears to be associated with several changes in the structural and chemical nature of the TM and it is thought that growth factors in the aqueous humour (AH) (the watery fluid that fills the front part of the eye) such as transforming growth factor-beta (TGF β) and matrix metalloproteinases (MMP) play a key role.

Matricellular proteins such as the thrombospondins (TSP-1 and TSP-2) are proteins that are secreted naturally and they have a role in influencing cell functions. They are important both in the development of the eye and in the constant renewal of the cells of the eye (known as tissue turnover) that goes on throughout life. They are important to the story of glaucoma because TSP-1 is known to be a potent activator of TGF β, both in life and under experimental laboratory conditions and TSP-2 (and to some extent TSP-1) are involved in MMP activity. It is also known that TSP-1 is present at increased levels in eyes with glaucoma and previous research has shown that both TSP-1 and TSP-2 are expressed in the TM. TSP-1 mRNA has been detected in both fresh and cultured TM cells and the levels are increased by TGF β and dexamethasone. It is therefore clear that both TSP-1 and TSP-2 interact with a variety of cell surface receptors including those associated with apoptosis (programmed cell suicide) and they are therefore likely to be implicated in playing a role in the development of POAG, which means that they may be good targets for new treatments in the future.

This research project is designed to examine the hypothesis that TSP-1 and TSP-2 cause changes in the extracellular matrix, the cytoskeletal organisation and the apoptosis of TM cells via TGF β and MMP pathways.

So far, the project has collected samples of both glaucoma and non glaucoma TM cells and has measured the levels of TSP-1 and

TSP-2. The cells were then exposed to either TGF β or dexamethasone (which are both factors known to cause glaucomatous changes) and the levels of TSP-1 and TSP-2 were again noted. Because MMP is known to play a key role in extracellular matrix deposition, its levels were also noted following exposure to TGF β or dexamethasone.

The results are interesting and encouraging:

- Before exposure to TGF β and dexamethasone both the glaucomatous and non glaucomatous cells were found to have TSP-1 and TSP-2 with a 1.1 fold change between the glaucomatous and non glaucomatous cells.

- After three day's incubation with dexamethasone the levels were measured again with the following results:

2.4 fold increase in expression of TSP-1 in non glaucomatous cells and a 6.1 fold increase in glaucomatous cells.

1.3 fold increase in expression of TSP-2 in non glaucomatous cells and a 1.97 fold increase in glaucomatous cells.

- After three day's incubation with TGF β the figures were:

2.7 fold increase in expression of TSP-1 in non glaucomatous cells and a 10.2 fold increase in glaucomatous cells. 221 fold increase in expression of TSP-2 in non glaucomatous cells and a 187 fold increase in glaucomatous cells.

- When looking at the expression of MMP it is important to note that the non glaucomatous eyes had higher levels to begin with than the glaucomatous eyes:

Following three days exposure to dexamethasone there was a 50% reduction in MMP levels in non glaucomatous eyes and the levels were undetectable in the glaucomatous eyes.

Following three days exposure to TGF β there was a 90% reduction in MMP levels in non glaucomatous eyes and the levels were undetectable in the glaucomatous eyes.

Results so far:

It has now been established that TSP-1 and TSP-2 are to be found in TM cells of both glaucomatous and non glaucomatous eyes, but that the levels tend to be higher in glaucoma. Incubation with either TGF β and dexamethasone causes an increase in the levels of both with the effect being more marked in glaucomatous cells. It is also noted that the increases in the levels of

TSP-2 were exponential following incubation with TGF β. With regard to MMP, it was noted that the MMP levels were lower in the glaucomatous cells before incubation with either TGF β or dexamethasone and that both caused significant drops in the non glaucomatous cells and for the glaucomatous cells, the levels were not measurable. Excessive levels of TSP-1 and TSP-2 would be expected to result in increased levels of tissue deposition in the trabecular meshwork which means that both are suitable candidates for new forms of glaucoma treatment in the future.

The research will continue and will examine the role of TSP-1 and TSP-2 on the expression of extracellular matrix formation, cytoskeletal organisation and cell apoptosis.

So what does this all mean?

In basic terms it means that this research is looking at the reasons for the development of glaucoma (the drainage system of the eye becoming less efficient with age) and for ways in which this can be prevented or reversed. It is at a very early stage, but it has potential for the future.