Progress Report (Brain Foundation Research Grant)

Do metallothioneins promote and direct regenerative growth of epidermal nerve fibres in diabetic peripheral neuropathy?

Chief Investigators:

Professor Adrian West, Professor Bruce Taylor and Dr Lisa Foa (School of Medicine and Menzies Research Institute, University of Tasmania)

Aims (from application):

We will establish whether administration of the protein, ***metallothionein (MT)*** *can promote regenerative growth of epidermal nerve fibres following the onset of diabetic neuropathy in a rat model of diabetes. We will assess separately the ability of MT to promote regenerative growth, and also, to chemoattract regrowing epidermal nerve fibres. We will establish an innovative biopsy model which will allow administration of MT so that its ability to regulate axonal pathfinding will be revealed in vivo.*

Progress

We have made substantial progress on both major aims. We have successfully developed the blister biopsy method in rats as a methodology for establishing the density of epidermal nerve fibres during diabetic neuropathy, although this technique was less useful for administering MT to animals. Instead, we developed a sensitive method of direct injection of MT and emtins into the epidermis and dermis. We have successfully developed our rat model of diabetic neuropathy, with animals allocated to the following groups:

5x High fat diet + STZ with Emtin injection
5x High fat diet + STZ with saline injection
5x High fat diet + STZ with MT injection
5x Normal diet, no STZ, with saline injection

We have collected nociception data for each of these groups using Von Frey fibres, which has confirmed functional allodynia in the STZ treated animals. The effects of MT and emtin treatment are being analysed at present.

We have collected biopsies from all animals (punch biopsies and blister biopsies), and these are undergoing histochemical analysis to i) confirm the pathological degeneration of epidermal nerve fibres in STZ treated animals and ii) to examine the effect of MT or emtin treatment. We have currently completed analysis of about 25% of the 160 biopsies we have collected.

Fig 1 shows some preliminary outcomes. The graph indicates the development of mechanical allodynia (a heightened sensitivity to touch), characteristic to some forms of diabetic neuropathy, in STZ treated animals. At the initiation of testing, all animals are sensitive to the Von Frey fibres, but in control animals

this sensitivity is rapidly lost. In contrast, STZ treated, diabetic animals retain the initial sensitivity. This outcome is confirmed at the histopathological level by a decrease in the density of epidermal nerve fibres, and an increase of inappropriate terminations in the dermis (thought to be the cause of the allodynia). This preliminary work will form the basis of an oral presentation at ANS (Adelaide, Jan 2014) by Ms Lila Landowski.

Our project was delayed by issues around the availability of suitable animals, and technical issues with the development of the blister biopsy. However, now that animal experimentation has been completed, we expect that analysis of biopsy specimens will be finished in the following 6-8 months.