Fatty acids - a potential treatment for spinal cord injury?

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What is the problem?

The spinal cord extends from the bottom of the brain to the lower back and carries messages between the brain and the rest of the body. Injury of the spinal cord leads to significant, often permanent, impairment of motor and sensory function which, in many cases, leads to severe disability. In the UK about 50,000 people live with the consequences of spinal cord injury (SCI), with an additional 1000 new cases each year. There is still no cure for SCI, so there is an urgent need for new treatments to improve functional recovery.

The spinal cord is a long thin tubular structure which is made up of nervous tissue, including nerve cells and cells which support the nerve cells. Following SCI injury, a complex process occurs which results in a hostile injury environment around the damaged nerve cells and scar tissue being formed. The scar tissue seals off the injury site and acts as a physical barrier to nerve regeneration and a chemical barrier, preventing movement of chemicals which are present at the site, and which inhibit regeneration, from moving away from the site of injury (see Figure 1). Promoting regeneration of nerve cells after SCI is, therefore, very challenging.

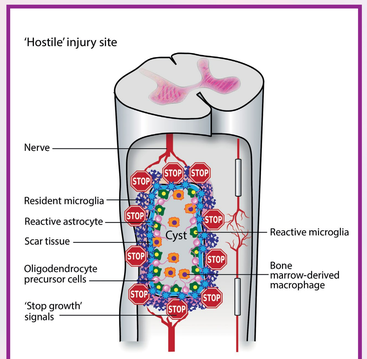


Figure 1 is a representation of a spinal cord injury site. Microglia, astrocytes, oligodendrocyte precursor cells and macrophages are different types of cells found at, and involved in, the injury site. Image source – Medical Illustration, University of Aberdeen.

What am I interested in?

A fatty acid called omega-3 docosahexaenoic acid (DHA) has been shown to promote nerve cell growth in a laboratory setting and could be an excelllent drug candidate for promoting nerve regeneration following SCI. A novel silk biomterial (called Spidrex®) has also shown promising results in promoting nerve cell growth in animal models following nerve injury in other parts of the body and it has been suggested that it might be used to bridge the scar and so overcome the physical barrier. My aim was to test whether DHA was able to promote nerve cell growth in an inhibitory environment, i.e. one that reflects the hostile injury environment seen in SCI, and, if so, whether this could be replicated with nerve cells grown on Spidrex® silk fibres.

What did I do?

I isolated nerve cells from the brains of postnatal rat pups and grew these cells on small thin flat pieces of glass (about 2cm by 2cm) called coverslips. These cells were then treated with a range of DHA concentrations in order to find out which doses were most effective at promoting growth of nerve cells. I also treated the cells with a range of concentration of a chemical called CSPG6, which is known to inhibit nerve cell growth to represent the SCI environment. I then chose the two most effective doses of DHA (8µM and 16µM (µM = micromolar)) and tested their effects when combined with the most effective dose of CSPG6. Finally, these DHA+CSPG6 conditions were tested on nerve cells grown in custom made Spidrex® dishes.

In order to see the effects of these treatments on the cells we looked for the presence of a protein which acts as a marker for nerve cells. Healthy growing nerve cells produce outgrowths from the body of the cell, called neurites, and we looked to see if our cells had a protein associated with neurites. We did this using an antibody that specifically recognises the neurite-associated protein and which had been labelled with a fluorescent green dye to allow us to clearly see the neurites using fluorescent microscopy. Images of the cells were taken and then specialist software was used to analyse these images, giving measurements of neurite growth and we observed nerve cell survival.

What did I find?

I found that DHA treatment alone showed increased neurite growth and nerve cell survival compared to the control cells grown without the addition of the test substances, whereas CSPG6 treatment showed decreased neurite growth and much more cell death (see Figure 2). A combination of DHA and CSPG6 treatments showed neurite growth and cell survival was similar to the control (the latter was not quantified), both for cells grown on coverslips (see Figure 2) and for those grown on Spidrex® silk fibres (see Figure 3).

I also found that CSPG6 reduced the average length of the total neurites of nerve cells and the average longest neurite, whereas DHA and CSPG6 together showed longer average length of the total neurites of nerve cells and a greater average longest neurite length compared to CSPG6 alone. The 8µM DHA and CSPG6 condition showed longer average neurite lengths than 16µM DHA and CSPG6, with the 8µMm DHA and CSPG6 condition approaching the average neurite lengths of the control cells (see Figure 4).

Figure 2 shows neurites of rat nerve cells (green) grown for 48 hours (A) without the test substances added (Control), (B) with CSPG6, and (C) with CSPG6 and DHA. White scale bars represent 50 thousandths of a millimetre.

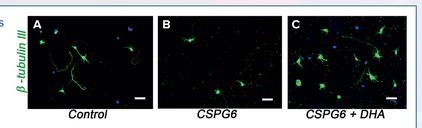


Figure 3 shows neurites of rat nerve cells (green) grown for 48 hours in the presence of (A) Spidrex® silk fibres, (B) Spidrex® silk fibres and CSPG6, and (C) Spidrex® silk fibres, CSPG6 and DHA. The blue band running across the field is the Spidrex® silk fibres. Cells found in the same focus plane as the fibres are considered to be growing on or interacting with the silk. White scale bars represent 50 thousandths of a millimetre.

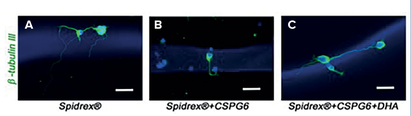
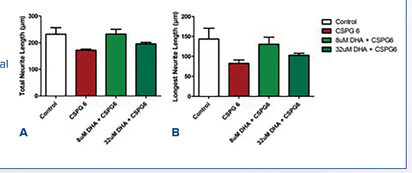


Figure 4 Column charts showing (A) the average overall length of neurites and (B) the average of the longest neurite length for each condition. The CSPG6 condition shows inhibition of growth, with shorter total neurite length and shorter longest neurite length. Both DHA+CSPG6 conditions show increased growth compared to CSPG6 alone, with the 8µM DHA condition and control cells showing no observable difference in total neurite length.



What does it mean?

This model of rat nerve cells grown in the lab shows that DHA can promote neurite growth in an environment with physiological relevance to the site of spinal cord injury, and suggests that this can be combined with Spidrex® silk fibres to promote growth across the injured spinal cord. This evidence will be used to inform future studies with the overall aim to eventually design an effective regenerative strategy for SCI patients.

Who am I?

I am in the final year of my BSc (Hons) Biomedical Sciences (Physiology) programme at the University of Aberdeen and am currently applying for postgraduate research programmes. This Medical Research Scotland Vacation Scholarship project was undertaken at the end of my 3rd year and provided me with valuable experience and insights into the research process. I would encourage anyone with an interest in science to consider a career in research as although it can seem very challenging it can also be highly rewarding.

Peter was supervised during his Vacation Scholarship project, *"Combining a novel silk biomaterial and omega-3 docosahexaenoic acid for spinal cord repair"* by Dr Wenlong Huang and Anna Varone.