CHIEF SCIENTIST OFFICE

CODE: ETM/439

RESEARCH PROJECT BRIEFING

Oligosaccharides as new therapeutics for CNS repair

AIMS

- The main aim of this project was to identify whether novel sugar molecules could be used as therapeutics for people with injury to their brain and spinal cord known as the central nervous system (CNS).

- The secondary aim was to identify their mechanism of action to enhance our understanding of using these molecules as novel therapeutics.

These modified sugars are made from heparins (mHeps) which have been changed so they express less sulphate groups (SO4-) on their surface. These heparins also lack the blood thinning properties of the unmodified versions making them potential candidates for therapies.

KEY FINDINGS

- We treated a range of cells in culture that mimic spinal cord injury using the library of modified sugars with different sulphate groups on them. We found that they promoted CNS repair, primarily resulting in increased myelination (the wrapping of nerves with their insulating material) as well as nerve process outgrowth after damage.

- We demonstrated that injured CNS tissue secrete factors that are negative for repair. Our hypothesis is that the modified sugars mop up these factors allowing repair to occur.

- We have identified several important candidates that may be the target of the modified sugars including amyloid-beta and some immune regulators known as chemokines/cytokines.

- We demonstrated that amyloid-beta inhibits nerve repair and this can be prevented by our modified sugars

WHAT DID THE STUDY INVOLVE?

In this investigation, we mainly used mixed rat brain cells grown in a dish, known as myelinating cultures to study spinal cord injury (SCI). These cultures develop to mimic a piece of spinal cord tissue, with nerves wrapped in their insulating sheath, called myelin. We mimic SCI by cutting the nerves which damages them and also causes the myelin sheath to degenerate (known as demyelination). These are similar characteristics as seen after spinal cord injury. In another assay we can use these myelinating cultures to study remyelination alone. This is carried out by adding reagents that cause the myelin sheaths to come off. Our modified sugars, mimic the complex sugars in the body that are found all around cells. These complex sugars are known to bind to many factors released by cells and regulate many cellular functions. We treated our injured and demyelinated cultures with a panel of modified sugars (different sulphate groups on them) and then measure nerve process outgrowth and remyelination. We studied in detail the factors secreted by the cells after injury. We then used a complex technique called mass spectrometry which allowed us to identify what the modified sugars were binding to after injury. We also used bench top assays called (chemokine arrays and enzyme-linked immunosorbent assays (ELISA)) to identify and quantify factors secreted by the damaged cells.

WHAT WERE THE RESULTS AND WHAT DO THEY MEAN?

Our results showed

1) Myelination and nerve process outgrowth was promoted after cultures were injured if treated with the low sulphated modified sugar. This is a new function for these molecules. See Fig. 1.

2) After a myelinating culture has been injured they secrete factors that inhibited myelination.

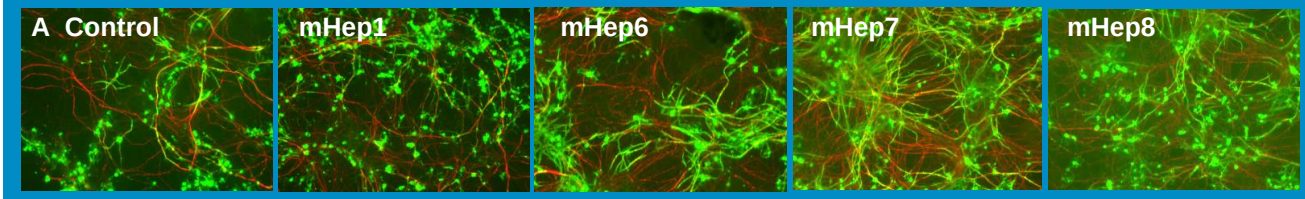
3) The secreted factor(s) that inhibited myelination can be overcome by treating with the low sulphated modified sugar (mHep7), suggesting they are binding to mHep7.

4) Modified sugars do not effect the development of myelination if the cultures are not injured. This supports the idea that the injured cultures are secreting something that stops repair.

5) We identified immune factors secreted by the cells after injury (known as chemokine and cytokines). These are known to be heparin binding and so are potential candidates for the modified sugars to bind to. This data suggests that many factors secreted by the cells after injury are immune factors and it is possible these may affect repair

6) We used a range of techniques to isolate factors that bind to the low sulphated modified sugar after injury and the subsequent mass spectrometry analysis allowed us to identify the factors based on their the physical properties. We identified amyloid-beta, alpha 2-macroglobulin, clusterin, ApoD, ApoB, thrombospondin 1, tenascin C and gelosin. Some of these factors have been suggested to be involved in Alzheimer's disease.

Example of myelinating cultures treated with the mHeps.



Green is myelin sheaths, red are nerve processes

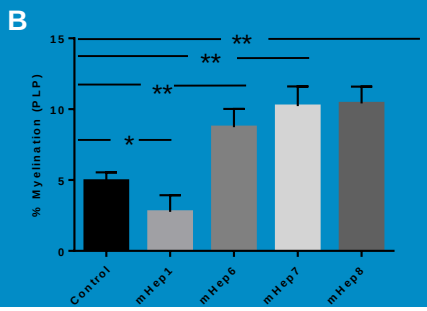


Fig. 1: A) Representative images of an area adjacent to the lesion in injured cultures, in untreated cultures (control) and after treatment with a range of modified sugars with different sulphation levels. We see a promotion of myelination (green) following a single treatment with the low sulphated modified sugars (6-8). In contrast the highly sulphated sugar showed a decrease in the levels of myelination. B) Quantification of the images measuring the percentage of myelinated fibres (overlap of green and red).

7) Amyloid-beta (Aβ) may be a factor that inhibits myelination. Aβ is thought to be involved in Alzheimer's disease specifically the 1-42 and 1-40 length forms. Aβ levels were confirmed to be secreted by cells after injury using a specialised ELISA assay that detects Aβ (Fig. 3). We proved it was a likely candidate by adding Aβ 1-42 to the myelinating cultures and showed that myelination was inhibited at day 24 (Fig 2C). This negative effect was rescued by the modified sugar, mHep7 co-treatment. This is the first recorded data of Aβ directly inhibiting myelination and that low sulphate sugar can negate the effect.

Points 1-7 are all novel findings and have been submitted to Glia for consideration and is under review. Overall we have shown that modified heparins with low sulphate groups can promote repair of damage

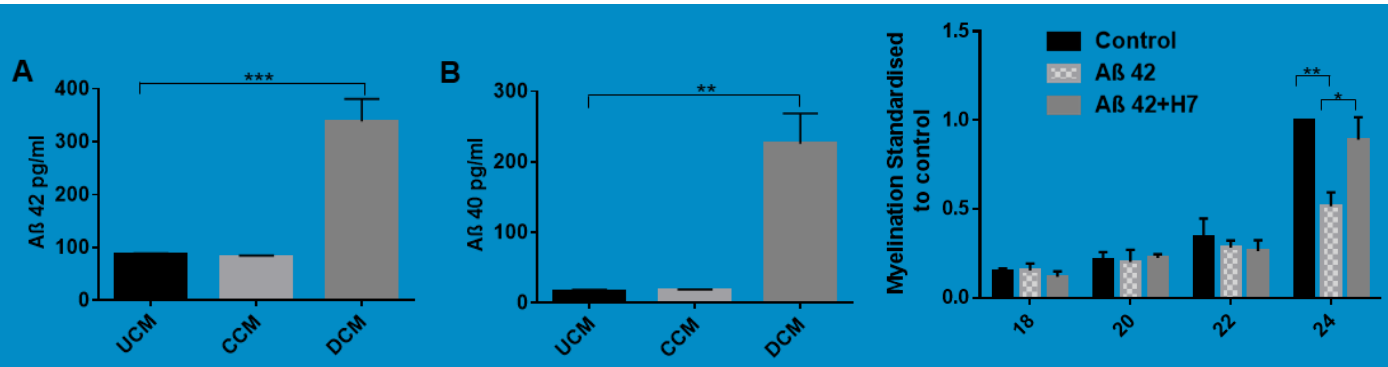


Fig 2: A,B) Using an ELISA assay that detects amyloid beta (1-42) and (1-40) forms we confirmed it was secreted by cells injured by demyelination only. Moreover when amyloid–beta was added to myelinating cultures the formation of new myelin sheaths was inhibited at day 24. This negative effect was overcome by the treatment with mHeps7 (Fig. 3C).

WHAT IMPACT COULD THE FINDINGS HAVE?

We now have strong evidence from our data obtained using cell cultures that our modified sugars are potentially a novel therapeutic for CNS repair. We now require further work using animal models to provide additional support for future translation to the clinic. Heparin is given to patients routinely, so the modified products would have a good safety record for future clinical use. Moreover they do not have blood thinning properties (which is imperative for translation). Identifying secretion of amyloid–beta using our cell model of CNS injury, may allow the cultures to be used successfully to study Alzheimer's disease.

HOW WILL THE OUTCOMES BE DISSEMINATED?

We have presented this data at many national and international conferences to inform and network with other scientists, namely:
Invited Speaker for the In Vitro Toxicology Society (IVTS). Glasgow 2016, the 13th ISN satellite meeting Myelin Biology: Island of Enbiez, 2017 and International networking meeting for the International Spinal Research Trust, London 2018.

I presented the data at seminars I was invited to give at the University of Aberdeen and University of Keele and also within Glasgow to University students at the Neuro Interest Group.

We have submitted the work to Glia and it is now under review. We will ensure that once accepted we will actively promote the publication through the University press offices

The intention is also to include the work in presentations to school children sitting Science Highers during public engagement events at the Glasgow Science centre in the coming year.

We have used the data to submit a grant to the BBSRC which is now under review.

CONCLUSION

We have shown that our modified sugars (low sulphated mHeps) have beneficial effects on CNS repair by binding to multiple factors important in regulating cell function in the body. After injury we observed changes in the cytokine and chemokine profiles, as well as factors involved in Alzheimer's disease suggesting damage alone can cause these factors to be released. Thus, these novel compounds could also have therapeutic potential in other neurological disorders.

RESEARCH TEAM & CONTACT

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Additional Information

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