



THE EFFECTS OF UNDEGRADED GLYCOSAMINOGLYCANS
FROM MUCOPOLYSACCHARIDOSES ON OSTEOBLAST
DIFFERENTIATION AND MINERALISATION *IN VITRO*

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Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Abstract

Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders which are characterised by a loss or decrease in one or more enzymes involved in the stepwise degradation of the glycosaminoglycans (GAG) heparan sulfate (HS), dermatan sulfate (DS), keratan sulfate (KS), chondroitin sulfate (CS) and hyaluronan. As a result, these GAGs accumulate within both cells and circulation and cause a range of pathologies depending on which GAGs are undegraded and accumulated. These include organomegaly, CNS degeneration, degenerative joint disorder, corneal clouding and dysostosis multiplex. Depending on the severity of the disease, patients can die as young as their early teens, usually from respiratory issues.

MPS I, MPS II and MPS VI present with dysostosis multiplex which consists of abnormal bone growth, short stature and decreased bone mass. The mechanisms behind decreased bone mass and short stature in MPS disorders is largely unknown. Bone formation is achieved through the bone forming cells, osteoblasts. This is achieved through the recruitment of mesenchymal stem cells (MSC) to the region of new bone formation where they differentiate into osteoblasts; excrete collagenous and non-collagenous proteins to form an extracellular matrix (ECM). Mature osteoblasts then secrete the calcium containing molecule hydroxyapatite which then mineralises the ECM by anchoring to non-collagenous proteins osteocalcin (OCN) and bone sialoprotein (IBSP), thus forming new bone.

The osteoblast differentiation and bone formation processes are regulated through multiple cellular pathways including the bone morphogenic protein (BMP) pathway and the canonical and non-canonical Wnt signalling pathways. Runx2 expression is essential for MSCs to commit to the osteoblast lineage and the Wnt5a non-canonical signalling pathway is believed

to drive initial differentiation while mineralisation occurs with the suppression of Wnt7b signalling.

This thesis details the results of MPS GAGs as well as commercially available GAGs, DS, HS and heparin on MSC differentiation into osteoblasts and osteoblasts maturation and mineralisation. Furthermore, GAG structural differences such as chain length and sulfation patterns have been found to play important roles in how GAGs interact with proteins. Different sulfation patterns can result in GAGs binding or not binding to proteins. In particular, HS binding sites called Weintraub motifs have been found on numerous proteins. The canonical Wnts are known to contain these Weintraub motifs. Therefore, how MPS GAGs affect Wnt signalling was analysed through signalling assays and gene expression.

Results showed that decreased bone mass due to MPS I, MPS II and MPS VI GAG addition was observed *In vitro* for normal MSCs and osteoblasts. MPS I GAG appeared to work through a different mechanism to MPS II and MPS VI and as a result, did not result in a decrease in mineralisation to an extent observed due to the other two GAGs. Furthermore MPS II and MPS VI GAG, although it does not prevent MSCs from differentiating into osteoblasts, they may keep osteoblasts in an immature state where they can produce an extra cellular matrix which is unable to be mineralised. Despite MPS I and MPS II GAG unable to increase Wnt3a signalling in a HEK293T Broad cell line, they may increase Wnt3a signalling in an SFRP2 dependant manner in MSCs and osteoblasts thus preventing terminal differentiation and mineralisation.

Hypothesis

That undegraded GAGs accumulating in MPS I, MPS II and MPS VI decrease *in vitro* MSC differentiation into osteoblasts and subsequent osteoblast mineralisation.

Aims

1. Determine the effects of undegraded GAGs from MPS I, MPS II and MPS VI patients on MSC differentiation into osteoblasts.
2. Determine the effects of undegraded GAGs from MPS I, MPS II and MPS VI on osteoblast mineralisation
3. Determine the effects of MPS I, MPS II and MPS VI GAG on canonical and non-canonical Wnt signalling during osteoblast differentiation and mineralisation.