Universitätsklinikum Carl Gustav Carus Dresden

UNIVERSITÄT LEIPZIG

www.trr67.de

TECHNISCHE UNIVERSITÄT DRESDEN

www.medizin.tu-dresden.de www.trr67.de http//tu-dresden.de

Glycosaminoglycans and their sulfate derivates differentially regulate the osteocytic phenotype of murine and rat osteocyte-like cell lines

Elena Tsourdi, Martina Rauner, Tilman D Rachner, Juliane Salbach-Hirsch, Claudia Richter and Lorenz C Hofbauer

Division of Endocrinology, Diabetes, and Bone Diseases, Department of Medicine III
Technical University, Dresden, Germany

Introduction

Extracellular matrix of bone consists primarily of collagen and glycosaminoglycans (GAGs) such as hyaluronan (HA) and chondroitin sulfate (CS). In view of the growing demand for bone replacement, collagen-GAG composites are under development for a wide range of applications in tissue engineering. Our aim was to characterize the molecular and cellular effects of GAGs on osteocytes, which are fundamental orchestrators of bone remodeling.

Methods

We manufactured native and sulfate-modified HA and CS as well as a low-molecular weight hyaluronan LMW and evaluated their effects on viability, necrosis, apoptosis, and gene expression of osteocyte markers in the murine osteocyte-like MLO-Y4 cell line and the rat osteogenic UMR 106-01 cell line, which both display properties of primary osteocytes. Cell necrosis and apoptosis were determined using an immunoassay to detect DNA fragmentation, and cell viabilty was evaluated with a fluometric assay. The gene expression profile was examined with real-time PCR.

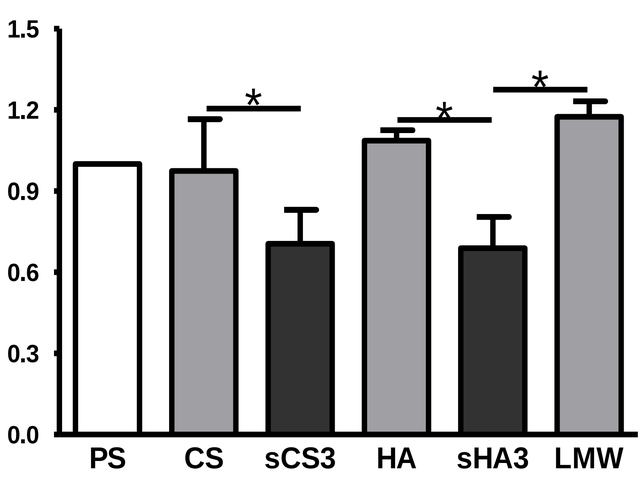
Results

Native and sulfated GAGs were stable and non-cytotoxic. Assessement of native and sulfate-modified GAGs at a concentration of 200 µg/ml in MLO-Y4 cells demonstrated that unsulfated HA did not significantly reduce apoptosis compared to control, whereas the highly sulfated form of HA led to a significant reduction of apoptosis both in comparison to control and unsulfated HA ($p<0.05$). Moreover, highly sulfated CS decreased apoptosis by 30% compared to its native form ($p<0.05$). The LMW product did not lead to a reduction of apoptosis (**A**). Similar results were observed for cell necrosis (**B**).

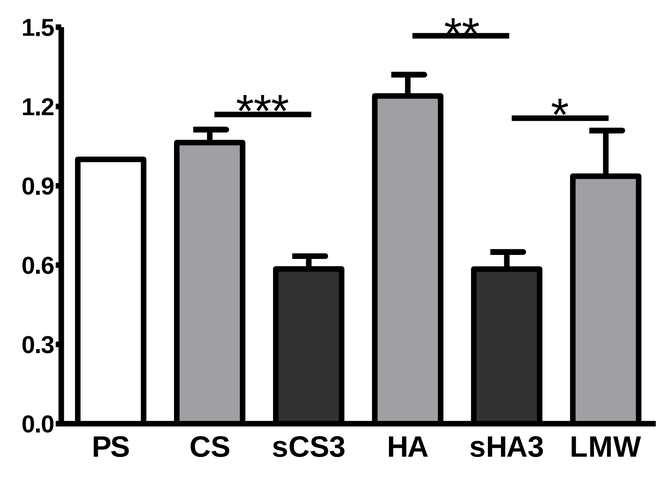
Using soluble GAGs at a concentration of 200 µg/ml in UMR-106 cells, we found that the unsulfated form of HA dose dependently increased levels of RANKL as well as the RANKL/OPG ratio in comparison to control, whereas highly sulfated HA led to a significant downregulation of both levels of RANKL and the RANKL/OPG ratio when compared to its native form (both $p<0.05$) (**C+D**). These results confirm the inhibitory effect of highly sulfated HA on osteoclastogenesis which has been displayed on our previous experiments on osteoclasts.

The expression of SOST, the gene encoding sclerostin was significantly reduced by the highly sulfated HA und CS products when compared to control ($p<0.05$) (**E**). The expression of PHEX, a gene regulating phosphate metabolism was significantly reduced by highly sulfated HA ($p<0.05$). Native HA and both CS compounds did not alter PHEX expression (**F**).

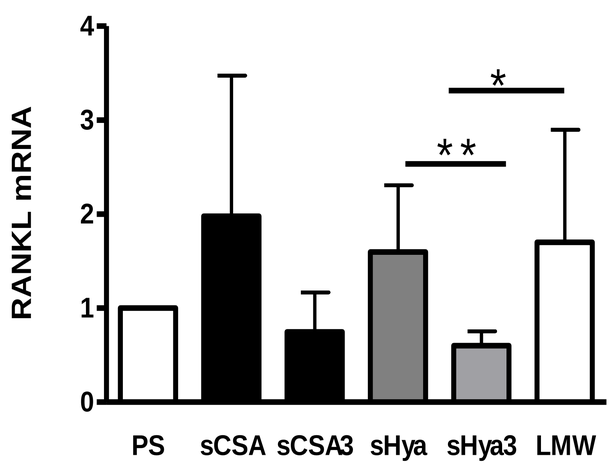
A. Apoptosis



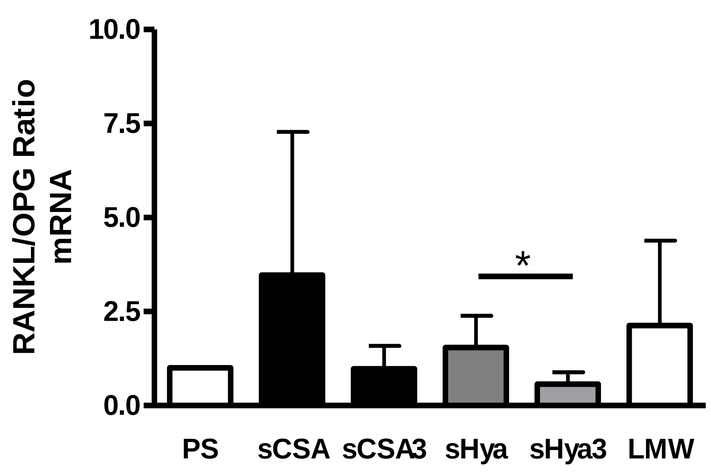
B. Necrosis



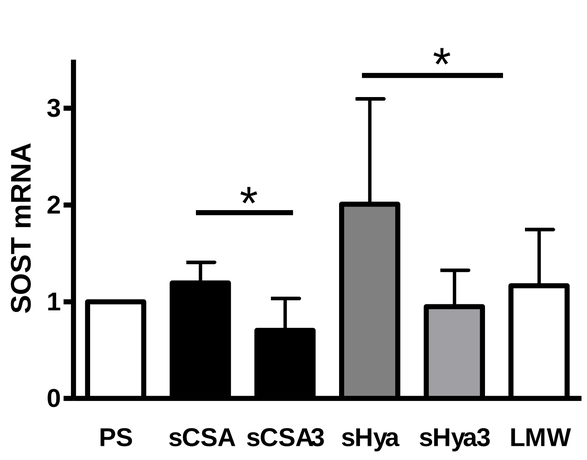
C. RANKL



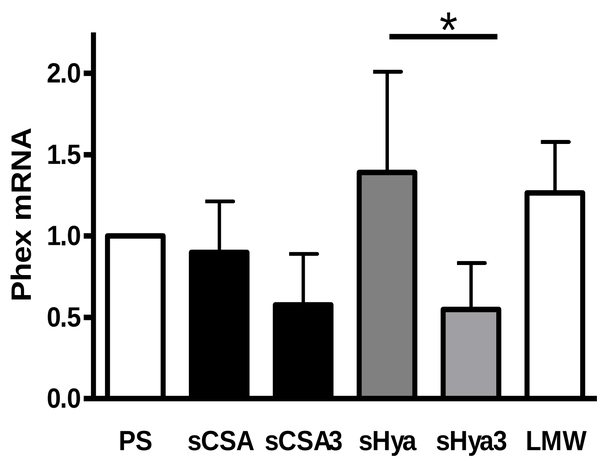
D. RANKL/OPG



E. SOST



F. PHEX



Conclusion

Highly sulfated GAGs and especially HA may contribute to the phenotype of healthy, viable and functional osteocytes while maintaining a physiological RANKL/OPG ratio. The clinical significance of these findings needs to be validated in vivo.

Acknowledgements

This work was supported by Grants of the Transregio Projekt TTR67 to ET and LCH. We thank Eva Schubert and Nicole Pacyna for technical support.