Mast cells in chitosan-mediated wound healing

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Introduction

Chitosan is a natural biopolymer made from glucosamine and sourced from crustaceans, which is chemically depolymerised and deacetylated in its manufacture. It is well known to contain pro-coagulant and wound healing properties with many studies involving whole blood or platelets; however, a systematic investigation of the response of a specific population of granulocytic cells, known as mast cells has not been performed. Mast cells are multifunctional secretory cells that control many inflammatory and wound healing processes by releasing mediators upon activation, which were bound to proteoglycans in intracellular granules.

The aim of this study was to investigate the adhesion and activation of mast cells to the biomaterial, chitosan, and to characterize the proteoglycans produced by mast cells in resting and activated states and examine their structures and roles in inflammation and wound healing.

Methods

Human mast cells, HMC-1, were incubated with chitosan or fibronectin coated glass slides for 3 hours at 37°C and observed using light microscopy to determine cell adhesion and morphology. Mast cell activation was investigated after exposure to chitosan for 3 hours at 37°C and measuring hexosaminidase release as a marker of activation. Proteoglycans from HMC-1 conditioned medium were purified by anion exchange chromatography and characterised using SDS-PAGE, Western blotting and ELISA with antibodies that reacted with either the glycosaminoglycan or protein components. Flow cytometry analyses were performed using the same antibodies on cells that were either activated or not to determine the intracellular and / or cell surface expression of proteoglycans.

Results

HMC-1 cells are usually non-adherent in culture, however, when cultured *in vitro* in the presence of fibronectin, were shown to adhere to the protein adsorbed onto the polystyrene surface (Fig 1A). When the cells were cultured with a surface coated with chitosan, more cells adhered to the surface, as well as to each other resulting in large cellular aggregates (Fig. 1B).

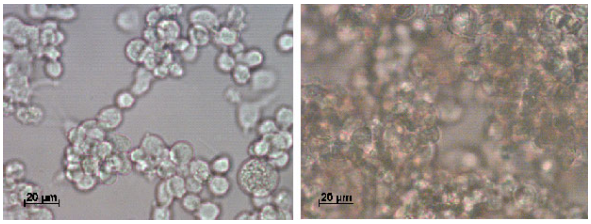

Figure 1. Mast cell adhesion to (A) fibronectin and (B) chitosan.

HMC-1 cells express chondroitin sulfate (CS), heparan sulfate (HS), heparin, and keratan sulfate (KS) in their resting state. The cells also produced various types of proteoglycan core proteins including serglycin, versican, bikunin and surprisingly, perlecan. The perlecan was produced as a full length molecule of >460kDa, which was cleaved into smaller fragments with molecular weights of 130 and 300 kDa. Probing with domain specific antibodies and N-terminal sequencing analyses confirmed that the 300 kDa fragment came from the C-terminus of the protein core and contained domains IV and V and was decorated with approximately 10kDa CS. The 130 kDa fragment was found to be a mixture of both N- and C-termini fragments. The cell-associated expression of HS, heparin, serglycin and VEGF was confirmed by FACS analyses whilst these experiments suggested that the perlecan was secreted constitutively into the conditioned medium in small amounts (Fig. 2) where it could be cleaved by secreted proteases.

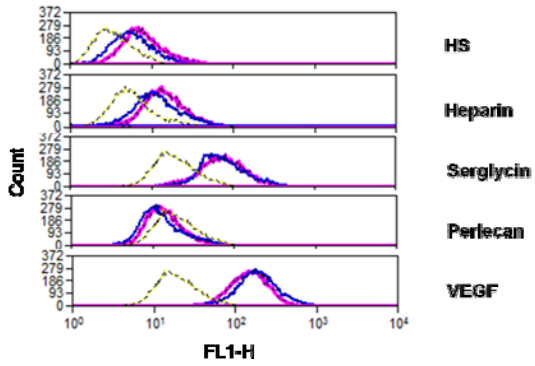

Figure 2. Flow cytometry analysis of the intracellular or on the membrane expression of mast cell mediators in resting (blue) and activated (pink) states.

Interestingly, HMC-1 cells produced VEGF growth factors, which can bind to the N-terminal HS chains of perlecan where it can bind to VEGF receptors and activate endothelial cell growth.

Conclusions

Our data shows for the first time that chitosan activates mast cells resulting in the release of mediators including proteases and growth factors, which can cleave the extracellular proteoglycan perlecan into fragments that may have downstream effects on wound healing by modulating VEGF binding and activation and cell adhesion via integrins. These events would have important effects on angiogenesis, a key step in wound healing.

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