<Regular Article>

Chondroitin Sulfate Disaccharide Enhances Extracellular Matrix-Related Gene and Protein Expression in Normal Human Dermal Fibroblasts in Vitro

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Abstract

The physiological effects of chondroitin sulfate (CS) depend on the extent of its sulfation. This study focused on the effects of highly sulfated CS with different molecular weights in normal human dermal fibroblasts (NHDFs). NHDFs were treated with each CS. The expression of various genes was assessed by real-time PCR. Type I collagen content and elastin protein expression levels were assessed by ELISA and Western blotting, respectively. Disaccharide CS significantly increased the expression of genes required for extracellular matrix (collagen, type I, decorin, elastin, lysyl oxidase, SMAD2 and SMAD3). In addition, we confirmed that collagen and elastin increased at the protein level. In contrast, high- and low-molecular-weight CS polymer had no significant effect. Therefore, highly sulfated disaccharide CS increased the expression of extracellular matrix-related genes and proteins. It may be possible that the highly sulfated disaccharide CS may increase firmness and elasticity of skin.

Key words: disaccharide chondroitin sulfate, type I collagen, decorin, elastin, lysyl oxidase.

1. Introduction

Chondroitin sulfate (CS) is a physiologically active substance that retains moisture and is present in various parts of the body. It is a water-soluble polymer, a linear chain formed by linking disaccharides of N-acetylgalactosamine and glucuronic acid. CS is present in the form of proteoglycans, which attach to core proteins in vivo. CS proteoglycans include decorin (DCN), versican and aglycan.

CS is a glycosaminoglycan, important in extracellular matrix-related proteins in the body. For example, it has been reported that CS increases the expression of elastin (ELN) in smooth muscle cells and that DCN (CS proteoglycan) is required for stabilizing of collagen fibers. This suggests that CS is an important factor in fibril formation.

Collagen fibers and elastic fibers are the main fibers in the skin, and type I collagen is the main component of collagen fiber in skin. As DCN is required to stabilize collagen fibers, type I collagen and DCN are important for collagen fiber formation in the skin. ELN is the main component of elastic fibers. ELN is a key extracellular matrix protein that is critical for elasticity and resilience. SMAD2 and SMAD3 are phosphorylated in response to TGF-β, inducing SMAD complex formation. The complex translocates into the nucleus to activate the transcription of ELN genes. Therefore, ELN, SMAD2 and SMAD3 are important for elastic fiber formation. Lysyl oxidase (LOX) plays an important role in cross-linking elastic fibers and collagen fibers.

This study investigated the effects of CS on the extracellular matrix-related genes collagen, type I, alpha 1 (COL1A1), DCN, ELN, LOX, SMAD2 and SMAD3. CS is present in the skin, and has a low degree of sulfation, affecting the viability of fibroblasts. However, the effects of CS vary depending on the degree of sulfation. For example, CS with a high degree of sulfation promotes neurite outgrowth and suppression of pits formed by osteoclasts, but these effects were not observed with CS having low sulfation. In addition, the interaction between CS and proteins varies depending on the extent of sulfation. Therefore, degree of sulfation is an important factor for effectiveness evaluation of CS, but there is little information on the effects of highly sulfated CS.

In contrast, hyaluronan is a member of the glycosaminoglycans, which includes the CS; its physiological effects differ depending on its molecular weight. It is therefore conceivable that the effects of CS are also dependent on molecular weight.

We aimed to elucidate physiological function of highly sulfated CS with different molecular weights. In this paper, we examine the effects of molecular weight of CS with 3 sulfate groups per unit (high degree of sulfation) by investigating the effects on gene expression of COL1A1, DCN, ELN, LOX, SMAD2 and SMAD3, and protein expression of type I collagen and ELN, which are important for fibril formation in normal human dermal fibroblasts.

2. Materials and Methods

2–1. Materials

High-molecular-weight CS (average M.W.: 47,000 Da, sulfate group content: 38.57%) and low-molecular-weight CS (average M.W.: 3,700 Da, sulfate group content: 38.74%) (high sulfation) were obtained from Maruho Co., Ltd. (Osaka, Japan).