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# Cyclodextrin polysulphates repress IL-1 and promote the accumulation of chondrocyte extracellular matrix[1]

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## Summary

*Objective*: To evaluate the influence of cyclodextrin polysulphate (CDPS) on the extracellular matrix (ECM) metabolism of human articular cartilage chondrocytes.

*Methods*: Isolated chondrocytes from femoral condyle cartilage of human knee joints were cultured in gelled alginate to maintain their differentiated phenotype. During 1 week of culture, the cells were exposed to different concentrations of CDPS. Synthesis of aggrecans was investigated in these cultures after using $Na_2^{35}SO_4$ as a radioactive precursor during the last 24 h of culture. The artificial matrix was then solubilised with Na-citrate and newly synthesised aggrecan aggregates, accumulated during culture, were liberated and assayed. The isolated chondrocytes were labelled with antibodies against aggrecan and type II collagen to analyse the ECM molecules in the cell-associated matrix (CAM). Plasma membrane levels of receptors for insulin-like growth factor-1 (IGF-1RI) and for interleukin-1 (IL-1RI and IL-1RII), as well as levels of IGF-1, IL-1$\alpha$ and -$\beta$ were determined after the cells had been permeabilized and stained with the appropriate antibodies. The release of IL-6 in the culture media was used as a variable reflecting auto/paracrine IL-1 activity of the cells in different experimental conditions.

*Results*: CDPS significantly increased total $^{35}$S-incorporation rates in ECM aggrecan. When compared with controls, CDPS-treated chondrocytes expressed significantly higher CAM aggrecan and type II collagen levels. As plasma membrane-bound IGFR1 and intracellular IGF-1 levels remained unchanged, this increase in accumulated CAM compounds may have resulted from suppressed catabolic activities by the chondrocytes in culture. CDPS-treated cells expressed significantly lower amounts of intracellular IL-1$\alpha$ and -$\beta$ levels. Plasma membrane-bound IL-1RI and decoy IL-1RII remained unchanged. $\beta$-cyclodextrin-treated chondrocytes released significantly less IL-6 in the supernatant culture media.

*Conclusion*: CDPS is a novel polysulfated polysaccharide showing cartilage structure modifying effects *in vitro* as it improves the synthesis of aggrecan and the accumulation of CAM macromolecules. This effect probably resulted in part from the downregulation of IL-1.


*Key words*: Chondrocytes, Aggrecan, IL-1$\beta$, Cyclodextrin polysulphate.

*Abbreviations*: CAM, cell-associated matrix, CPS, chondroitin polysulphate, ECM, extracellular matrix, IL, interleukin, MFI, median fluorescence intensity, OA, osteoarthritis, CDPS, cyclodextrin polysulphate.

## Introduction

Sulphated polysaccharides have been licensed for the use in different pathophysiological conditions, e.g., to modulate plasma anticoagulant and fibrinolytic activities, to exert hypolipaemic effects, to inhibit retroviral multiplication. Interestingly, most of these distinct effects are shared by different polysulphated polysaccharides.

Where degenerative joint diseases are concerned, the most interesting biological activities of these sulphated substances are their effects on synthesis and turnover of important compounds of the extracellular matrix (ECM) of connective tissues. The first reports already dated from the early 1970s[1–4] but extended reviews on the potential use of these polysaccharides have appeared more recently[5,6]. Amongst other glycosaminoglycans, chondroitin sulphate was shown to increase aggrecan synthesis by articular cartilage cells[7] and both chondroitin sulphate and chondroitin polysulphate (CPS) increased hyaluronan synthesis by synovial lining cells *in vitro*[8] and *in vivo*[9,10]. Interestingly, hyaluronan molecular weight significantly increased when synovial cells were treated with CPS and other sulphated polysaccharides[9–11]. Similarly, human articular chondrocytes in monolayer culture responded with an increased synthesis of highly polymerised hyaluronan when CPS was supplemented to the culture medium[12]. Under the same conditions these articular cartilage cells increased their production of aggrecans in the aggregate form[13]. Electron microscopic studies confirmed the immobilisation of higher

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numbers of aggrecans on longer hyaluronan filaments synthesised by differentiated human cartilage cells exposed to CPS and other sulphated polysaccharides such as xylosan polysulphate[13].

Considering their capability of increasing synthesis rates and improving the immobilisation of physiologically important ECM molecules, some of these sulphated polysaccharides have been considered to be important therapeutical tools in osteoarthritis (OA), where declining aggrecan synthesis rates and the decreased capability of assembling large molecular size aggregates illustrate the progressive failure of the repair function of articular cartilage cells. A damaged articular cartilage can affect the normal function of the joint and this ultimately results in clinical symptoms in a proportion of the patients[14]. Structure/disease modifying OA drugs (DMOADs) are expected to prevent structural damage in normal joints at risk for development of OA, or to retard the progression of structural damage in joints already affected by OA[15].

A major flaw when these substances are introduced for pharmacological use in humans is their poor chemical definition. The exact molecular weight is varying. The degree and type of sulphation is different on each of the individual molecules. One class of biologically occurring polysaccharides with well-defined molecular structure and weight are the cyclodextrins. α-, β- and γ-cyclodextrins are cyclic polysaccharides containing 6, 7 and 8 glucopyranose units, respectively, linked through α1-4 glycosidic bonds. Classical sulphation procedures result in the sulphation of the three free hydroxyl groups of the glucopyranoses.

α-, β- and γ-cyclodextrins have been fully sulphated and the influence of these cyclodextrin polysulphates (CDPSs) on the ECM metabolism of human articular cartilage chondrocytes has been evaluated. The incorporation of radiosulphate in proteoglycan was used as a variable to assess which concentrations of the cyclodextrins would result in an effect on chondrocyte metabolism. Once the optimal working concentrations were defined, the effects on factors known to affect the homeostasis of the ECM by chondrocytes from intact articular cartilage were assessed in more detail.

## Materials and methods

### PREPARATION OF SULPHATED α-, β-, AND γ-CYCLODEXTRINS

Commercially available α-, β-, and γ-cyclodextrins (Sigma Chemical Company, St. Louis, USA) were sulphated according to the procedure described by Astrup *et al.*[16]. Briefly, drop-by-drop, one volume of chlorosulphonic acid was added to 6.6 volumes of ice-cold pyridine. Approximately, 300 mg of the polysaccharides was added to 5 ml of the chlorosulphonic/pyridine mixture. The solution was then kept at 100°C for 5 h. After cooling, 25 ml of distilled water was added. One hundred milliliters of 10% (w/v) Na-acetate in methanol was then added to this solution to precipitate the polysaccharide polysulphuric acids. The precipitate was washed two times in methanol and dissolved in an appropriate amount of distilled water before being applied to a Sephadex G10 gel permeation chromatography column to elute the remaining chlorosulphonic acid, pyridine and buffer salts. The void volume fractions containing the polysaccharides were lyophilised. The cyclodextrin polysulphates were recovered as a dry white powder. Electrophoresis on cellulose acetate in a LiCl/HCl (0.01/0.06 N) buffer pH 3.5, at 1 mA/cm kept for 20 min was done to evaluate the degree of sulphation of the cyclodextrins and showed the polysulphated cyclodextrins migrating as homogeneous bands and with the same velocity as the CPS or xylosan polysulphate standards (Fig. 1). Mass Spectrometry was done in the Institut de Recherche SERVIER Laboratories (Orleans, France; Dr F Lefoulon) and confirmed complete sulphating of all cyclodextrin glucopyranoses. Considering initial molecular weights of 973, 1135 and 1297 Da for the unsulphated α-, β-, and γ-cyclodextrins, respectively, on sulphation of the three free hydroxyl groups of the glucopyranoses their molecular weights increased to 2413, 2815 and 3217. The CDPSs were used as such in our experimental *in vitro* models.

### ISOLATION OF ARTICULAR CHONDROCYTES

Human articular chondrocytes were isolated as described elsewhere[17]. Briefly, human articular cartilage was obtained

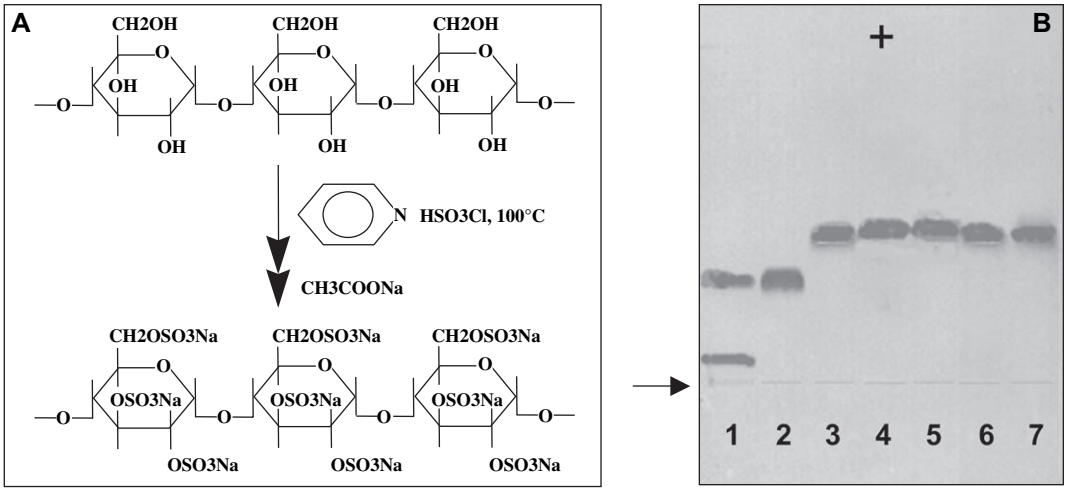


Fig. 1. A: Synthesis of CDPSs – schematic representation. B: Cellulose acetate electrophoresis of the sulphated polysaccharides. +: Positive pool; →: start; lane 1: hyaluronan, chondroitin sulphate; lane 2: chondroitin sulphate; lane 3; CPS; lane 4: α-CDPS; lane 5: β-CDPS; lane 6: γ-CDPS; lane 7: xylosan polysulphate.