EFFECTS OF HYALURONAN ON CELL PROLIFERATION AND GENE EXPRESSION OF ADHESION-RELATED COLLAGEN AND CYTOKINES IN GLENOHUMERAL SYNOVIAL/CAPSULAR FIBROBLASTS DERIVED FROM PATIENTS WITH ADHESIVE CAPSULITIS

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Introduction: Adhesive capsulitis of the shoulder is characterized by spontaneous onset of gradually progressive shoulder pain and passive glenohumeral movement in all planes. Primary adhesive capsulitis develops insidiously, following minimal or no trauma. Adhesive capsulitis may also occur secondary to intrinsic shoulder pathology such as rotator cuff disease.

Understanding of the cause of adhesive capsulitis has been advanced in recent years by several studies of inflammatory cellular mediators [1, 2]. Rodeo et al. compared capsular samples from patients with adhesive capsulitis and normal subjects using a monoclonal antibody technique [2]. They noted an increase in cytokines such as transforming growth factor β (TGF-β) and platelet-derived growth factor (PDGF).

Type III collagen is a molecule that occurs normally, but it is also synthesized as a repair response to tissue injury. In adhesive tissue, type III collagen is produced in larger amounts, and this can inhibit the growth of collagen fibrils and lead to adhesion formation [3]. In addition to an increase of type III collagen production, cell proliferation may be associated with the development of adhesion [4].

Hyaluronan (HA), a high-molecular-weight polysaccharide, is present in large amounts in the extracellular matrix of soft connective tissue and synovial fluid. It has been found to exert beneficial effects for prevention of adhesion in controlled experimental studies [4, 5]. Some investigators have studied the effect of HA in preventing adhesion of flexor tendons. In patients with painful adhesive capsulitis, some studies have demonstrated that exogenously applied HA promotes recovery of range of shoulder motion [6, 7]. However, the mechanism responsible for the clinical improvement seen after HA injection is not fully understood.

In this study, we examined the effects of HA on cell proliferation and the mRNA expression of procollagen α1(III) as an adhesion-related collagen, and that of TGF-β and PDGF as adhesion-related cytokines, in glenohumeral synovial/capsular fibroblasts (GSCF) derived from patients with adhesive capsulitis.

Materials and Methods: Five patients with rotator cuff disease, who satisfied the Codman criteria for adhesive capsulitis, were entered into the study. The average age of the patients was 55 years (range 42-65 years), and the average duration of symptoms was 8 months (3 months to 14 months). Synovial/capsular specimens were harvested from these patients with primary or secondary adhesive capsulitis during arthroscopy. Informed consent was obtained from all patients, and the study protocol was approved by the ethics committee of our hospital. Various concentrations of HA (1.0–4.0 mg/ml) were added to monolayer-cultured GSCF derived from patients with adhesive capsulitis.

The effects of HA on cell proliferation and the mRNA expression of procollagen α1(III) as an adhesion-related collagen, and that of TGF-β and PDGF as adhesion-related cytokines, in glenohumeral synovial/capsular fibroblasts (GSCF) derived from patients with adhesive capsulitis.

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Results: Immunofluorescence cytochemistry showed that GSCF constitutively expressed CD44. Fluorescent HA also showed similar labeling on GSCF. When we used subclass-matched mouse IgG1 instead of OS/37, fluorescence was scarcely seen.

HA decreased cell proliferation significantly in a dose-dependent manner. In addition, HA significantly and dose-dependently decreased the mRNA expression of procollagen α1(III), TGF-β, and PDGF in GSCF derived from patients with adhesive capsulitis.

Discussion: There is a growing body of evidence supporting the use of intra-articular injection of HA in patients with adhesive capsulitis [6, 7], although the mechanism of the effect has not yet been clarified. This in vitro study examined the effects of HA in GSCF derived from patients with adhesive capsulitis, and revealed that HA significantly and dose-dependently decreased cell proliferation and mRNA expression of adhesion-related collagen and cytokines. These results suggest that HA may prevent the progression of adhesion formation in patients with adhesive capsulitis.

HA plays an important role during repair of injured tissue, leading to improved healing with minimal formation of adhesion. The effects of HA may be mediated by its ability to inhibit fibroblast proliferation and concentration of the collagen matrix. Our findings show that exogenously applied HA may act as a modulator of cell proliferation and synthesis of collagen III, inducing minimal formation of adhesion.

TGF-β and PDGF produced in the glenohumeral joint are involved in the inflammatory and fibrotic processes in adhesive capsulitis of the shoulder [2]. In the present study, HA decreased the mRNA expression of TGF-β and PDGF in GSCF derived from patients with adhesive capsulitis, indicating that HA may improve and/or inhibit the development of adhesion in these patients.

CD 44, which is a major receptor for HA, plays an important role in multiple physiological and pathological functions. Our immunohistochemical study identified binding of HA and expression of CD44 on GSCF from patients with adhesive capsulitis. It is therefore considered that these effects may be exerted through the CD44 pathway.

In conclusion, the present study has demonstrated that HA modulates cell proliferation and mRNA expression of procollagen α1(III), TGF-β, and PDGF in GSCF from patients with adhesive capsulitis. Our data may at least partly explain why HA is effective in patients with adhesive capsulitis, suggesting its clinical utility for treatment of the disease.

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