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Basic Science

Experimental chemonucleolysis with recombinant human matrix metalloproteinase 7 in human herniated discs and dogs

Hirotaka Haro, MD, PhD[a,*], Miyuki Nishiga, PhD[b], Daisuke Ishii, MS[b], Takasumi Shimomoto, DVM[b], Tsuyoshi Kato, MD, PhD[c], Osami Takenouchi, MS[b], Satoshi Koyanagi, PhD[d], Tetsuro Ohba, MD, PhD[a], Hiromichi Komori, MD, PhD[e]

[a]*Department of Orthopaedic Surgery, Graduate School of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan*
[b]*Teijin Institute for Bio-Medical Research, Teijin Pharma Limited, Tokyo, Japan*
[c]*Department of Orthopaedic and Spinal Surgery, Tokyo Medical and Dental University, Tokyo, Japan*
[d]*The Chemo-Sero-Therapeutic Research Institute (Kaketsuken), Kumamoto, Japan*
[e]*Department of Orthopaedic Surgery, Yokohama City Minato Red Cross Hospital, Kanagawa, Japan*

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Abstract

BACKGROUND CONTEXT: Chemonucleolysis has been proposed as a less invasive technique than surgery for patients with lumbar disc herniation. Once chymopapain had been approved as a chemonucleolysis drug, it was withdrawn because of serious complications. A novel agent with fewer complications would be desirable.

PURPOSE: The purpose of this study was to investigate the effects of recombinant human matrix metalloproteinase 7 (rhMMP-7) in experimental chemonucleolysis in vitro and in vivo and examine its effects on tissue damage.

STUDY DESIGN: The study design is the experimental study using human herniated discs and enzyme substrates in vitro and dogs in vivo.

METHODS: The effects of rhMMP-7 on the degradation of human herniated discs were examined by measuring the wet weight in vitro. The correlations between the decrease in wet weight by rhMMP-7 and the conditions associated with herniated discs were also analyzed. The effects of rhMMP-7 on the proteoglycan and water contents were respectively examined with alcian blue staining and T_2-weighted magnetic resonance imaging at 7 days after intradiscal injection in dogs. The distribution of [^{125}I]-labeled rhMMP-7 was investigated by autoradioluminography at 7 days after intradiscal injection in dogs. An epidural injection study with rhMMP-7 was performed to evaluate the effects on the tissue damage around the discs at 1 and 13 weeks after the treatment in dogs. The Type 1 and 2 collagen cleavage rates were measured and compared with those of aggrecan in vitro.

RESULTS: Recombinant human matrix metalloproteinase 7 concentration dependently decreased the wet weight of herniated discs in vitro. The decrease in wet weight of the discs by rhMMP-7 did not significantly correlate with the conditions associated with herniated discs. Intradiscal injection of rhMMP-7 reduced the proteoglycan and water contents, with an increase in the serum keratan sulfate levels. Radioactivity of [^{125}I]-labeled rhMMP-7 was detected in the nucleus pulposus and annulus fibrosus but not in the muscle. Epidural injection of rhMMP-7 had no effect on the injection site or the nerve tissues. The Type 1 and 2 collagen cleavage rates of rhMMP-7 were 1,000-fold weaker than those of aggrecan.

CONCLUSIONS: This study demonstrated experimental chemonucleolysis with rhMMP-7 in vitro and in vivo. The effects of rhMMP-7 were not affected by the conditions associated with herniated discs. The epidural injection study together with the autoradioluminography and

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* Corresponding author. Department of Orthopaedic Surgery, Graduate School of Medicine, University of Yamanashi, 1110 Shimokato, Chuo, Yamanashi 409-3898, Japan. Tel.: (81) 55-273-6768; fax: (81) 55-273-9241.

E-mail address: haro@yamanashi.ac.jp (H. Haro)

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in vitro enzyme assay suggests that intradiscal injection of rhMMP-7 may not induce tissue damage around the discs because of its distribution and substrate selectivity. Recombinant human matrix metalloproteinase 7 may be a novel and promising chemonucleolysis agent.

Keywords: Chemonucleolysis; Matrix metalloproteinase 7; Intervertebral disc; Herniation; Minimally invasive treatment; Herniated disc resorption

Introduction

Lumbar disc herniation commonly occurs through the annular disruption associated with degenerative disc disease. In the United States, 2.8 million patients suffer from lumbar herniated discs each year [1]. Patients with lumbar herniated discs are usually 20 to 40 years old and show acute onset of lower extremity pain and/or low back pain. This can result in absenteeism from work because of extensive limitations of daily activity. Intervertebral disc herniation is currently treated with conservative therapy or surgery, including microdiskectomy. Although microdiskectomy is less invasive than traditional herniated discectomy [2], it remains minimally invasive because a skin incision under general anesthesia is needed, and hospitalization is sometimes required. In addition, unavoidable complications such as dural tear and postoperative hematoma are also reported [3].

Chemonucleolysis, a medical procedure involving enzyme injection into a herniated disc, has been proposed as a simpler and less invasive approach for patients who wish to avoid surgery. The hypothetical mechanism of action of chemonucleolysis by the injected enzyme is as follows. First, the intradiscally injected enzyme degrades aggrecan, a major component of the extracellular matrix in the disc. The degradation of aggrecan also results in the reduction of water content in the herniated disc. These processes lead to a decrease in herniated disc volume and reduce the pressure of the herniated disc against nerve tissues on the spinal cord or nerve root. As a result, pain relief will be achieved. Smith et al. [4] administered chymopapain derived from papaya latex into lumbar herniated discs in 1963. The therapeutic effects have been well documented, but chymopapain was withdrawn because of its serious complications, such as anaphylaxis, paraplegia, and subarachnoid hemorrhage. Thus, no drugs for chemonucleolysis are currently available anywhere in the world, and a novel agent with fewer complications would be desirable.

Matrix metalloproteinases (MMPs) belong to a family of neutral secreted Zn^{2+} proteases, and they degrade various components of the extracellular matrix. In particular, both MMP-3 (stromelysin 1) and MMP-7 (matrilysin), which degrade cartilage proteoglycans [5,6], are strongly expressed in surgical samples of herniated discs, and they play a crucial role in the natural resorption process of herniated discs, especially the noncontained type [7–12]. We previously demonstrated that recombinant human matrix metalloproteinase 7 (rhMMP-7) decreased the wet weight of surgical samples of herniated discs more extensively than MMP-3 and the control not treated with either MMP, and it decreased the protruded mass at 1 week after injection in naturally occurring herniation in dogs [13]. The above results suggested that rhMMP-7 might be an ideal candidate as a chemonucleolysis agent.

In this study, the effects of rhMMP-7 on the degradation of human herniated discs were further investigated in vitro, and the effects of the conditions associated with herniated discs, such as patients' ages, degeneration grade, or interval between the onset of symptoms and surgery, on the wet weight reduction of the herniated discs by rhMMP-7 were examined. In addition, the in vivo pharmacologic effects and distribution of rhMMP-7 after intradiscal injection were examined in normal dogs because there is no reproducible animal model of intervertebral disc herniation that accurately reflects the clinical condition in vivo. An epidural injection study with rhMMP-7 was performed to evaluate tissue damage around the discs of dogs. The substrate selectivity of rhMMP-7 was also examined in an in vitro enzyme assay in a mechanistic analysis.

Materials and methods

The in vitro study with surgical samples from herniated patients was approved by the Institutional Review Board of our Institute (approval No. 392, HB11-001). The animal experimental protocols were approved by the Animal Care and Use Committee of our Institute (approval No. B08-001, B09-001, B09-004).

In vitro chemonucleolysis in human herniated discs

All subjects provided their informed consent for use of surgically removed samples for the experiments.

Concentration-dependent effects

Ten surgical samples of herniated discs were obtained from patients undergoing primary lumbar herniotomy. These subjects were nine men and one woman, with an average age of 46 years (range, 18–85 years) at the time of surgery.

Surgical samples were sliced into several pieces, and their weights were measured. Each sample was divided into 10- to 100-mg pieces. Each piece was placed in a separate well of a 24-well plate (Nunc; Thermo Fisher Scientific Inc., Waltham, MA, USA) and incubated with or without rhMMP-7 (0.0198, 0.0781, 0.310, 1.24, and 4.96 U/mL;