

Therapeutic effects of high molecular weight hyaluronan injections for tendinopathy in a rat model

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Abstract

Background Tendinopathy is the most common tendon disorder. The etiology is still uncertain, and the disorder poses many therapeutic problems. In a few clinical studies, analgesic effects of high molecular weight hyaluronan (HMW HA) injections were observed, but the underlying mechanisms were not elucidated. In the present study, we analyzed the therapeutic effects of hyaluronan injections for tendinopathy in an animal model.

Methods We made the tendinopathy rat model using a rodent treadmill machine. Rats with tendinopathy were injected with HMW HA (HA group), normal saline (NS group), or nothing (control group) into the space between the patellar tendon and the fat pad bilaterally, or were injected with HMW HA into the right knees and with saline to the left knees (HA/NS group), 5 times every 4 days. To assess the pain-relieving effect of HA, the spontaneous locomotor activities at night (12 h) and weight bearing of hind paws were measured every day. Histological sections of the patellar tendon stained with hematoxylin–eosin or prepared by TdT-mediated dUTP nick end labeling were microscopically analyzed.

Results The number of spontaneous locomotor activities in the HA group was significantly larger than those in NS or control groups, and in the HA group they recovered up to a healthy level. The percent weight distribution of the right hind paws was significantly increased along with the number of injections. On histologic examinations, the numbers of microtears, laminations, or apoptotic cells in the

patellar tendons in the HA group were significantly lower than those in the NS or the control groups.

Conclusions The injections of HMW HA were effective for pain relief and for partial restoration of the patellar tendon in our tendinopathy rat model, and thus may become an effective therapeutic modality for the disease.

Abbreviations

HMW HA	High molecular weight hyaluronan
NS	Normal saline
EDTA	Ethylenediamine tetra-acetic acid
H&E	Hematoxylin–eosin
TUNEL	TdT-mediated dUTP nick end labeling

Introduction

Tendinopathy (often called tendinitis, tendinosis, or enthesopathy) is the most common tendon disorder characterized by activity-related pain, focal tendon tenderness and decreased strength and movement in the affected area. Tendinopathy can occur in almost any tendon, and if developed, it impairs active performance in athletes and workers of many occupations that involve repetitive movements. Some of the most common tendinopathies are tennis elbow, jumper's knee, and Achilles tendinitis.

Increased production or activation of matrix metalloproteinases and stimulated regeneration of free nerve endings around vessels in affected tendons is found by histological or biochemical studies [1]. Moreover, the finding of apoptosis of tendon cells is a the specific feature of the tendinopathy [1]. It has been suggested that oxidative stress facilitated by high levels of cyclical strain and subsequent apoptosis are involved in the pathogenesis of tendinopathy [1]; however, the detailed pathophysiological mechanisms

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are not understood, and therefore, an effective therapy for tendinopathy has not yet been established.

Muneta et al. recently reported that injections of high molecular weight hyaluronan (HMW HA) into or near tendinopathy-affected tendons produce relief from pain; the treated tendons were the patellar tendon, biceps tendon of the thigh, medial hamstring tendon, lateral gastrocnemius tendon, ilio-tibial band, or the Achilles tendon (reported in a Japanese journal). A similar effect of HMW HA injections for tendinopathy of the plantar fascia was also observed by Higashiyama et al. [2]. In addition, it has been recently reported that peri-articular HA treatment for tennis elbow produced significantly better pain relief at rest and after maximal grip testing than a control therapy [3]. Although a few clinical reports such as those described above have been published, there has been no study scientifically evaluating the pain-relieving effect of HMW HA for tendinopathy or that has histopathologically analyzed tendon tissues.

In order to prove the therapeutic effects of HMW HA injections for tendinopathy, we created an experimental animal model of patellar tendinopathy and scientifically examined the pain-relieving effect of HMW HA and histologically analyzed the tendinopathy-affected tendons. The rat model was prepared using a repetitive running exercise on a rodent treadmill machine. The pain-relieving effect was assessed by measurement of rats' spontaneous locomotor activities for 12 h following the exercise and by evaluation of weight-bearing percent distribution in hind paws injected with HMW HA. Furthermore, histological examinations were performed to evaluate the effects of HA injections on suppression and acceleration of tendon tissue-repair.

Materials and methods

Reagents and materials

Suvenyl[®] (SVE), a preparation of HMW HA for injections into tendons was purchased from Chugai Pharmaceutical Co., Ltd., Tokyo, Japan. The concentration of HMW HA was 10 mg/mL with an average molecular weight of 2700 kDa (viscosity average molecular weight was 1900 kDa). The normal saline for injections was from Otsuka Pharmaceutical Factory, Inc., Naruto, Japan. A rodent treadmill, KN-73, was purchased from Natsume Seisakusho, Co., Ltd., Tokyo, Japan. The Supermex system for measurement of spontaneous locomotor activities of rats was from Muromachi Kikai, Tokyo, Japan. The Incapacitance Tester for measurement of weight bearing distributions in hind paws in rats was from Linton Instrumentation, Norfolk, England. The ApopTag Peroxidase In Situ

Apoptosis Detection Kit was from EMD Millipore Corporation, Billerica, MA, USA.

Animals

Male Wistar rats (16–18 weeks of age, weighing 350–400 g) were purchased from Nippon SLC, Hamamatsu, Shizuoka, Japan, and served as subjects in the study. Rats were housed in an environmentally controlled animal facility on a 12:12 light/dark cycle with food and water available ad libitum in the laboratory animal section.

The animal model

All experiments were conducted in accordance with the institutional guidelines for the care and use of experimental animals after an institutional approval for the study was obtained. A rat model of patellar tendinopathy was made according to the method of Flatow and colleagues [4–6]. A running exercise was performed on a rodent treadmill with 10 % inclination of the running protocol. The rats were acclimated to the treadmill by gradually increasing running speed and time for 2 weeks before the running exercise. Running distance reached up to 40 km during 40 days and the mean running speed was 0.5 km/h. The spontaneous locomotor activities at night were measured for 12 h with the Supermex system on the day before the running exercise and on the day after exercise. The reduction in spontaneous locomotor activity after exercise was different among rats, and it was 26 ± 18 % (3–36 %) for 43 rats. The rats showing >25 % reduction (25–36 %) in spontaneous locomotor activity after exercise were then selected for later study as the tendinopathy rats. Approximately 85 % of the rats ran the whole distance and approximately 90 % were selected for further analyses. Histological sections from the patellar tendons or rotator cuffs in the shoulders of the tendinopathy rats were prepared. Tendinopathy-specific histopathological changes [1] were confirmed with light microscopy at the insertion site of the patellar tendon, while less tendinopathy-specific histopathological changes were observed at the insertion site of the rotator cuffs in shoulders.

Group division

The rats with tendinopathy were divided into four groups. The rats in the HA group, the normal saline (NS) group, or the control group were injected with HMW HA, normal saline, or nothing, respectively, into the patellar tendons bilaterally. Each group consisted of 10 rats. Rats in the fourth, HA/NS group, were injected with HMW HA into the right patellar tendon and with normal saline into the left patellar tendon at the same time and in the same manner. The HA/NS group consisted of 6 rats.

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