A Thrombin-injection Model of Disc Degeneration in the Rabbit

Kunihiro Asanuma,1,3, Yumiko Abe1, Carol Muehleman2, Howard An1, John Sandy2, Atsumasa Uchida1, Koichi Masuda1,2
1Orthopedic Surgery, Rush Medical College at Rush University Medical Center, Chicago, IL; 2Biochemistry, Rush Medical College at Rush University Medical Center, Chicago, IL; 3Orthopedic Surgery, Mie University, Tsu, Japan
Kasanum@gmail.com

Introduction: Several small animal models have been proposed to study the mechanisms of intervertebral disc (IVD) degradation and/or reparative capacity using surgical interventions or the injection of matrix-degrading enzymes. However, most of these models do not mimic the natural course of IVD degeneration in the human [1].

Thrombin, a serine protease that is a key enzyme in the coagulation cascade, has been shown to be a multifunctional protein involved in a variety of biologic functions. Recent studies have suggested that thrombin plays a critical role in matrix degradation by inducing matrix metalloproteinases (MMPs) and by cleaving matrix components, such as syndecan and fibronectin [2]. In articular cartilage, thrombin has been shown to stimulate proteoglycan (PG) degradation [3]. In humans, the presence of thrombin and the thrombin receptor (PAR1) has been demonstrated in the degenerative disc [4]. In bovine IVD cells, thrombin induced MMP production and accelerated proteoglycan (PG) turnover [4]. The fact that a degenerative disc is increasingly vascularized may be a factor responsible for the intradiscal presence of thrombin [4]. These studies lead us to hypothesize that thrombin may have a potential involvement in the degradation of the matrix in IVD tissues [4].

The purpose of this study was to identify the in vivo effects of an intradiscal thrombin injection and to develop an animal model that mimics human disc degeneration.

Materials and Methods: Intradiscal thrombin injection: Eight adolescent NZW rabbits (3.5–4 kg) received an injection of bovine thrombin (1, 10, 100 U in 10 μl of saline) into the center of the nucleus pulposus (NP) at the L2/3, L3/4 and L4/5 levels (randomly assigned). Radiological/MRI assessments: The disc height was radiographically monitored biweekly. The percent disc height index was calculated as previously described [5]. After sacrifice, T2W MRI scans of the spinal columns were obtained to grade the level of degeneration based on a modified Thompson grade (MRI: 1=normal, 4=severely degenerated) [5]. Histological analysis: The discs were graded on four parameters using an established grading scale ranging from a normal score (4) to a severely degenerated (12)/5). Immunoblotting for PG fragments: NP and anulus fibrosus (AF) tissues were extracted with 4 M guanidine hydrochloride [6]. PGs were deglycosylated [6], and separated by 4–12% SDS-PAGE. Immunoblotting was performed with an anti-G1 antibody and anti-EAR antibody (against the C-terminal neo-epitope NITEGEAR, the plasmin cleavage site). Statistical Analysis: Two-way ANOVA and Fisher’s PLSD post hoc test or the Mann-Whitney test.

Results: Disc Height Analysis (Fig. 1): Thrombin treatment resulted in a significant decrease of %DH1 in a dose-dependent manner (-15.6% at 1 U/disc, -27.0% at 10 U/disc, -44.7% at 100 U/disc vs. non-injected control at 12 weeks, P<0.005, repeated ANOVA). The higher doses of thrombin-induced changes in %DH1 at earlier time points (100 U/disc 2W, p<0.05, 10 U/disc 4W, p<0.05) than the lower dose-induced (1 U/disc, 6W, p<0.05). MRI Grading (Table 1): Thrombin treatment significantly increased MRI grade scores (more degeneration) in a dose-dependent manner (Grade: P<0.0001 at all doses). Histological Grading and Appearance (Figs 2, Table 1): The total score for the histological grading scale of the thrombin group (10 and 100 U/disc) at the 12-week time point was significantly higher (more degeneration) than that of the non-injected control group (P<0.005). A dose-dependent histological change of the NP and AF was observed (Fig. 2). Aggrecan fragmentation (Fig. 3): In the NP and AF of the thrombin-injected discs, the aggrecan was fragmented into small-size fragments (see anti-G1). The smallest G1 positive fragment (55 kDa) was confirmed to be reactive to the anti-EAR antibody, corresponding to the G1-EAR fragment.

Discussion: We have shown for the first time that an injection of thrombin into the rabbit NP-induced IVD degeneration. This was confirmed by radiographical, MRI and histological analyses. The analysis of aggrecan fragmentation in the disc indicated that there were several G1-bearing fragments of aggrecan in the thrombin-injected discs. Our preliminary study of the in vitro effects of thrombin on aggrecan degradation also indicated that thrombin cleaves aggrecan within the interglobular region. Because thrombin has been shown to stimulate the production of MMPs by disc cells and to accelerate PG turnover in vitro [4], the cell-mediated stimulation of disc degeneration may occur to some degree; this needs further study. Because the rabbit aggrecan fragmentation pattern was similar to that in the human [7] and the degree of degeneration can be manipulated by using different doses of thrombin, this model may resemble the natural degeneration of the disc in the human and may be utilized to study disc degeneration and regeneration using therapeutic interventions, such as the injections of growth factors, protease inhibitors, or cytokine inhibitors.


Acknowledgements: NIH grants (P01-AR48152 and P50-AR39329).

Table 1: MRI and Histological grade (Thrombin: U/ml)

<table>
<thead>
<tr>
<th>MRI grade</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

Fig. 1 Change in disc height index (%DH1) after thrombin injection

Fig. 2 Histological appearance in non-injected and thrombin-injected IVDs. (HE: top, Safranin-O bottom)

Fig. 3 Aggrecan fragmentation profile: western blot by anti-G1 antibody (left) and anti-EAR antibody (right). Thrombin (U/disc)