CATHEPSIN B AND NEUTROPHIL ELASTASE DEGRADE LUBRICIN AND INCREASE FRICTION IN EXCISED MURINE JOINTS

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INTRODUCTION

Lubricin[1] and SZP[2], are heavily glycosylated mucinous proteins, secreted by synovial fibroblasts and superficial zone articular chondrocytes. These factors are responsible for boundary lubrication of apposed and pressurized articular cartilage surfaces. Compromised boundary lubrication, due to the proteolytic degradation of lubricin, was evidenced in synovial fluid (SF) aspirates from patients with rheumatoid arthritis (RA) and knee injuries[3]. The loss of boundary lubrication can potentially enhance articular cartilage damage[4] as a result of increased friction. Cysteine proteases e.g. cathepsins B, L, and serine proteases e.g. neutrophil elastase (NE)[5], have previously been observed in synovial fluids from patients with RA and OA. The implication is that either or both of these enzymes classes play a role in the proteolytic damage to lubricin and subsequent loss of boundary lubrication.

The purpose of this study is to examine the effects of a time-dependent digestion of normal bovine SF (BSF) by cathepsin B, and NE on lubricating ability *in vitro*, and whether these observations could be reproduced in mammalian joints *ex vivo*.

METHODS

A- Proteolytic digestion of BSF and *in-vitro* friction assay

BSF was aspirated percutaneously from the lateral aspect of radiocarpal joints of freshly slaughtered cattle. Aliquots of BSF (1 ml) were incubated with NE (Sigma) at 0.05 U/ml, reconstituted in 100mM Tris-HCl, 100mM $CaCl_2$, pH 8.8, and cathepsin B (Sigma) at 0.05 U/ml, reconstituted in 0.25 M Na acetate, pH 5.5 for 24 hours at 37°C. Aliquots of digested BSF (200 μl) were removed after 6, 12, and 24 hours, and the reaction was stopped by adding phenylmethanesulfonyl fluoride at a final concentration of 1mM, and E-64 at a final concentration of 100 μM. The integrity of lubricin band in BSF was analyzed by 4-15% SDS-PAGE, followed by Western blotting and probing with peanut agglutinin-peroxidase. The friction analysis was performed as previously described[3], and the change in BSF μ in reference to normal saline was expressed as Δμ. A negative Δμ indicates lubrication and a positive Δμ indicates friction. The ability of cathepsin B and NE to degrade lubricin and cause a loss of lubrication was compared to two other proteolytic enzymes (trypsin and α-chymotrypsin) previously shown to digest lubricin.

B- *Ex-vivo* μ of excised murine joints using Stanton modified pendulum technique

Excised murine joints were stripped of supporting connective tissue and musculature, while the synovium was left undisturbed. The femur and tibia were severed mid-length and covered with connecting plexiglass tubing. The center of the joint served as the axis of rotation of a 1 Hz pendulum. The joints were loaded with 20 grams and allowed to oscillate. The pendulum motion was videotaped and post-hoc analysis was performed to establish baseline μ. A 5 μl protease solution containing 0.05 U of each of chymotrypsin, cathepsin B, and NE was delivered intra-articularlly. The limbs were incubated at room temperature for 2 hours and the limbs were subsequently allowed to oscillate to estimate μ following treatment with proteases.

RESULTS

NE and Cathepsin B in particular exhibited an ability to degrade lubricin in BSF as demonstrated by a decreasing lubricin concentrations in the digested BSF compared to control and previously used enzymes (Fig. **1**). A loss of lubricating ability was observed for all enzymes. Cathepsin B appeared to result in a more rapid loss and greater amount of friction in the latex-glass bearing (Table **1**). The intra-articular injection of cathepsin B, and NE increased friction in excised murine joints. Cathepsin B resulted in an approximately 75% increase in μ of excised murine joints compared to the normal saline sham injection (Table **2**). By contrast, NE resulted in an approximately 25% increase in μ.

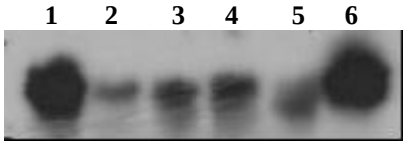

Figure 1 Western Blot Analysis of **1**- BSF, **2**- BSF+0.05 U/ml cathepsin B, 24 hr, **3**- BSF+0.05 U/ml NE, 24 hr, **4**- BSF+0.05 U/ml chymotrypsin, 24 hr, **5**- BSF+0.05 U/ml trypsin, 24 hr, **6**- BSF following incubation at 37°C for 24 hr.

Table 1 Changes in μ (Δμ±S.D.) following time-dependent proteolytic digestion by 0.05 U/ml cathepsin B, NE, chymotrypsin, and trypsin.

	BSF	BSF+Cathepsin B (0.05 U/ml) (n=8)	BSF+NE (0.05 U/ml) (n=8)	BSF+Chymotrypsin (0.05 U/ml) (n=8)	BSF+Trypsin (0.05 U/ml) (n=8)
0 Hours	-0.061±0.005	-0.061±0.005	-0.061±0.005	-0.061±0.005	-0.061±0.005
6 Hours	-0.056±0.002	-0.017±0.007	0.009±0.001	0.006±0.001	0.005±0.001
12 Hours	-0.052±0.003	0.004±0.016	0.010±0.001	0.01±0.004	0.009±0.003
24 Hours	-0.049±0.001	0.048±0.006	0.013±0.002	0.012±0.007	0.012±0.004

Table 2 Excised murine joints (n=4) μ before and after intra-articular injection of 0.05 U of chymotrypsin, cathepsin B, and NE.

	Saline			Chymotrypsin		
	Before	After	% Δμ	Before	After	% Δμ
μ±S.D.	0.0017±0.0004	0.0017±0.0002	2.89	0.0021±0.0005	0.0048±0.0032	137.80
	Cathepsin B			NE		
	Before	After	% Δμ	Before	After	% Δμ
μ±S.D.	0.0019±0.0005	0.0033±0.0005	78.57	0.0022±0.0005	0.0028±0.0008	27.91

DISCUSSION

BSF digested by either Cathepsin B, or NE are capable of proteolytically degrading lubricin, resulting in an elevation in μ in an artificial bearing system. These same results were replicated *ex vivo* following intra-articular injections of cathepsin B and NE. Given the elevated levels of these two enzymes in RA and Cathepsin B in early OA, they likely play a role in the loss of boundary lubrication and may be important targets in mitigating early joint damage.

REFERENCES

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