INHIBITION OF CATHEPSIN B, AND L RETARDS THE LOSS OF BOUNDARY LUBRICATION OF RHEUMATOID ARTHRITIS SYNOVIAL FLUID ASPIRATES *IN VITRO*

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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. Previously, we have reported the boundary lubricating ability of synovial fluid (SF) aspirates from patients with osteoarthritis (OA), and rheumatoid arthritis (RA)[3]. The SF aspirates from RA populations have exhibited a compromised boundary lubricating ability compared to OA and normal SF aspirates[3]. Lubricin has been shown to be proteolytically degraded by a host of proteases, which are generally up-regulated in inflammatory diseases[4].

The aim of this research is to study the effect of supplementing pooled RA SF aspirates with purified human lubricin in restoring SF's lubricating ability and the effects of Z-LLL-FMK, a specific cathepsin B, and L inhibitor in retarding the loss of boundary lubrication in RA SF aspirates.

METHODS

A- Supplementing OA, and RA pooled SF with purified human lubricin and subsequent friction analysis

Human lubricin was purified from the SF aspirates from patients undergoing total knee replacement as described previously[1]. The SF aspirates from OA (n=60) and RA (n=45) were pooled in equal proportions and subsequently supplemented with purified human lubricin until normal lubricating values, comparable to that of normal human SF were obtained. The, lubricin-supplemented, pooled SF aspirates were incubated at 37°C, and assayed in the friction apparatus at 24, 48, and 96 hours. The friction apparatus recorded changes in coefficient of friction ($\Delta\mu$) of SF compared to normal saline as described previously[1]. A negative $\Delta\mu$ value indicates lubrication, while a positive $\Delta\mu$ value indicates friction. Normal HSF aspirates were from allograft cartilage donors, generously provided by Martin Lotz, PhD.

B- Inhibition of loss of boundary lubrication of RA SF aspirates following purified human lubricin supplementation by Z-LLL-FMK and EDTA

The RA pooled SF aspirates were supplemented with purified human lubricin as described above. Two inhibitors were utilized in this study, Z-LLL-FMK (Sigma), a specific inhibitor of cathepsins B, and L at a final concentration of 20 μM, and ethylenediamine tetra acetate (EDTA) to a final concentration of 10 mM. Pooled SF was incubated with the two inhibitors separately at 37°C and sampled at 24, 48, and 96 hours. The friction analysis of the SF aspirates was performed as described above. Specific cathepsin B activity was determined colorimetrically in a manner similar to the one described previously[5].

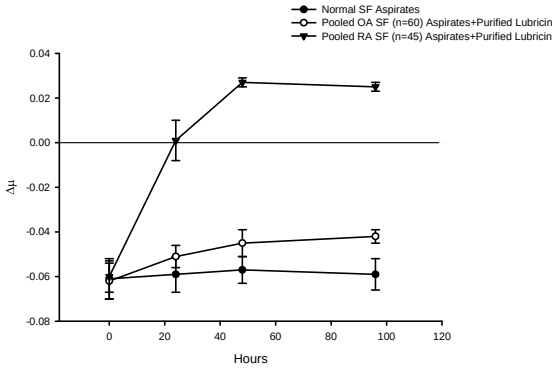

Fig. 1. $\Delta\mu$ of pooled normal, OA, and RA SF supplemented with purified human lubricin and assayed at 24, 48, and 96 hours in friction apparatus. Each $\Delta\mu$ value is an average of two experiments, each with 4 distinct measurements of μ.

RESULTS

The pooled RA SF aspirates exhibited a time-dependent loss of lubricating ability following lubricin supplementation (**Fig. 1**). Addition of Z-LLL-FMK significantly reduced the loss of the boundary lubrication of lubricin-supplemented pooled RA SF aspirates (**Table 1**). By contrast, addition of EDTA failed to preserve lubrication as well. Cathepsin B activity levels are elevated in the RA SF aspirates compared to OA SF aspirates ($p < 0.001$) indicating increased proteolytic activity attributable to this enzyme (**Fig. 2**).

Table 1 $\Delta\mu$ of pooled RA SF supplemented with purified human lubricin and Z-LLL-FMK and EDTA.

	Pooled RA SF (n=45)+Purified Lubricin	*Pooled RA SF (n=45)+Purified Lubricin+ Z-LLL-FMK*	*Pooled RA SF (n=45)+Purified Lubricin+EDTA*
	$\Delta\mu$ ±S.D.	*$\Delta\mu$ ±S.D.*	*$\Delta\mu$ ±S.D.*
0 Hours	-0.060±0.002	-0.060±0.002	-0.060±0.002
24 Hours	0.001±0.009	-0.049±0.002	-0.006±0.004
48 Hours	0.027±0.002	-0.019±0.001	0.007±0.004
96 Hours	0.025±0.002	-0.005±0.003	0.017±0.001

Each $\Delta\mu$ value is an average of two experiments, each with 4 distinct measurements of μ.

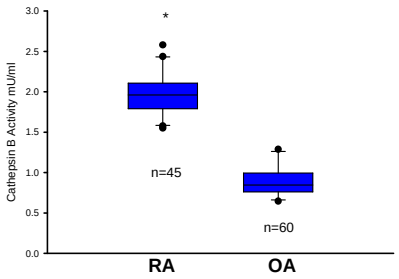

Fig. 2. Cathepsin B Activity in the SF Aspirates from RA (n=45), and OA (n=60).

DISCUSSION

The time-dependent declining lubricating ability of pooled RA SF aspirates, following lubricin supplementation indicates that lubricin is being degraded by the proteases resident in the RA SF aspirates. Z-LLL-FMK, a specific inhibitor of cathepsins B, and L demonstrated a significant ability to retard the loss of boundary lubrication of lubricin-supplemented RA SF aspirates, which exceeded that of EDTA. The increased cathepsin B activity in the RA SF aspirates, and the ability of cathepsin B inhibition to retard the loss of boundary lubrication point to an important role by cathepsin B in the loss of SF boundary lubrication in RA populations.

REFERENCES

1- Jay *et al*, 2000, J. Rheumatol., 27, 594-600.
2- Flannery *et al*, 1999, Biochem. Biophys. Res. Comm., 254, 535-541.
3- Jay *et al*, 2004, J. Rheumatol., 31, 557-564.
4- Jones *et al*, 2003, Trans. Orthop. Res. Soc., 28, 31.
5- Mantle *et al*, 1999, Clin. Chim. Acta, 284, 45-58.