HEPARAN SULFATE MIMETICS INCREASE THE CONCENTRATION OF MACROPHAGES, BUT NOT NEUTROPHILS FOLLOWING ACHILLES TENDON INJURY.

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Introduction

Heparan sulfate analogue, called regenerating agent (RGTA) has been shown to improve tissue healing in several animal models. For instance, RGTA preserves the basal lamina structure, favors the maturation of myosin isoforms and improves reinervation following crushed muscle injury. In other tissues, RGTA can reduce myocardial injury and accelerate osteogenesis after craniotomy. Recent results strongly suggest that RGTA would promote healing and prevent damage by increasing the bioavailability of growth factors and by modulating the activity of proteinases involved in tissue degeneration and regeneration. However, the effect of RGTA on the inflammatory response following an injury has never been investigated.

Tendons are hypocellular and hypovascular tissues that are known to heal very slowly as functional deficits can remain even after 1 year of healing. Therefore, the goal of this study was to evaluate the effect of RGTA on the accumulation of key subsets of leukocytes that participate in the degenerating and regenerating phases of tendon healing; namely neutrophils (PMNs) and ED1+ macrophages (Mφs), after a tendon injury. We hypothesized that RGTA would modulate the profile of inflammatory cell accumulation since some heparan binding growth factors (HBGF) are known to influence this process.

Methods:

RGTA: (d120) is a dextran based heparan sulfamate kindly provided by OTR3 SARL, Creteil, France.

Surgical procedure: Female adult Wistar rats weighing approximately 200 g were used in this study. Rats were anesthetized with a cocktail of ketamine and xylazine at doses of 87.5 mg/kg and 12.5 mg/kg, respectively. Skin was disinfected using classical surgical procedures in order to avoid any inflammation due to infection. The sham procedure consisted in a percutaneous injection of 15 µl of sterile phosphate buffered saline (PBS), into the Achilles tendon. Tendon injury was induced by adding 5 mg/ml of crude collagenase into PBS solution. Ambulatory controls (CTR) were allowed to normal cage activity. Animals were sacrificed 1 and 3 days following the experimental procedures [3]. The Laval University Research Center Animal Care and Use Committee approved all animal care and handling.

Experimental treatment: RGTA was administered 1 day following the injury to avoid any interference with the activity of exogenous collagenase. RGTA was administered in two sites. Thirty µl of RGTA (100 µg/ml) were injected into the tendon. Simultaneously, 1.5 mg/kg of RGTA dissolved in saline was administered IM in the contralateral hindlimb.

Tissue Preparation and Immunohistochemistry: After sacrifice, hindlimbs were fixed overnight in formalin 10% in a dorsiflexion position and excised tendons were prepared for paraffin sectioning. Two longitudinal sections separated by 0.5mm were withheld and labeled for PMNs and ED1+ Mφs. Positive cells were counted blindly under a light microscope and cell concentration was expressed per volume of tissue examined.

Statistical analysis: The concentration of inflammatory cells from sham-operated tendons was compared to CTR to evaluate the effect of the surgery. Collagenase injected tendons were compared to sham-operated animals from the same time point. A Fisher’s PLSD test was used to compare means when a significant F ratio was obtained following the analysis of variance. The level of significance was set at p< 0.05.

Results:

Sham-operated tendons showed a very small, although significant, accumulation of ED1+ Mφs on day 1 when compared to CTR. The concentration of PMNs was slightly affected by the sham procedure. Percutaneous injection of collagenase produced significant tissue degradation and inflammatory cell accumulation when compared to the sham procedure. The concentration of PMNs peaked on day 1 at a concentration of 175 000 cells per mm3 and rapidly decreased to approximately 45 000 cells per mm3 on day 3. ED1+ Mφs also peaked on day 1 but slightly decreased 3 days post-trauma. The administration of RGTA on day 1 induced a 2-fold increase of ED1+ Mφs at day 3. Surprisingly, RGTA did not change the concentration of PMNs.

Discussion:

Current evidence indicates that Mφs play more subtle functions than phagocytosis during the resolution of inflammation [4]. Mφs can release potent growth factors that can influence the proliferation, differentiation and recruitment of fibroblasts which will contribute substantially to the extracellular matrix formation. The results of the present study indicate that RGTA increased the infiltration of ED1+ Mφs in injured tendons at 3 days post-injury. However, the concentration of PMNs in tendons did not differ between placebo and treated groups. One possible explanation for the selective increase in ED1+ Mφs is that HBGF such as Transforming Growth Factor-β1 or Platelet Derived Growth Factor are chemoattractant molecules for Mφs. On the other hand, PMNs are known to respond particularly to CXC chemokines. The impact of ED1+ Mφs accumulation in injured tendons is not known but cumulative evidences indicate that chemotraction and activation of Mφs at the site of injury would accelerate tissue healing. Additional histomorphological analyses and biomechanical testing are required to clarify the contribution of RGTA in promoting tendon healing.

References:


This investigation was supported by Natural Sciences and Engineering Research Council of Canada (NSERC), Fonds de Recherche en Santé du Québec (FRSQ), Fonds Concertés pour l’Aide à la Recherche (FCAR).

Poster #0816