Low Molecular Weight Hyaluronic Acid-Methotrexate Conjugates for Targeted Drug Delivery in the Treatment of Rheumatoid Arthritis

N. Zhang, K.L. Wagoner, R.A. Bader

Department of Biomedical & Chemical Engineering, Syracuse University, Syracuse, NY 13244

Statement of Purpose: Despite over expression of the hyaluronic acid/CD44 receptor by inflamed synovial tissue,[1] the use of hyaluronic acid (HA) as a targeted drug delivery vehicle in the treatment of rheumatoid arthritis has been relatively unexplored. The nature of the disease necessitates systemic delivery; however, native hyaluronic acid is too large to safely be administered systemically, and the half life of HA is short due to rapid clearance by the HARE receptor of liver endothelial cells.[2] As a first step in generating a systemically administrable HA based drug delivery vehicle, we degraded HA to a molecular weight that will permit passive targeting through the highly permeable vasculature of the inflamed synovial tissue. The resultant low molecular weight HA was then conjugated to methotrexate (MTX) through an adipic dihydrazide linker using known methodology. The goal of the current study was to demonstrate that MTX conjugated to HA (HA-MTX) is more effective at inducing an anti-inflammatory response in rheumatoid arthritis synovial fibroblasts (RASFs) than MTX alone or MTX conjugated to a synthetic polymer that cannot bind to the CD44 receptor.

Methods: Adipic dihydrazide modified HA was obtained following known methods.[3] Briefly, high molecular weight HA was degraded with hyaluronidase. The resultant low molecular weight HA was coupled to adipic dihydrazide following activation of the carboxylic acid moiety with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI). In an analogous manner, adipic dihydrazide was coupled to the EDCI activated glutamic acid group of MTX to yield HA-MTX (Fig. 1). The bioconjugates were fully characterized by ^{1}H NMR and GPC coupled with a static and dynamic light scattering detector. ^{1}H NMR showed modification of approximately 68% of the disaccharide monomers with MTX. GPC-light scattering yielded a molecular weight (58 kDa) and hydrodynamic radius (48 nm) that were both above the threshold necessary for passive targeting.

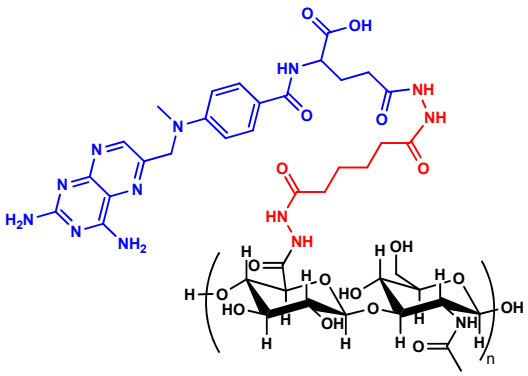

Fig. 1. Structure of HA-MTX

MTX and HA-MTX were tested *in vitro* by administration of doses of 0.1 mg/ml to a TNF-α stimulated RASF cell line. For comparison, poly(ethylene glycol) conjugated MTX (PEG-MTX),[4] which will not bind to the CD44 receptor, but that may be internalized via the reduced folate carrier, was also evaluated. 24 hours after administration, the supernatant was removed (N=3 wells for each group), and the concentrations of the following pro-inflammatory mediators that have been implicated in rheumatoid arthritis pathogenesis were determined using multiplex immunoassay on the Luminex® platform: IL-6, IL-8, and VEGF.

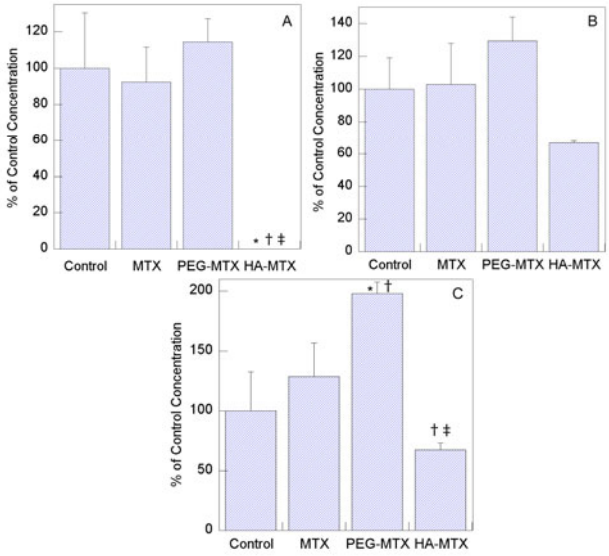

Fig. 2. Effect of MTX, PEG-MTX and HA-MTX on RASFs with regards to secretion of (A) VEGF, (B) IL-6, and (C) IL-8. * Indicates $p < 0.05$ with respect to control cells, † indicates $p < 0.05$ with respect to cells treated with MTX, and ‡.indicates $p<0.05$ with respect to cells treated with PEG-MTX.

Results: As shown in Fig. 2, MTX alone elicited no response, while PEG-MTX was pro-inflammatory in regards to the secretion of IL-6 and IL-8. In contrast, HA-MTX significantly reduced the secretion of VEGF and IL-8. IL-6 concentrations were also decreased for cells treated with HA-MTX relative to the other treatment groups, but the difference was not significant. The levels of VEGF were so low as to be undetectable after administration of HA-MTX.

Conclusions: The results obtained thus far suggest that HA can be used as an effective tool in improving the effectiveness of existing therapeutics in the treatment of rheumatoid arthritis by targeting the CD44 receptor. Although the effects of HA alone were not evaluated in the current study, fragmented HA has been shown to simulate release of IL-6 and IL-8;[5-6] therefore, the anti-inflammatory response observed is believed to be a result of more effective uptake of MTX.

References:

1. Haynes BF. Arthritis Rheum. 1991;34:1434-1443.
2. McGary CT. Biochem J. 1989;257:875-884.
3. Luo Y. Biomacromolecules. 1999;10:755-763.
4. Vortherms AR. Oncology. 2009;3:39-36.
5. Fujii K. Lab Invest. 1999;79:1439-446.
6. McKee CM. Clin Invest. 1996;98:2403-2413.