A Novel Nanoparticle Delivery Vehicle for Transfection of microRNA-122 in Rat Articular Chondrocyte Model of Osteoarthritis In Vitro

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INTRODUCTION: microRNA (miR) are 19-24 nucleotides in length and act as non-coding RNA that differentially regulate expression of messenger RNA. Previously, we showed that lipofectamine transfection of miR-122 mimic in rat articular chondrocytes (rArCs) increased total cellular DNA and attenuated the inflammatory response to interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which mimics the inflammatory environment found in osteoarthritis. However, lipofectamine demonstrates limited utility in vivo. As such, alternative miR delivery mechanisms are needed. Amino-poly-amido saccharide (AmPAS) is a carbohydrate-derived, aminated, cationic polymer that is cyto-compatible and forms nanoparticles in the presence of nucleotides, including those found with microRNA, due to ionic interactions between the amines along AmPAS backbone and the phosphate groups along the nucleotide backbone. Therefore, the aim of this study is to develop and evaluate the utility of the AmPAS polymer to deliver miR-122 to regulate osteoarthritis in the in vitro rArC model.

METHODS: rArCs were harvested from 100-150g male Sprague Dawley rats following VCU IACUC approval. Cells were plated at 15,000 cells/cm^2 and allowed to grow to 60% confluency before treatment with 29 nM AmPAS complexed with miR-122-5p mimic (AmPAS-miR122) or triphosphate negative control (AmPAS-TPP) for 72 hours. A third and fourth group received transfection media without AmPAS nanoparticles. After 48 hours, group 3 was transfected with 14.5 nM miR-122-5p mimic using Lipofectamine RNAiMAX for an additional 24 hours. After 72 hours of AmPAS treatment or lipofectamine transfection, cells were treated with 10 ng/mL of rat IL-1β or vehicle for 24 hours. Cells were harvested followed by quantification of DNA, prostaglandin E2 (PGE2), matrix metalloproteinase 13 (MMP-13), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS). In a second set of experiments, the IL-1β treatment was replaced with TNF-α treatment with the same outcome measurements. One-way ANOVA with Bonferroni correction was used for significance ($p < 0.05$).

RESULTS: DNA significantly increased with lipofectamine transfection of miR-122-5p regardless of IL-1β or TNF-α treatment. AmPAS-miR-122 increased DNA above non-treated controls and AmPAS-TPP with IL-1β treatment, but not to the level of lipofectamine transfection. AmPAS-miR-122 mitigated the inflammatory response to both IL-1β and TNF-α as measured by MMP-13 (Figure A). A similar reduction was seen in IL-6 with IL-1β induction, not reaching the level of lipofectamine. However, AmPAS-122 significantly decreased the inflammatory response to TNF-α as measured by IL-6, even more so than lipofectamine transfection (Figure B). No effect was found with treatment of AmPAS-miR122 at the level of PGE2 or iNOS, unlike lipofectamine, which significantly attenuated both inflammatory markers.

DISCUSSION: There is a growing demand for biopolymers and biomaterials to deliver signaling molecules, such as miR. The results of the present study indicate that AmPAS, with its biocompatible nanoparticle formation, is a viable delivery mechanism for miR. It did not exhibit cellular toxicity, even with higher dosing than lipofectamine transfection. However, the inflammatory response to IL-1β and TNF-α is only partially attenuated with two markers demonstrating significant decreases and two markers at the same level as the untreated control following AmPAS-miR122 treatment in contrast to the lipofectamine treatment. Ongoing studies are directed at varying the physical characteristics of the AmPAS-miR122 nanoparticle to increase the release kinetics of miR delivery into cells, allowing for greater utility of this biomaterial in vivo.

SIGNIFICANCE/CLINICAL RELEVANCE: IL-1β or TNF-α treatment of rArCs serves as an in vitro model of the inflammatory environment of osteoarthritis. As such, potential treatments and their associated delivery can be assessed. The current study evaluates the use of a carbohydrate-derived polymer to aid in the delivery of miRNA-122 as a possible therapeutic for osteoarthritis with results suggesting a viable delivery mechanism without cellular toxicity.

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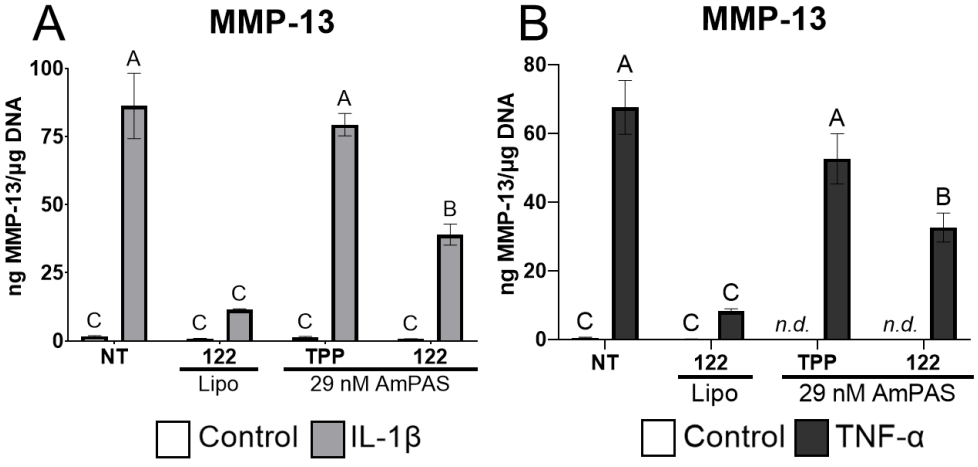

FIGURE 1: Matrix metalloproteinase 13 (MMP-13) significantly decreased with AmPAS-miR-122 treatment following inflammatory induction with IL-1β (A) and TNF-α (B) compared to untreated (NT) control.