Melittin-GST fusion protein and hyaluronic acid have augmented anti-inflammatory properties when combined

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ABSTRACT SUMMARY
We explore the anti-inflammatory therapeutic potential of a melittin GST-fusion protein and hyaluronic acid. We show that the combination of these components results in augmented anti-inflammatory activity. Our results are a preliminary showcase for the development of a controlled delivery system for protein delivery in which the delivery vehicle is pharmacologically active and aids in arthritis therapy.

INTRODUCTION
With a growing population of osteoarthritis sufferers, and no disease modifying drugs, looking into combinations of traditional and alternative medicine may help elucidate new therapeutic options. We have explored melittin, a component of bee venom, which has been used in complementary/alternative medicine to treat arthritis for hundreds of years (1). Although it is thought to have potent anti-inflammatory effects, it is relatively cytotoxic, forming membrane pores at less than nanomolar concentrations (1-3). This limits its therapeutic window and thus its clinical utility for arthritis. We have created a “latent” melittin, by recombinantly fusing a glutathione s-transferase at the N-terminus of the peptide. This modification allows melittin a much wider therapeutic window with non-toxic concentrations that far exceed those of the traditional peptide. Additionally, GST has also been shown to have anti-inflammatory effect (4). We have looked at GST-melittin in combination with hyaluronic acid. High molecular weight hyaluronic acid is routinely used in arthritis therapy, can be incorporated into controlled delivery systems because of its favorable biologic properties, has the ability to modulate macrophage phenotype, and has potent anti-inflammatory properties (5). We show that in inflammation due to LPS, a combination of the traditionally used polymer and GST-melittin results in a decrease in inflammation at the doses tested to a greater extent than either component alone. This finding suggests that exploring combinations of anti-inflammatory peptides, proteins, and polymers may result in novel clinically applicable therapeutic options for osteoarthritis sufferers.

EXPERIMENTAL METHODS
GST-melittin was purified by a method previously described (2). In vitro toxicity of the components was assessed using J774A.1 macrophages via metabolic analysis using MTS reagent as well as changes in membrane permeability visualized with propidium iodide. Evaluation of anti-inflammation of both components, alone and in combination, was done using quantitative real time PCR for common inflammatory markers. Production of nitric oxide, which was quantified using the Griess reagent, was also used to assess extent of inflammation. Current studies are evaluating the effect of these components on inflammation due to LPS using the chorioallantoic membrane assay.

RESULTS AND DISCUSSION
Neither hyaluronic acid nor GST-melittin have toxicity on J774A.1 cells (Figure 1). This is especially significant in the case of melittin, as the native form of the peptide is known to have cell-lytic properties. The fusion of GST and melittin attenuates this toxicity and allows us a much wider therapeutic window for its use.

In cells that have been activated with LPS, both components have the ability to modulate the inflammatory response by reducing nitric oxide synthesis, which corroborates with their potential to reduce iNOS gene expression (Figures 2 and 3). Reduction of iNOS and nitric oxide is significant as
once activated iNOS can synthesize significantly more nitric oxide than physiologic concentrations produced by the constitutive enzymes over longer periods of time, and this can lead to excessive tissue toxicity and damage.

Each of these also has the ability to significantly reduce COX-2 and TNF-alpha gene expression in inflamed macrophages (Figure 3). COX-2 is responsible for prostaglandin synthesis, which has a role in modulating pain. Similarly, TNF-alpha, a central regulator of the inflammatory response, has widespread functions in tissue damage and pain. Therefore, the downregulation of both of these genes has significant implications in not only inflammation due to osteoarthritis, but also other disease pathologies.

The anti-inflammatory activity of the combination of both hyaluronic acid as well as GST-melittin is differential at early and late timepoints (Figure 4). Early inflammation time-points (4 hours) show that a combination of both results in an augmented reduction in COX-2 expression compared to either component alone. Later time-points (24 hours) show superior reduction of iNOS and TNF-alpha expression, but not COX-2 expression. At all time-points, all genes of interest are reduced compared to the untreated inflamed control.

Current studies are underway to elucidate if this augmented anti-inflammatory action holds in an in vivo LPS inflammation model using the chorioallantoic membrane assay.

CONCLUSION

GST-melittin is a non-toxic alternative to native melittin that maintains anti-inflammation over several logs of concentration. In combination with hyaluronic acid, augmentation of anti-inflammation can be achieved. The findings of this study suggest: (I) exploring the fusion protein complement to native proteins may offer alternative therapeutically relevant, non-toxic, options, and (II) that exploring other combinations of natural therapeutic polymers and proteins can lead to therapeutics that have superior pharmacodynamic qualities.

REFERENCES


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