Signal Transduction of Hyaluronic Acid - Peptide Conjugate for Formyl Peptide Receptor Like 1 Receptor

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Statement of Purpose: Hyaluronic acid (HA) has been investigated as a novel drug carrier for various protein and peptide drugs [1,2]. HA is a natural linear polysaccharide composed of alternating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine with β (1→4) interglycosidic linkage. Bioconjugation of the peptide drug to HA increases its half life in circulation contributing for a high efficacy. In this work, a novel bioconjugation protocol for formyl peptide receptor like 1 (FPRL1) peptide therapeutics was developed to make them suitable for *in vivo* applications.

Methods: Aminoethyl methacrylated HA (HA-AEMA) was synthesized in DMSO using tetra-n-butylammonium salt of HA (HA-TBA) and benzotriazol-1-yloxy-tris(dimethyl-amino)phosphonium hexafluorophosphate (BOP), and N,N-diisopropylethylamine (DIPEA) (Sigma-Aldrich, St. Louis, MO). Agonistic peptide for FPRL1 receptor (CWRYMVm) and HA-AEMA were dissolved in distilled water, respectively. 10-fold molar excess of TCEP to disulfide bond was dissolved in the peptide solution and incubated for 10 min. Then, the peptide solution was added to the HA-AEMA solution, mixed immediately, and incubated at 37℃ for 16 hours. The formation of HA-peptide conjugate was confirmed by ^{1}H-NMR and GPC. The stability of peptide in fetal bovine serum (FBS) was investigated before and after its conjugation to HA. Since the binding of agonistic peptide to FPRL1 receptor induces the elevation of phospho-extracellular signal-regulated kinase (pERK) and calcium ion levels [3], the biological activity of HA-peptide conjugates was assessed from the intracellular level of pERK and calcium ion in FPRL1 over-expressing RBL-2H3 cells. *In vivo* study was carried out in cecal ligation puncture mouse model of sepsis (n = 10).

Results: HA-AEMA was successfully synthesized and conjugated with CWRYMVm, one of agonistic peptide drugs for FPRL1 receptor, via Michael addition between methacryloyl group of AEMA and thiol group of cysteine. The formation of HA-peptide conjugate was confirmed by ^{1}H-NMR and GPC. HA-peptide conjugate could be prepared to have 5~23 peptide molecules per single HA chain by changing the initial feeding ratio of peptide to HA repeating unit.

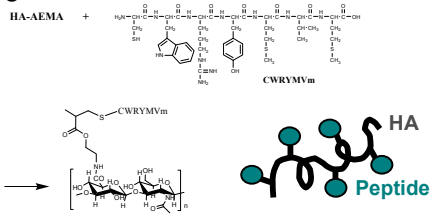

Figure 1. Schematic representation for the conjugation of HA-AEMA with the peptide (CWRYMVm) for FPRL1 receptor.

The conjugation of peptide to HA molecules resulted in significantly enhanced serum stability. The signal transduction activity of HA-peptide conjugate was confirmed by measuring the elevation level of pERK and calcium ion in FPRL1 over-expressing RBL-2H3 cells.

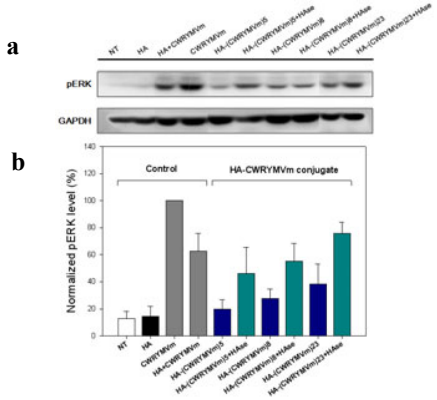

Figure 2. (a) Western blots for pERK levels in the FPRL1 over-expressing RBL-2H3 cells. (b) Densitometry of Western blot bands for the samples in (b). The results were presented as means ± S.E. of three independent experiments.

In vivo study of HA-peptide conjugate for FPRL1 receptor in murine model of sepsis showed the improved therapeutic effect and survival rate of mice.

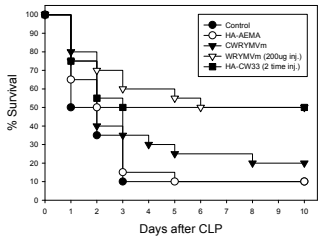

Figure 3. Survival after CLP in mice after treatment with HA-(CWRYMVm)33 for FPRL1.

Conclusions: A novel bioconjugation protocol of peptide drugs using HA-AEMA was successfully developed. The resulting HA-peptide conjugate showed an elongated half-life and a feasible biological activity. Further *in vivo* tests of HA-peptide conjugate for FPRL1 receptor will be carried out in murine model of inflammatory diseases such as sepsis, asthma and rheumatoid arthritis.

References:

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