Hyaluronic Acid Nanoparticles for Delivery of $\gamma$-Secretase inhibitor

Roun Heo, Jong-Sung Park, Soyoung Son, Jueun Jeon, Jae Hyung Park

Sungkyunkwan University, Suwon 440-746, Republic of Korea
virtuousie@naver.com

ABSTRACT SUMMARY
The hyaluronic acid nanoparticles (HA-NPs) bearing a $\gamma$-secretase inhibitor (DAPT) was prepared as potential therapeutics for treatment of rheumatoid arthritis. In this study, we demonstrated that DAPT-HA-NPs have the good targetability to inflamed joints, resulting in high therapeutic efficacy.

INTRODUCTION
Rheumatoid arthritis (RA) is a chronic inflammatory disease that is involved in hyperplasia of the synovial membrane and the destruction of cartilage and bone. The inflamed joints by RA are characterized by the infiltration of chemokines, pro-inflammatory cytokines, and growth factors. Also, they show an intensive angiogenesis and continuous trans-endothelial migration of leukocytes from the blood vessels into the synovial tissue.

$\gamma$-Secretase inhibitors which prevent Notch activation are emerging as potent therapeutics for diverse inflammatory diseases, such as ischemic stroke and rheumatoid arthritis. Nevertheless, their indiscriminate distribution in the body causes serious side effects after systemic administration, because Notch proteins are ubiquitous receptors that play an important role in cellular functions like differentiation, proliferation, and apoptosis. Therefore, targeted delivery of $\gamma$-secretase inhibitors to the inflamed joints of RA is required to minimize side effects.

Hyaluronic acid (HA), a naturally occurring polysaccharide abundant in the extracellular matrix of the body, has received increasing attention as an anticancer drug carrier since it is biocompatible and specifically binds to the CD44 receptor, which is over-expressed in cancer cells. Recently, it has been demonstrated that the CD44 receptor is also over-expressed in the synovial tissue of RA patients, and an anti-CD44 antibody has potential for the treatment of RA. Therefore, HA-NPs would be useful for targeted drug delivery into the inflamed joints of RA. In this study, we investigated DAPT-bearing HA-NPs (DNPs) as the potential therapeutics for treatment of RA.

EXPERIMENTAL METHODS
Amphiphilic HA conjugates, composed of 11 cholic acid (CA) moieties per 100 sugar residues of HA, were synthesized. Briefly, the carboxylic group of CA was modified to aminoethyl 5$\beta$-cholanoamide (EtCA) in the presence of ethylenediamine. Then, the hydrophobic EtCA was chemically conjugated to the backbone of HA in the presence of EDC and NHS.

DAPT was encapsulated in the hydrophobic core of HA-NPs using the dialysis method. In brief, HA-CA conjugates (20 mg) in 10 ml of distilled water were mixed with DAPT (2 mg) in 2 ml of dimethyl sulfoxide. The mixture was treated with a bath-type sonicator three times for 15 s each in ice bath. The mixture was dialyzed against an excess amount of distilled water for 1 day, filtered with a 0.8-$\mu$m syringe filter, and lyophilized.

To induce collagen-induced arthritis (CIA), DBA/1J mice were injected intradermally at the base of the tail with 200 $\mu$g of bovine type II collagen (CII) (2 mg/ml, Chondrex, Redmond, WA, USA) emulsified in 100 $\mu$l of Complete Freund’s Adjuvant (4 mg/ml, Chondrex). Mice were subjected to a second immunization 21 days later with CII emulsified in Incomplete Freund’s Adjuvant (Chondrex). After 42 days, Cy5.5-labeled DNPs were injected into the tail vein of wild-type (WT) or CIA mice in order to observe the biodistribution in vivo using the Optix MS3 system (ART Advanced Research Technologies Inc., Montreal, Canada).
For *in vivo* RA therapy and histological analysis, CIA mice were treated with PBS, DAPT (2 mg/kg), or DNPs (1 mg/kg DAPT) every 3 days starting on the day of the booster injection, intravenously. Mice were examined every other day for signs of joint inflammation. Paw scores were allocated as follows: 0 = normal, 1 = slight swelling and/or erythema, 2 = pronounced swelling, 3 = ankylosis. These paw scores were summed for each mouse, giving a maximum possible score of 16 per mouse.

For histological analysis, the dissected knee joints were fixed in 10 % (v/v) buffered formalin solution, decalcified for 8 h using decalcified solution-lite (Sigma-Aldrich Co.), embedded in paraffin, and sectioned. The paraffin tissue was sliced to 4-mm thickness, stained with H&E, and examined using a fluorescent microscope (BX51, Olympus, Optical Co. Ltd., Tokyo, Japan).

**RESULTS AND DISCUSSION**

The therapeutic effects of the DNPs on the development and pathogenesis of RA were investigated after their intravenous injection into the CIA mice.

![Fig. 1. In vivo biodistribution of DNPs in WT and CIA mice. (a) The hind leg fluorescence images of WT and CIA mice treated with DNPs. (b) Quantitative analysis of DNPs. Values are mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 versus WT.](image)

In this study, we have demonstrated that DNPs significantly accumulated at the inflamed joints of the CIA mice (Fig. 1). For evaluation of the therapeutic efficacy, the CIA mice were treated once every 3 days with vehicle, DAPT (2 mg/kg), or DNP (1 mg DAPT/kg). Fig. 2a shows images of the paws 42 days after immunization. Compared to the WT mice, vehicle- and DAPT-treated CIA mice showed significant paw swelling, erythema, and joint rigidity. Of the samples tested, DNP-treated mice exhibited the lowest morphological change.

**CONCLUSION**

HA-NPs were investigated as a potential carrier for γ-secretase inhibitor for the treatment of RA. The results suggested that DNPs had high targetability to the inflamed joint of CIA mice, resulting in high therapeutic efficacy with minimal production of pro-inflammatory cytokines and autoantibodies.

**REFERENCES**