Therapeutic Effect of Kartogenin-Loaded Nanosomes on Arthritic Chondrocytes

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INTRODUCTION: Osteoarthritis (OA) is a chronic degenerative joint disease that affects over 527 million individuals worldwide. It is characterized by progressive cartilage degradation and fibrosis of the joint tissue. As cartilage tissue is avascular and relies on diffusion within the synovial fluid space to receive metabolic support, it has a limited capacity to heal itself through cellular repair mechanisms. Current OA therapies include NSAIDs, corticosteroids, and hyaluronic acid injections, but these only provide symptomatic relief rather than targeting the underlying pathology of OA. Kartogenin (KGN) is a small hydrophobic molecule that acts through the CBFβ–RUNX1 pathway. It has been shown to promote chondrogenic gene expression (COL2A1, ACAN) and suppress catabolic enzymes (MMP13, ADAMTS5). However, KGN faces three major limitations: poor solubility, lack of tissue specificity, and risk of dose-dependent toxicity. We hypothesized that encapsulating KGN into lipid nanosomes would overcome these limitations by enhancing solubility, improving uptake in inflamed chondrocytes, and reducing the required therapeutic dose.

METHODS*: Culture of Primary Chondrocytes:* Articular cartilage was harvested from the femorotibial joints of 3 month old porcine. Cartilage slices were digested with Pronase followed by Collagenase under gentle agitation. Isolated chondrocytes were filtered, washed, and cultured in DMEM supplemented with 10% fetal bovine serum (FBS), streptomycin, penicillin G, L-glutamine, and L-ascorbic acid. Cells were incubated at 37°C and used at passage 3 for all experiments. *Establishment of In Vitro OA Model:* To model an OA catabolic phenotype, chondrocytes were seeded at 2.5×10^5 cells/well in 6-well plates and treated with 10 ng/mL of TNF-α for 24 hours. *Preparation of KGN-Nanosomes:* Lipids consisting of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, DSPE-PEG, and DSPE-PEG-maleimide were combined in a glass tube and dissolved in chloroform. The solvent was evaporated under a stream of air for 45 minutes to form a thin lipid film. It was vacuum dried for 15 minutes to remove residual solvent. The dried lipid film was rehydrated with PBS containing 500 μM Kartogenin. The suspension was then extruded through 200 nm polycarbonate membranes to obtain uniform nanosomes. Unencapsulated KGN was removed by size-exclusion chromatography. Dynamic light scattering confirmed a mean diameter of 178.7 nm and a polydispersity index of 0.023. Encapsulation efficiency was around 10%. The final external concentration was 40 nM/mL after 1:1000 dilution for cell treatment. *Experimental Design:* Chondrocytes were divided into five groups (n = 3 wells per group): (a) untreated control, (b) TNF-α only, (c) TNF-α + free KGN (1 μM), (d) TNF-α + free KGN (100 μM), and (e) TNF-α + KGN-loaded nanosomes (40 nM external concentration). Treatments were administered at the same time as TNF-α stimulation, and the cells were incubated at 37°C for 24 hours. *Gene Expression Analysis (RT-qPCR):* Total RNA was extracted using TRIzol reagent and quantified with a NanoDrop spectrophotometer. cDNA synthesis was performed. Quantitative PCR was conducted and targeted anabolic genes (COL2A1, ACAN) and the catabolic marker (MMP13). GAPDH was used as the internal reference housekeeping gene. Relative gene expression was calculated. *Statistical Analysis:* Data is expressed as mean ± standard deviation (SD). Statistical comparisons were made using one-way ANOVA. A p-value < 0.05 was considered statistically significant.

RESULTS: *Establishment of an In Vitro OA Model:* Upon exposure to TNF-α (10 ng/mL) for 24 hours, the chondrocytes transitioned towards an increased inflammatory and catabolic state. RT-qPCR analysis revealed a statistically significant upregulation of MMP13 of a 100-fold increase compared to untreated controls. Additionally, expression of the key anabolic markers COL2A1 and ACAN was significantly downregulated (Fig. X). These results confirm that TNF-α treatment reliably induces OA-like conditions in vitro. *Effect of KGN on Healthy Chondrocytes:* To assess potential off-target effects, KGN was applied to unstimulated, healthy chondrocytes. RT-qPCR demonstrated that COL2A1 and ACAN levels remained unchanged compared to control, and MMP13 expression did not increase (Fig. 1). This suggests that KGN does not disrupt baseline anabolic or catabolic signaling in non-inflamed cartilage cells. The absence of a catabolic response by KGN supports its safety profile for targeted therapeutic use. *KGN-Nanosome Characterization:* Dynamic light scattering measurements confirmed the successful formulation of KGN-loaded lipid nanosomes with a mean particle diameter of 178.7 nm and a polydispersity index (PDI) of 0.023 (Fig. 2). The particle size distribution fell within the optimal range for endocytosis-mediated uptake and reduced the likelihood of rapid clearance or macrophage recognition of the nanosome particles. After dilution to a 1:1000 ratio, the final external treatment concentration of the KGN-nanosomes was only 40 nM/mL, enabling delivery of a high local dose intracellularly while minimizing systemic exposure. *Impact of Free KGN and KGN-Loaded Nanosomes on Inflamed Chondrocytes:* Treatment of TNF-α–stimulated chondrocytes with free KGN at 1 μM produced negligible changes in gene expression. Increasing the dosage to 100 μM restored cartilage anabolism, as evidenced by a significant increase in COL2A1 expression, and suppressed MMP13 (Fig. 3). In comparison, KGN-loaded nanosomes administered at only 40 nM/mL achieved markedly similar outcomes of the 100 μM treatment group. RT-qPCR demonstrated significant upregulation of COL2A1 and significant suppression of MMP13. These results indicate that nanosome-mediated delivery not only enhances the bioactivity of KGN but also enables potent therapeutic effects at a concentration of 2,500 times less of the free KGN dose.

DISCUSSION: This study shows that delivering Kartogenin through lipid nanosomes improves its effectiveness in an in vitro model of osteoarthritis. TNF-α treatment reliably induced an OA-like phenotype in chondrocytes: high MMP13 expression and reduced COL2A1 and ACAN. Free KGN at high concentrations partially improved this imbalance, but only at 100 μM. In contrast, KGN-loaded nanosomes achieved similar anabolic and anti-catabolic effects at 2,500 times less concentration of just 40 nM/mL. Encapsulating KGN in nanosomes overcame key limitations to its use: poor solubility, lack of tissue specificity, and the risk of dosage-dependent systemic toxicity. The nanosomes allowed KGN to be delivered directly into inflamed chondrocytes at a high local drug concentration while keeping overall systemic exposure low, which reduces the risk of toxicity. Despite the nanomolar external dose, we saw a clear increase in COL2A1 and strong suppression of MMP13. The consistent size (~179 nm) and low polydispersity of our nanosomes indicate they are well-suited for cellular uptake and could be retained within the joint if tested in vivo. Future studies will need to evaluate their performance in murine models of OA and assess long-term effects, but these results provide a strong foundation for further development.

SIGNIFICANCE: KGN-loaded nanosomes represent a promising approach for OA treatment by delivering a potent regenerative molecule directly to inflamed cartilage cells. Our results achieved robust therapeutic effects at low doses. This strategy may pave the way for developing clinically viable disease-modifying osteoarthritis drugs that can halt or reverse disease progression.

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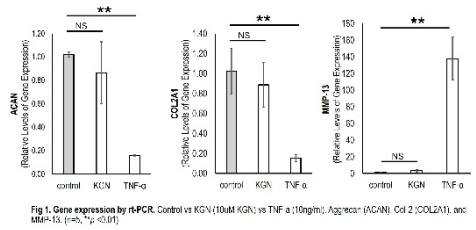

Fig 1. Gene expression by rt-PCR. Control vs KGN (10uM KGN) vs TNF-α (10ng/ml). Aggrecan (ACAN), Col 2 (COL2A1), and MMP-13. (n=5, **p <0.01)

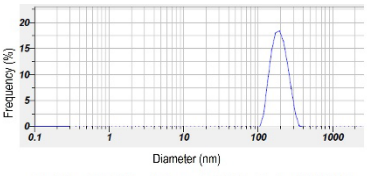

Fig 2. Size distribution by Dynamic Light Scattering (DLS). KGN encapsulated nanosome's diameter is 178.7nm and a polydispersity index (PDI) is 0.023

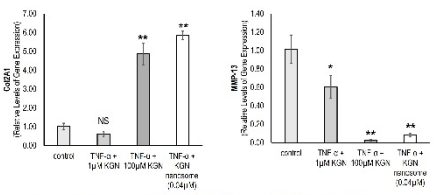

Fig 3. Gene expression by rt-PCR. Control vs 1uM KGN vs 100uM KGN vs 0.04uM KGN-Nanosomes. All groups were treated with TNF-α (10ng/ml) except normal control group. Gene expression of the Col 2 (COL2A1), and MMP-13. all groups are comparison to control group (n=5. N.S: no statistically difference. *p <0.05, **p <0.01)