sPLA$_2$ Inhibitor-Loaded Nanoparticles for The Treatment of Spontaneous Osteoarthritis in Guinea Pigs

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INTRODUCTION: Osteoarthritis (OA) is a degenerative joint disease characterized by chronic inflammation, cartilage degradation, and joint pain. Despite its high prevalence and substantial societal burden, there are currently no approved disease-modifying therapies. Secretory phospholipase A$_2$ (sPLA$_2$) recently emerged as a promising therapeutic target for OA. Thioetheramide-PC (Thio-PC), a sPLA$_2$ inhibitor (sPLA$_2$i), was recently shown to have potent therapeutic efficiency in murine OA models (1). However, these findings are limited to mouse studies, which differ significantly from human clinical conditions. The Dunkin Hartley guinea pig naturally develops OA as early as three months of age and represents a promising but underutilized spontaneous OA model for evaluating therapeutic interventions. Herein, we first used Thio-PC to synthesize sPLA$_2$i-loaded nanoparticles (sPLA$_2$i-NPs) and then evaluated their therapeutic efficacy in the spontaneous OA of guinea pigs.

METHODS: *sPLA$_2$i-NP generation*– sPLA$_2$i-NPs were formulated using the thin-film hydration method by combining Thio-PC (25 mol%), DSPE-PEG2K (65 mol%), and DOTAP (10 mol%). The total amount of Thio-PC was 1 mg in each preparation. *Explant study*- Cartilage explants (diameter: 2 mm; thickness: 2 mm) were biopsied from the joints of 3-month-old guinea pigs and cultured in chemically defined medium [DMEM, 1% insulin-transferrinselenous acid (ITS) + premix, l-proline (50 µg/ml), 0.1 µM dexamethasone, 0.9 mM sodium pyruvate, and ascorbate 2-phosphate (50 µg/ml)] with or without IL-1β (10 ng/ml) and sPLA$_2$i-NPs (0.1 mg/ml) for 7 days. *Animals*- All animal work was approved by the Institutional Animal Care and Use Committee at the University of Pennsylvania. Female Dunkin Hartley guinea pigs (Charles River) at 3 months of age received intra-articular (IA) injections of 100 µl sPLA$_2$i-NPs (0.25 mg/ml) at the right knee and Ctrl-NPs (i.e., no sPLA$_2$i) at the left knee. Injections are performed once every 2-3 weeks until the animals reach 7 months of age. *Gait analysis*- Guinea pigs were walked down a custom-made plexiglass walkway with integrated force plates, mirrors positioned at 45° along the tunnel and a video camera directly underneath (2). Kinematic and kinetic gait parameters were quantified. *Histology*- Knee joints and cartilage explants were processed for paraffin sections, followed by H&E, Safarin O/fast green, and immunohistochemical (IHC) staining. *Statistics*- Data are expressed as means±SD and analyzed by one-way ANOVA or paired, two-tailed Student's t-test.

RESULTS: The knee joints of female Dunkin Hartley guinea pigs exhibited healthy articular cartilage at 2.5 months of age, developed cartilage degeneration at 6 months of age, and displayed severe cartilage surface erosion at 12 months of age (Figure 1, top). H&E staining revealed that synovitis is not evident until 12 months of age (Figure 1, middle). IHC staining for 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of reactive oxygen species (ROS), showed progressive elevation during aging (Figure 1, bottom, n=1/group). sPLA$_2$i-NPs were synthesized using a micelle formulation composed of DSPE-PEG2K and DOTAP, a cationic lipid that enhances electrostatic interactions with the negatively charged cartilage matrix and promotes retention within the inflamed joint (Figure 2). Cartilage explants were harvested from knees of 3-month-old guinea pigs and cultured with IL-1β for 7 days to induce OA-like degeneration. Addition of sPLA$_2$i-NPs, but not Ctrl-NPs or free sPLA$_2$i, remarkably restored extracellular matrix integrity (Figure 3, n=3/group). To examine the in vivo efficacy of sPLA$_2$i-NPs, 3-month-old guinea pigs received IA injections of Ctrl-NPs in their left knees and sPLA$_2$i-NPs in their right knees once every 2-3 weeks (Figure 4A). Gait analysis was conducted before the treatment (3M), 2 months (5M) and 4 months (7M) after the first injection (Figure 4B, n=8-10/time point). By 7 months of age, sPLA$_2$i-NP-treated legs exhibited significantly enhanced stride length, stride speed, and peak normal force compared to Ctrl-NP-treated legs, suggesting that sPLA$_2$i-NP treatment mitigates OA-induced gait abnormalities (Figure 4C). Bilateral knees were harvested at 7 months of age. Histological analyses demonstrated that sPLA$_2$i-NPs significantly inhibit cartilage degeneration and synovitis progression (Figure 5, n=3/group). Notably, the cartilage integrity in sPLA$_2$i-NP-treated joints was comparable to that of healthy joints from 3-month-old animals.

DISCUSSION: Our data confirm guinea pigs as a promising spontaneous OA model and demonstrate that they are suitable for gait analysis and histopathological evaluation. Our findings highlight the therapeutic potential of targeting sPLA$_2$ for OA treatment. Further experiments should be conducted with an increased sample size and quantitative analyses of OA-related markers, such as matrix metalloproteinases, and inflammatory markers, such as p-p65 and p100, for mechanistic insight. In the future, large animal studies will be performed to translate this novel nanomedicine into the clinic for OA treatment.

SIGNIFICANCE: This study demonstrates nanoparticles carrying sPLA$_2$ inhibitor as an effective treatment for spontaneous OA in guinea pigs.

REFERENCES: (1) Wei, Y., et al., *Sci Adv*, 2021. 7(15). (2) Sarver, J., et al., *J Biomech*, 2010. 43(4).

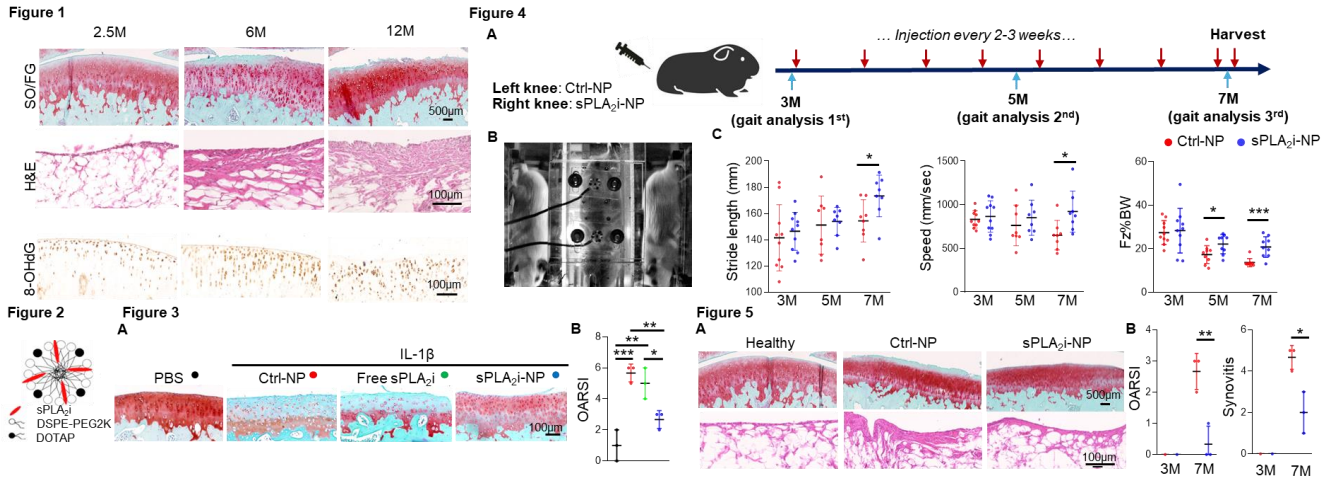

Figure 1. Dunkin Hartley guinea pigs develop spontaneous OA. SO/FG staining of knee joints, H&E staining of the synovium, and IHC staining of 8-OHdG in the articular cartilage from guinea pigs at 3, 6 and 12 months of age (n=1/group). **Figure 2. Schematic diagram of sPLA$_2$i-NPs.** Thin-film hydration was applied to fabricate sPLA$_2$i-NP using 25 mol% Thio-PC, 65 mol% DSPE-PEG2K, and 10 mol% DOTAP. **Figure 3. Chondroprotective effects of sPLA2i-NPs on cartilage explants from 3-month-old guinea pigs.** Left: SO/FG staining cartilage explants. Right: quantification of OARSI score (n=3/group). **Figure 4. sPLA$_2$i-NPs ameliorate OA-induced gait abnormalities.** (A) Experimental design of in vivo treatment. (B) The customized system used for gait analysis. (C) Quantification of gait parameters (n=8-10/group). **Figure 5. sPLA$_2$i-NPs attenuate OA progression.** Left: SO/FG and H&E staining of healthy joints from three-month-old animals, and the left (Ctrl-NP) and right (sPLA$_2$i-NP) knees of seven-month-old animals . Right: OARSI scores and synovitis scores for left (Ctrl-NP) and right (sPLA$_2$i-NP) knees (n=3/group). *P < 0.05, **P < 0.01, ***P < 0.001.