**Inhibitory effects of ginger-derived extracellular vesicles on the heterotopic ossification of Achilles tendon**

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INTRODUCTION: Achilles tendon ossification is characterized by ectopic bone formation within the tendon, leading to pain and tendon rupture. Currently, few effective pharmacological treatments exist, and new therapeutic strategies are needed. Plant-derived extracellular vesicles (PDEVs) contain nucleic acids such as microRNAs (miRNAs) and growth factors, and regulate gene expression in animals [1]. Ginger has been reported to exert anti-inflammatory effects [2], and ginger-derived extracellular vesicles (GDEVs) are likewise suggested to possess anti-inflammatory properties, with the potential to attenuate inflammation-induced heterotopic ossification. The objective of the present study is to elucidate the inhibitory effects of GDEVs on Achilles tendon heterotopic ossification

METHODS: In vitro, GDEVs were added to tendon fibroblasts derived from injured mouse Achilles tendons to evaluate cell proliferation, migration, and anti-inflammatory effects. In vivo, Achilles tendon transection was performed in C57BL/6 mice to induce ossification, and GDEVs (EV group: n=14) or PBS (control group: n=14) were injected into the transected site once a week. Mice were sacrificed at 4 and 8 weeks for micro-CT and histological evaluation. Expression analysis of GDEV contents was also performed.

RESULTS: In vitro, GDEVs significantly suppressed proliferation and migration of tendon fibroblasts, and reduced IL-6 expression in IL-1β–stimulated tendon fibroblasts. In vivo, immunohistochemistry at 4 weeks showed that the EV group had a significantly smaller type II collagen–positive area compared with controls ($p < 0.05$). Safranin-O staining revealed significantly lower chondrification scores in the EV group at both 4 and 8 weeks ($p < 0.05$) (Fig 1). At 8 weeks, micro-CT demonstrated a significantly smaller ossified volume in the EV group compared with controls ($p < 0.05$) (Fig 2). Molecular analysis showed that GDEVs contained high levels of the anti-inflammatory compound 6-gingerol and several miRNAs which target cartilage specific genes including SOX-9.

DISCUSSION: GDEVs suppressed ossification of the Achilles tendon by inhibiting scar formation through anti-inflammatory effects and suppression of cell proliferation and migration. Notably, several miRNAs targets cartilage specific genes, suggesting that inhibition of them by GDEV may have contributed to the suppression of endochondral ossification within the tendon.

SIGNIFICANCE/CLINICAL RELEVANCE: Natural product–derived GDEVs may represent a promising novel therapeutic option for Achilles tendon ossification.

REFERENCES:

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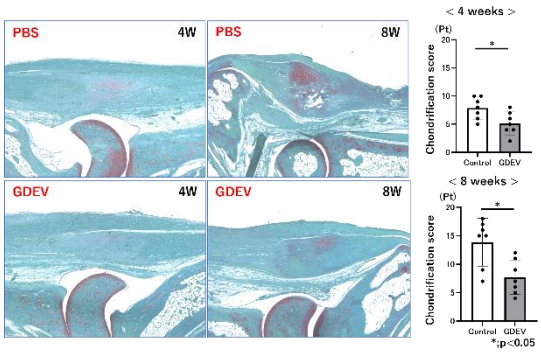


Fig 1. Histological findings of Achilles tendon after infection of GDEV or PBS. Local injection of GDEV could inhibit ossification of Achilles tendon.

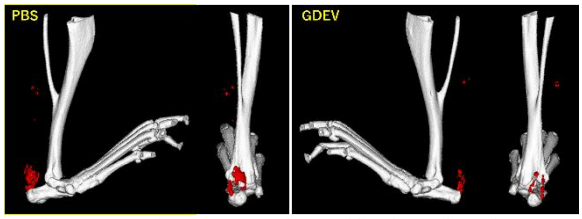


Fig. 2. 3D-CT image at 8 weeks. Ossification volume in GDEV group was significantly smaller than that in control.