Piperlongumine Attenuates Osteoarthritis Progression By Clearing Senescent Chondrocytes And Meniscus Cells

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INTRODUCTION: Knee osteoarthritis (OA) is a prevalent joint disease characterized by progressive degeneration of cartilage and meniscus. One emerging factor contributing to OA pathogenesis is the accumulation of senescent cells in cartilage. Senescent cells not only lose their optimal function but also release pro-inflammatory factors known as senescence-associated secretory phenotype (SASP) to exacerbate the degenerative process. Recent studies have revealed that selective elimination of senescent chondrocytes by senolytic drugs attenuated OA progression. However, the relationship between meniscus cell senescence and meniscus degeneration remains unclear. This study investigated whether the senescent cells accumulate in the degenerated meniscus and explored the drug that can eliminate both senescent chondrocytes and meniscus cells.

METHODS: This study was approved by the Animal Committee and Medical Research Ethics Committee of Tokyo Medical and Dental University. Lewis rats received anterior cruciate ligament transection (ACLT) or sham surgery. After 6 weeks, the meniscus was collected to evaluate the expression of senescence markers (p16, SASPs, and senescence-associated β-galactosidase [SA-β-gal] activity). Subsequently, we assessed the effect of six known senolytic agents on both rat normal and H_2O_2-induced senescent chondrocytes and meniscus cells. The effect on human OA chondrocytes and meniscus cells was also investigated. The drug that showed efficacy in removing both senescent chondrocytes and meniscus cells of rat and human was injected into the knee of ACLT rats to evaluate inhibitory effect on OA progression.

RESULTS: Significant upregulation of the expression of p16, SASP, and SA-β-gal was observed in meniscus cells of ACLT rats compared with those of sham rats (Fig. 1A-D). H_2O_2 treatment increased cell size, SA-β-gal activity, DNA damage, and SASPs, and decreased glycosaminoglycan (GAG)-producing ability in both rat chondrocytes and meniscus cells. Of 6 senolytic drugs, piperlongumine (PL) was able to selectively kill both rat senescent chondrocytes and meniscus cells (Fig. 2A). PL showed similar effects on human chondrocytes and meniscus cells from OA patients (Fig. 2B). PL administration to the rat ACLT model significantly improved the histological cartilage and meniscus degeneration scores (Fig. 3A and B).

DISCUSSION: The expression of senescence markers was higher in the meniscus of ACLT rats. This provides the evidence that senescent meniscus cells accumulate in degenerated meniscus. PL is a natural alkaloid from long peppers which has been reported to target oxidation resistance 1 (OXR1) in senescent human WI-38 cells. Our results demonstrated that PL had senolytic effects on both chondrocytes and meniscus cells and could inhibit the progression of OA in rats. Although further studies using larger animal models are necessary, PL will be a promising drug for OA.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides a novel strategy for OA drug discovery that targets both senescent chondrocytes and meniscus cells.

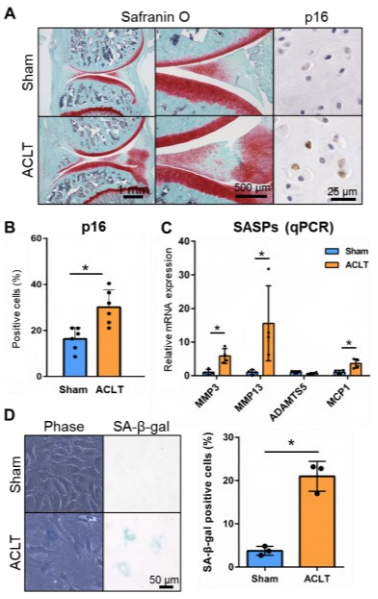

Fig. 1 (A) Safranin O staining and p16 immunostaining of the meniscus of sham and ACLT rats. (B) Percentage of p16-positive cells in the meniscus. (C) Relative gene expression of SASPs in the meniscus. (D) SA-β-gal staining of primary meniscus cells derived from sham and ACLT rats.

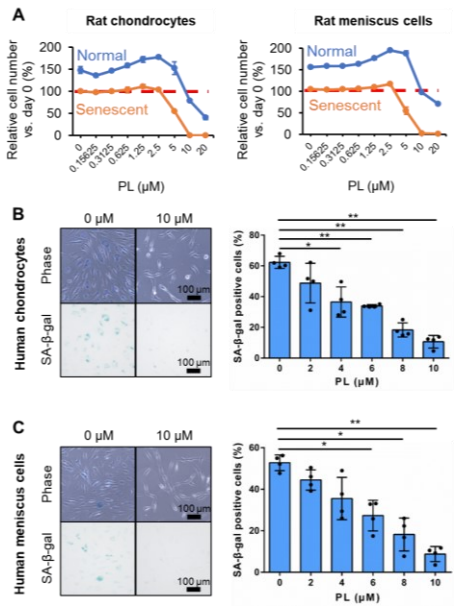

Fig. 2 (A) Relative viable cell number of rat normal/senescent chondrocytes and meniscus cells after treatment with 0-20 μM PL. The red dotted line represents the cell number before the treatment (set as 100%). (B) SA-β-gal staining of primary human OA chondrocytes after treatment with 0-10 μM PL. (C) SA-β-gal staining of primary human OA meniscus cells after treatment with 0-10 μM PL.

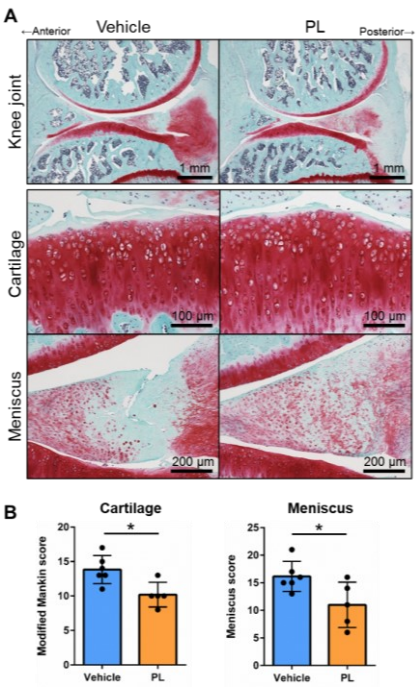

Fig. 3 (A) Safranin O staining of the knee joints (B) Histological scores for cartilage and meniscus.