

Senotherapeutic effect of fisetin, resveratrol and tocotrienol on senescent chondrogenic progenitors of knee osteoarthritis patients

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INTRODUCTION: The development of late-stage osteoarthritis is a complex, multifactorial process that involves irreversible damage to the whole knee joint. The central focus of osteoarthritis research is the articular cartilage that is susceptible to damage and is irreparable when full thickness lesions reach subchondral bone. Moreover, chondrocytes are terminally differentiated cells and rarely proliferate and thus lack regenerative potential due to scarcity of stem cells. Previous research has identified the presence of chondrogenic progenitor cells in the adult articular cartilage of knee OA patients. These CPCs have a potential for chondrogenesis. The deterioration of the cartilage has been linked to the cellular senescence resulting from the chronic stressed microenvironment. However, in this constant oxidative micro-environment of aging knee, CPCs are susceptible to cellular senescence impeding their regenerative function. Additionally, the senescent cells accumulate and propagate the cellular senescence in the form of secretory phenotype or SASP. Therefore, it is necessary to identify and combat the cellular senescence of CPCs in aging knee of OA patients. The use and mechanism of natural compounds such as fisetin, resveratrol and tocotrienol as senotherapeutics in the CPCs of articular cartilage has not been explored so far and thus presents an emerging area for the development of new therapeutics.

METHODS: In the present study, 12 patients clinically diagnosed with knee osteoarthritis were recruited and the knee articular was obtained from the discarded tibial plateaus of the patients after total knee replacement surgery with written informed consent and approval from the Institutional Ethics Committee. The primary culture of CPCs was established and evaluated for cellular senescence as described previously [1]. Cell viability of CPCs after treatment with increasing doses of fisetin, resveratrol and tocotrienol (5µM-100µM) for 24 hours was performed to assess their toxicity followed by apoptotic activity evaluation by PI-Annexin Assay. Effect of these drugs on senescence of CPCs was evaluated by SA-β Gal assay, gene and expression of cellular senescence markers and SASP related matrix degradation markers using real time PCR and western Blotting. The data was statistically analyzed using Graph pad prism.

RESULTS SECTION: Cell viability was not affected at all doses of both fisetin (86.03%) and resveratrol (83.11%) upto 100 µM without inducing apoptosis, but tocotrienol significantly decreased the cells viability to 62.42% at the highest concentration. Further it was revealed by apoptosis assay that both fisetin and resveratrol did not induce the apoptosis while tocotrienol at the highest concentration induced early apoptosis in CPCs thus suggesting the senolytic effect of tocotrienol while a senomorphic effect of both fisetin and resveratrol. Fisetin significantly decreased senescence index by 42.93% at 100µM (p=0.01) while resveratrol decreased it by 30.21% at 50µM (p<0.05). Tocotrienol exerted its senotherapeutic activity at the highest dose of 100 µM by reducing the senescence index to 19.3% (p=0.002). This was further supported by significant downregulation of senescence effector genes p53 and p38MAPK of all three drugs. In addition, tocotrienol suppressed the expression of p21 gene and protein at 50 µM concentration. Additionally, secretion of SASP comprising of pro-inflammatory cytokine IL-1β and matrix-degrading enzymes MMP-9 and MMP13 from the senescent CPC were also significantly downregulated. Overall, these potential senotherapeutic drugs ameliorate the senescence by downregulating the p53 senescence effector protein. This indicates the action of these potential senotherapeutics converged at the p53 pathway. Furthermore, these potential senotherapeutics can also target SASP (p38, MMP-9 and MMP-13) and can be utilized as potent senescence modulator.

DISCUSSION: This study demonstrates that native chondrogenic progenitors exist in the late-stage osteoarthritic cartilage have potential for chondrogenesis. However, it was observed that these cells are undergoing cellular senescence that might limit their use for future therapies. Nonetheless, treatment of these cells with senotherapeutics (fisetin, resveratrol, and tocotrienol) aid in overcoming cellular senescence and might lead to restoration of the functional capacity of the cartilage. Our results indicate that all three drugs i.e., fisetin, resveratrol and tocotrienol target the transient state of senescence and leads to downregulation of p53 related senescence pathway. These results suggest the promising role of senotherapeutics in targeting the senescence associated secretory phenotype for future treatment of knee osteoarthritis. Senotherapeutic-pretreated chondrogenic progenitor cells therefore can either be differentiated into mature cartilage or be directly seeded onto scaffold for future transplants. This will require robust in vitro and in vivo studies that might be used in conjunction with novel drugs or independently to reduce the onset of knee osteoarthritis.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings can be well extrapolated to in vivo models and future clinical trials. Though these chondrogenic progenitor cells and senotherapeutics hold a future for novel treatment of osteoarthritis, a deep insight into their action on these cells is required to realize their use from “bench to bedside”.

RefErence

1. Jacob, J., Aggarwal, A., Aggarwal, A., Bhattacharyya, S., Kumar, V., Sharma, V. & Sahni, D. (2022) Senescent chondrogenic progenitor cells derived from articular cartilage of knee osteoarthritis patients contributes to senescence-associated secretory phenotype via release of IL-6 and IL-8, *Acta Histochem.* **124**, 151867.