6-shogaol treatment improves experimental knee OA exerting a pleiotropic effect over immune innate signaling response in chondrocytes

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Abstract

Background:The pathogenesis of osteoarthritis (OA) implicates a low-grade inflammation associated to the activation of the innate immune system. Toll like receptor (TLR) stimulation triggers the release of inflammatory mediators, which aggravate OA severity. The aim was to study the preventive effect of 6-shogaol (6S), a potential TLR4 inhibitor, on the treatment of experimental knee OA. **Experimental approach:**OA was induced in C57BL6 mice by surgical section of the medial meniscotibial ligament, which received 6S for eight weeks. Cartilage damage, inflammatory mediator presence, and disease markers were assessed in the joint tissues by immunohistochemistry. Computational modeling was used to predict binding modes of 6S into the TLR4/MD2 receptor and its permeability across cellular membranes. Employing LPS-stimulated chondrocytes and MAPK assay we clarified 6S action mechanisms. **Results:**6S treatment was able to prevent articular cartilage lesions, synovitis, and the presence of pro-inflammatory mediators and disease markers in OA animals. Molecular modeling studies predicted 6S interaction with the TLR4/MD-2 heterodimer in an antagonist conformation through its binding into the MD-2 pocket. In cell culture, we confirmed that 6S reduced LPS-induced TLR4 inflammatory signaling pathways. Besides, MAPK assay demonstrated that 6S directly inhibits the ERK1/2 phosphorylation activity. **Conclusion:**6S evoked a preventive action on cartilage and synovial inflammation in OA mice. 6S effect may take place not only by hindering the interaction between TLR4 ligands and the TLR4/MD-2 complex in chondrocytes, but also through inhibition of ERK phosphorylation, implying a pleiotropic effect on different mediators activated during OA, which proposes it as an attractive drug for OA treatment.

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A

B

C

A

B

A

SUPPLEMENTARY FIGURE 1

SUPPLEMENTARY FIGURE 2

SUPPLEMENTARY FIGURE 3

6S extended mode

6S lipid IVa-like mode

One docked molecule

Two docked molecules

Three docked molecules

SUPPLEMENTARY FIGURE 4

A

B

C

SUPPLEMENTARY FIGURE 5

A

B

C

SUPPLEMENTARY FIGURE 6

A

B

SUPPLEMENTARY FIGURE 7

SUPPLEMENTARY FIGURE 8

A

B

	Tak-242	6-shogaol	SSi6
Log P	3.082	3.835	3.856
Caco-2 permeability (nm s⁻¹)	2256	1181	54
MDCK permeability (nm s⁻¹)	4457	592	21