Desmethylanhydroicaritin Exerts Anti-inflammatory Effects in LPS-stimulated RAW264.7 macrophages

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Desmethylanhydroicaritin (DMAI), a flavonoid extracted from Sophorae Radix, has been known as a potent in vitro free radical and peroxynitrite scavenger. A multitude of flavonoids have been known to inhibit various inflammatory diseases. In the present study, we observed that DMAI reduced the release of nitrogen monoxide (NO), TNF-α, IL-1β, and prostaglandin E2 (PGE2), and also the expression of iNOS and COX-2 in LPS-stimulated RAW264.7 cells. Moreover, DMAI decreased iNOS and NF-κB promoter genes activities, and these data were supplemented by the inhibitions of IKK activity, IκB-α phosphorylation, NF-κB translocation to the nucleus, and NF-κB B-DNA binding activity. These results suggest that DMAI lowered the production of the inflammatory mediators by inhibiting upstream activators of the IKK pathway and subsequent NF-κB transactivation.

Discovery of a new small molecule targeting thioredoxin redox system and its anti-angiogenic activity

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Antioxidant defense systems protect cells or organisms from environmental oxidative stresses. One of major cellular antioxidant defense systems in cells is thioredoxin (Trx) redox system containing thioredoxin reductase (TR)/thioredoxin (Trx)/peroxiredoxin (Prx), which mediates the transfer of electrons from NADPH to hydrogen peroxide leading to reduction of cellular oxidative stress. To discover new small molecules targeting Trx redox system, chemical genomics-based screening of small molecules was conducted. As a result, CGC83 was identified as an active compound from our in-lab small molecule library. CGC83 accelerates NADPH oxidation and reduces H₂O₂ efficiently. CGC83 reduces H₂O₂ to H₂O by replacing the function of peroxiredoxin. In HeLa cells, intracellular level of H₂O₂ was decreased after treatment of CGC83. The anti-oxidant activity of CGC83 was further validated in cellular phenotype including its effect on angiogenesis. The proliferation of endothelial cells were inhibited by the compound at nanomolar range. In addition, H₂O₂-induced tube formation and invasion of the cells were blocked by CGC83. Overall, these results demonstrate that a new antioxidant small molecule, CGC83, could be a molecular probe targeting Trx redox system.

Ectopic expression of COX-2 induces dedifferentiation in rabbit articular chondrocytes

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Arthritic joints produce large amounts of prostaglandins (PGs) that are involved in cartilage inflammation. The rate-limiting step of PG production is the initial conversion of arachidonic acid to PGH2 by cyclooxygenases(COX). Therefore, we have investigated the role of COX-2 protein on differentiation in rabbit articular chondrocyte. A previous study from our lab identified that IL-1β increased expression of COX-2 and induced dedifferentiation. Ectopic expression of COX-2 was sufficient to causes dedifferentiation in articular chondrocytes as determined by the expression of type II collagen via Alcian Blue staining and Western blot. Also, COX-2 overexpression caused suppression of SOX-9 expression, a major transcription factor that regulates type II collagen expression, as identified by the Western blot and RT-PCR. And then, we were identified that COX-2 overexpression inhibited type II collagen expression in articular chondrocytes tissues by immunocytochemistry. However, Pyrrolidine dithiocarbamate(PDTC), NF-κB inhibitor, inhibited COX-2 causes dedifferentiation as demonstrated by the enhancement type II collagen and SOX-9 expression. Our results collectively suggest that COX-2 overexpression causes dedifferentiation in articular chondrocytes through NF-κB pathway.

ERK and Akt prevent osteoclasts apoptosis through blockage caspase-9 and -3

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Osteoclasts are multinucleated cells with the unique ability to resorb bone. The regulation of osteoclast apoptosis is important in bone homeostasis. The activated caspase-9 catalyzes cleavage of procaspase-3, which is inhibited by a caspase-9 inhibitor such as ILP-2 and XIAP. Here we show that the JAK2 inhibitor, AG490, inhibits osteoclasts apoptosis through blockage of caspase-9 and -3 activation. Also, AG490 stimulated the phosphorylation of ERK and Akt. Osteoclast survival by AG490 was dependent on ERK and Akt activation, which inhibit caspase-9 activities. AG490-induced caspase-9 inhibition and survival were blocked in DN-RAS or DN-Akt infected osteoclasts. Moreover, overexpression of CA-MEK or CA-Akt inhibited etoposide-induced caspase-3 activation and apoptosis in osteoclasts and RAW 264.7 cells. These results suggest that ERK and Akt can inhibit apoptosis of osteoclasts, which was associated to caspase-9 and -3 inhibition.