osteogenic period. Cellular (synthesized) ALP activity was increased during 15 days of culture, and was confirmed by the staining of ALP activity on cell matrix layers for matrix calcification. SD stimulatory effect for cell mineralization we observed in 14 and 21 days. Elevated mRNA or protein levels of bone morphogenetic protein-2(BMP 2), the differentiation marker osteocalcin, osteopontin, collgen I, and a master osteogenic transcription factor, Runx2, were observed in SD-treated cells.
Conclusions: These results suggest that SD may increase osteogenic effect by stimulating cell ALP activity and affect the BMP signaling pathway cascades in osteoblastic cells, then promotes osteoblast differentiation, mineralization, and bone formation.

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THE EFFECT OF PALMITATE ON HUMAN ARTICULAR CHONDROCYTES IS PARTIALLY REVERSED BY OLEIC ACID

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Purpose: Osteoarthritis is a heterogeneous disease and metabolic factors contribute substantially to its pathogenesis. Conditions such as obesity and metabolic syndrome have been directly related to osteoarthritis regardless of the mechanical damage. In this regard, saturated fatty acids such as palmitate are particularly interesting, since their circulating levels are elevated in these conditions. Palmitate causes apoptosis, inflammation and mitochondrial damage in peripheral tissues. Thus, the aim of this work is to analyze the effects of palmitate and oleate (monoinsaturated fatty acid) on human chondrocytes
Methods: The TC28a2 chondrocyte cell line was used to analyze the effect of 0.4mM and 0.7mM palmitate (PA) and palmitate/oleate (PA/O) 1:2 for 12 hours. Isolated RNA was retrotranscribed to cDNA to analyze the gene expression of metalloproteinases (MMP-1, MMP-3, MMP-13), Interleukin 6 (IL6) and Cyclooxygenase 2 (COX2). We also analyzed reactive oxygen species (ROS) production by flow cytometry using MitosoxTM. Total and mitochondrial ATP were measured using ATP Bioluminescence Assay Kit CLS II. Apoptosis was analyzed using the Annexin V FITC/IP Apoptosis detection kit and Caspase 3,7 and 9 activity by means of a luminescense assay (Promega). Data analysis was performed with SPSS software(v19) and qBase plus (Biogazelle). Statistical significance was declared at $p<0.05$
Results: PA stimulation induced a significant overexpression of MMP-3 (0.4mM/basal=4.70±0.37; 0.7mM/basal=5.62±1.87), MMP-13 (0.4mM/basal=1.2±0.13; 0.7mM/basal=2.04±0.63), IL6 (0.4mM/basal=4.07±0.35; 0.7mM/basal=2.87±0.23) and COX2(0.4mM/basal=1.6±0.65; 0.7mM/basal=2.2±0.85) ($p<0.05$)
Mitochondrial superoxide anion was significantly increased by 6.68-fold (0.4mM PA) and 6.46-fold (0.7mM PA)compared tothe non-stimulated (PA-free) cells ($p<0.05$). Both total and mitochondrial ATP production were significantly decreased in PA-stimulated cells: PA/basal ratio 0.45 ± 0.04 (0.4mM PA) and 0.67± 0.1 (0.7mM PA)for total ATP and 0.55±0.07 (0.4mM PA) and 0.31±0.29 (0.7mM PA)for mitochondrial ATP ($p<0.05$)
A significant increase of apoptosis was also detected (0.4mM PA/basal ratio=2.32±0.04; 0.7Mm PA/basal ratio=2.7±0.22) ($p<0.05$); besidescaspases3/7 (0.4mM PA/basal ratio=2.37±0.19; 0.7mM PA/basal ratio=4.32±1.07) and caspase 9 activity (0.4mM PA/basal ratio=2.3±0.24; 0.7mM PA/basal ratio=2.83±0.5) significantly increased after PA stimulation too ($p<0.05$). After pre-incubation with 5mM NAC (N-Acetylcisteine) the apoptotic effects of PA stimulation were significantly reversed ($p<0.05$)
Co-stimulation with PA/O 1:2 partially reversed the effects of PA; the expression levels of MMP-3, MMP-13 and IL-6 as well as mitochondrial superoxide production andcaspases 3/7 and 9 activity were significantly reduced in relation to PA-stimulated cells ($p<0.05$); on the contrary, total and mitochondrial ATP showed a non-significant increase in relation to PA-stimulated cells
Conclusions: Palmitate overload causes an increased inflammatory response and mitochondrial superoxide anion productionas well as decreased levels of total and mitochondrial ATP. Besides, palmitate-induced apoptosis is mediated by increased mitochondrial oxidative stress.The effects of saturated fatty acids,related to obesity and metabolic syndrome, are partially reversed by monoinsaturated fatty acids such as Oleic acid and could help understand the metabolic phenotype of osteoarthritis

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WATER-SOLUBLE C60-(OH)24 FULLERENE HYDROXIDE AS A THERAPEUTIC AGENT AGAINST THE DEGENERATION OF ARTICULAR CARTILAGE IN OSTEOARTHRITIS

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Purpose: During the development of osteoarthritis (OA), mechanical stress on articular cartilage downregulates the stable cellular activities of chondrocytes and induce the production of catabolic factors, such as proinflammatory cytokines and chemokines. In addition, it is well known that chondrocytes produce excess amounts of reactive oxygen species (ROS; superoxide, nitric oxide, hydrogen peroxide and peroxynitrite) in response to mechanical stress. Recent reports have demonstrated that mechanical stress to articular cartilage stimulates excess production of ROS from chondrocytes, consequently resulting in the depolymerization of hyaluronic acid and chondrocyte death. It has been suggested that the generation of ROS and the depletion of cellular antioxidants may be involved in the pathogenesis of OA.
C60 fullerene is a molecule composed completely of carbon in a unique spherical structure that has a high reactivity with oxygen free radicals and can potentially act as a free radical scavenger. It has been reported that the antioxidant level of C60 is several hundred-fold higher than that of other antioxidants. Recently, C60 fullerene has shown to inhibit neuronal apoptosis by scavenging oxygen reactive species and protects human skin keratinocytes from ROS-induced cell death after UV stress, suggesting that C60 is a useful agent to protect against the oxygen free radical-induced pathological features in a variety of diseases. Use of C60 could lead to the development of novel therapeutic strategies in the prevention of both chondrocyte aging and cartilage degeneration.
To prevent the degeneration of articular cartilage in OA, we have newly developed on water-soluble C60(OH)24 fullerene hydroxide, a strong free radical scavenger, as an anti-oxidative agent. In this study, we examined the therapeutic effects of water-soluble C60(OH)24 on chondrocyte activity and energy metabolism, which were monitored by the production of cartilage component (proteoglycan), matrix degrading enzyme MMP-3, and the uptake of glucose in the cells, respectively.
Methods: In the presence or absence of C60(OH)24 (1.0 nM, 10.0 nM, 100.0 nM), human OA chondrocytes were incubated with IL-1β (1.0, 10.0 ng/ml). After the 24-hour incubation period, chondrocyte activities (production of proteoglycan and MMP-3, glucose metabolism) were analyzed.
Our previous study revealed that expression of DNA repair enzyme, apurinic/apyrimidinic endonuclease (Apex) 2, in chondrocytes was associated with the degeneration of articular cartilages and was induced by OA-relating catabolic factors. Thus, we also studied effects of C60(OH)24 on the expression of Apex 2 in osteoarthritic chondrocytes.
Results: IL-1β significantly inhibited the production of proteoglycan and the glucose uptake in chondrocytes, and accelerated the secretion of MMP-3 from chondrocytes. Even in the presence of IL-1β (1.0 ng/ml, but not 10.0 ng/ml), C60(OH)24 inhibited the IL-1β-induced production of MMP-3, the IL-1β-induced down-regulation of proteoglycan production and glucose metabolism in OA chondrocytes. C60(OH)24 fullerene hydroxide also reduced the OA-relating catabolic factor-induced up-regulation of DNA repair enzyme, Apex 2, in OA chondrocytes.
Conclusions: Our data indicated that nM order of water-soluble C60(OH)24 fullerene hydroxide may have a potential to protect against the catabolic factor-induced down-regulation of chondrocyte activities in OA. Our findings provide a novel pathogenic mechanism linking oxidant-mediated degenerative process of articular cartilage in OA.

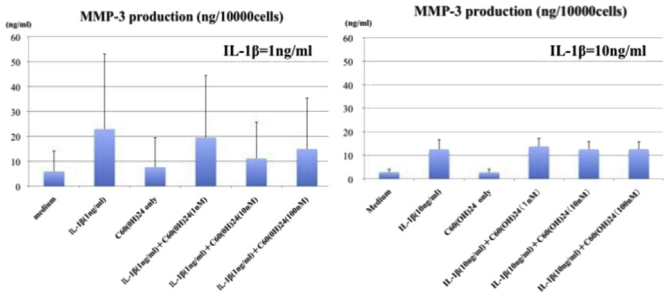

Figure 1. Effect of $C60(OH)_{24}$ on MMP-3 production in chondrocytes