on female rat knee joint cartilage properties under extreme condition simulating for example obesity in postmenopausal women. It arises the question on the difference of ranelate vs chloride form of strontium salt and what part of these salts actively works within joint cartilage, cation of strontium or strontium plus anion carrier.

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**STUDY OF ANTIAPOPTOTIC EFFECT OF TGF-β1 ON HUMAN ARTICULAR CHONDROCYTES: ROLE OF PHOSPHATASE PP2A**


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**Purpose:** Death of chondrocyte cells by apoptosis is a hallmark of osteoarthritis (OA). Combination of Tumour Necrosis Factor α (TNF-α) and Ro 31-8220 (Ro) have been proved to induce apoptosis in chondrocytes. Transforming Growth Factor β-1 (TGF-β1) is a pleiotropic cytokine that provides signals for cell survival and apoptosis. Protein Phosphatase type 2A (PP2A) is a major Ser/Thr phosphatase involved in several cellular signal transduction pathways, being considered, a positive regulator of apoptosis. The ratio of the apoptotic-related proteins bcl-2 and bax determines the susceptibility of many types of cells to apoptosis.

To study whether TGF-β1 is able to protect human chondrocytes from apoptosis induced by an in vitro model (TNF-α + Ro).

**Methods:** Human osteoarthritic (OA) and normal (N) cartilage was obtained from the femoral heads of 8 patients each. OA cartilage was obtained from patients who were undergoing joint replacement while normal cartilage was obtained from cadavers who had no history of joint disease and who had macroscopically normal cartilage. Apoptosis was assessed by flow cytometry (propidium iodide) and ELISA cell death, while nuclear morphology was evaluated using the fluorescent stain DAPI (4',6-diamidino-2-phenylindole, dihydrochloride). PP2A activity was estimated by measuring the absorbance of a molybdate: malachite green: phosphate reaction complex. Bcl-2 and bax expression were determined by Western blot analysis.

**Results:** It was established two groups of cells, one group was preincubated with TGF-β1 for 120 hours and another group without incubation (both in OA cells and normal cells). Both groups were stimulated with TNF-α and Ro for 16h. In OA cells, TGF-β1 significantly reduced the percentage of hypodiploid chondrocytes (TNF-α+Ro 18.6% vs TGF-β1+ TNF-α+Ro 8.9%, p < 0.05), as well as the percentage of internucleosomal DNA breakage (TGF-β1 + TNF-α+Ro 40.3% vs TNF-α+Ro 100%; p < 0.05). However, in normal chondrocytes, TGF-β1 did not reduce apoptosis as we assed both the percentage of hypodiploid chondrocytes (TNF-α+Ro 20.3% vs TGF-β1+ TNF-α+Ro 25.2%, p = 0.381) and internucleosomal DNA breakage (TGF-β1+ TNF-α+Ro 83.0% vs TNF-α+Ro 100% p = 0.391). Furthermore, nuclear morphology using DAPI fits well with previous results, both in OA and normal chondrocytes. In normal chondrocytes, preincubation of TGF-β1 plus PP2A Inhibitor Protein (i2PP2A) reduced internucleosomal DNA breakage as compared with TGF-β1 alone (TGF-β1 + i2PP2A + TNF-α+Ro = 59.0% vs TGF-β1 + TNF-α+Ro = 100%; p < 0.05). In addition, bcl-2/bax ratio was significantly higher in TGF-β1 + i2PP2A preincubated normal chondrocytes than in TGF-β1 alone (1.21 vs 0.72).

**Conclusions:** These results show that TGF-β1 alone is able to reduce the apoptosis induced by TNF-α+Ro 31-8220 in OA, but not in normal, chondrocytes. In normal chondrocytes, TGF-β1 exerts an antiapoptotic effect when PP2A is specifically inhibited. These results show the major role that PP2A plays in the outcome of TGF-β1 signal transduction, giving the potential of modulate TGF-β1 pathway, by manipulating the degree of PP2A activity, to produce a particular desired therapeutic outcome.

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**CELL DEATH IN OSTEOARTHRITIS: APOPTOSIS OR AUTOPHAGOCYTOSIS?**

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**Purpose:** Previous studies have shown that in osteoarthritic cartilage the number of apoptotic chondrocytes is higher than normal cartilage. Different studies have shown up until now that programmed cell death is not necessarily synonymous with apoptosis. Autophagy has been proposed as a cellular death involving to chondrocytes. It has been reported that SNP, but no NOC-12, induces apoptosis in human articular chondrocytes. To analyse the cellular changes induced by NOC-12 in human articular chondrocytes. To compare these morphologic alterations with those found in chondrocytes stimulated with SNP and in OA chondrocytes.

**Methods:** Normal and osteoarthritic (OA) human cartilages were obtained from patients with joint replacement (femoral and knee joint) and from autopsy cases (knee joint). After enzymatic digestion, chondrocytes were kept in DMEM with 10% SBF at 37°C in a humidified atmosphere provided by an incubator until the first subculture was reached. Cells were analysed by flow cytometry to measure the mitochondrial membrane potential using the probe JC-1 and the propidium iodide method to assess apoptosis. To characterize the cell death process that NOC-12 induces on normal chondrocytes, we carried out cytochemical techniques to reject the existence of lipidic drops, like hematokline-eosine, oil-red-o and black sudan B; on the other hand, chondrocytes were stained with different probes: DAPI (visualization of apoptotic bodies), JC-1 (visualization of mitochondrias) and DAF-FM (detection of NO gas) and subsequently they were analysed with fluorescent microscopy. Last of all, we proceeded to study the ultrastructure of OA, normal and NOC-12 stimulated normal chondrocytes by mean of transmission electronic microscopy (TEM).

**Results:** NOC-12 2 mM induces mitochondrial depolarization in normal chondrocytes at 24 hours (23.5±10.04% vs. 12.2±6.6%; P = 0.05), and hypodiploid cells in 21.6±8.5% vs. 0.8±0.5%; P < 0.05, at 48 hours. Direct observation by means of optic microscopy of NOC-12 stimulated chondrocytes induced cytoplasmatic vacuoles. Lipidic nature of these structures was discard by cytochemical techniques. Mitochondrias (JC-1 stain) and NO (DAF-FM) were distributed around the vacuoles. Ultrastructural study of chondrocytes showed that vacuoles were not empty; there was granular degraded material inside. TEM showed that nuclei of cells were entire and in the cytoplasm were observed mielinic bodies. DAPI experiments showed not fragmentation of nuclei and apoptotic bodies were not detected. Immunofluorescence and TEM study of OA chondrocytes showed the presence of vacuoles with morphologic characteristics similar to the vacuoles induced by NOC-12 in normal chondrocytes.

**Conclusions:** These results suggest that NOC-12 induces morphologic changes in normal articular chondrocytes more characteristic of autophagy than apoptosis. These findings are similar to the results found in some chondrocytes from OA cartilages.