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ABSTRACT BOOK

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PULSED SIGNAL THERAPY (PST) ENHANCES PROTEOGLYCAN CONCENTRATION IN HUMAN CHONDROCYTE CULTURES

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INTRODUCTION

Osteoarthritis (OA) is one of the most common rheumatic diseases directly involving the articular cartilage. The biomechanical properties of the cartilage depend upon the extracellular matrix produced by the chondrocytes, cells which are capable of maintaining a dynamic equilibrium between the anabolic and catabolic processes. The metabolic activity of these cells is regulated by several mediators, such as cytokines, hormones and growth factors. Interleukin 1 (IL-1 β) is a cytokine involved in cartilage degradation processes. It is produced by several types of cells and can be found in the synovial fluid of osteoarthritic subjects.

The clinical use of Pulsed Signal Therapy (PSTTM) has proven effective in the treatment of 70,000 patients suffering from osteoarthritis. To explain this efficacy, it is hypothesized that the PST-induced Faradic potentials serve to mimic intrinsic pressure-generated streaming potentials.

In this work we have studied the *in vitro* effects of PST on cultures of human articular chondrocytes cultivated in the presence or in the absence of IL-1 β . Under these conditions we studied the effect of PST through metabolic activity evaluated by proteoglycans (PG) levels in the culture medium and morphologic assessments carried out with a transmission electron microscope (TEM) and a scanning electron microscope (SEM).

MATERIALS AND METHODS

Human articular cartilage was obtained from the femoral heads of eight OA subjects undergoing surgery for total hip prostheses. Immediately after surgery, macroscopically healthy cartilage were cut aseptically and minced into 2-mm³ pieces. The cartilage fragments were washed in saline solution and then digested by clostridial collagenase.

Chondrocytes were cultivated in alginate gel on Petri dishes for 72 hours with and without IL-1 β (5ng/ml). Some dishes were exposed for 3 hours a day to PST. Control cultures were maintained under identical conditions to the treated cells, but in the absence of PST. After the culture period the medium was removed and collected for PG determination by immunoenzymatic method on microplates for the quantitative measurement of human PG. Cells in alginate gel were immediately fixed for transmission electron microscopy (TEM) and for scanning electron microscopy (SEM).

The data were expressed as the mean \pm SD of PG release into the culture medium per micrograms of DNA in the eight tested cultures. The Student's test was used for the statistical analysis; $p < 0.05$ was considered significant. When data were not normally distributed, the Mann-Whitney U test was used.

RESULTS

The PG concentration in the culture medium at baseline conditions, in the presence of IL-1 β at a concentration of 5ng/ml without and with PST stimulation, is shown in Fig.1. The presence of IL-1 β determines a significant decrease ($p < 0.05$) in PG levels, but when the cells are cultured in the presence of IL-1 β and submitted to PST stimulation statistically significant restoration ($p < 0.05$) of PG production is observed.

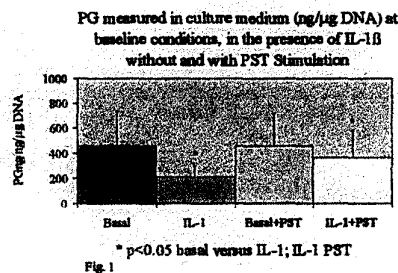


Fig. 1

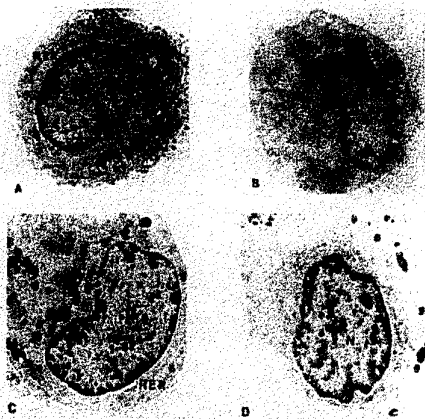


Fig. 2 A,B,C,D

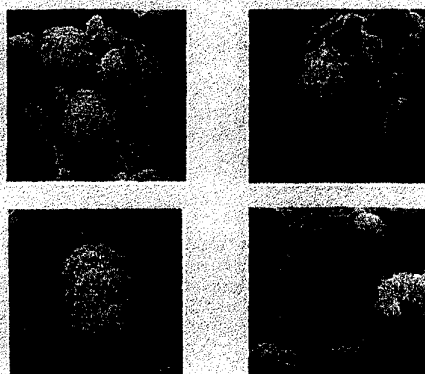


Fig. 3 A, B, C, D

The results concerning metabolic production are further confirmed by the morphologic findings obtained by TEM and SEM. Fig. 2 shows chondrocytes photographed by TEM. Fig. 2A shows a cultured cell at basal conditions: the nucleus (N) appears euchromatic, the cytoplasm contains a fair amount of rough endoplasmic reticulum (RER) and lipid droplets. Fig. 2B shows a cell cultured in the presence of IL-1 β and its damage is evident: several vacuoles (V) in the cytoplasm are devoid of typical structures.

Fig. 2C shows a cell cultured in basal condition and submitted to PST stimulation: the cell shows a good state of health. Fig. 2D shows a cell cultured in the presence of IL-1 β and submitted to PST stimulation; a clear restoration of the cell structures can be observed: the cytoplasm contains rough and smooth endoplasmic reticuli, and lipid droplets.

Fig. 3 shows the SEM image of cells. Fig. 3A shows cells cultured at baseline conditions: it is interesting to observe that it has retained its spherical shape, some secretion granules and the thick network of collagen fibrils. Fig. 3B shows a cell cultured in the presence of IL-1 β : its damage is clearly evident and this is further confirmed by the loss of cytoplasmic processes and by the presence of superficial alterations. Fig. 3C shows a cell cultured at basal conditions and submitted to PST stimulation: its SEM image shows the presence of abundant matrix fibers and secretion granules. Fig. 3D shows a cell cultured in the presence of IL-1 β and submitted to PST stimulation: a clear restoration of the cell structures can be observed as confirmed by the presence of several surface granules.

DISCUSSION

Chondrocyte cultures represent a valid and simplified biological model in which the effects and influence of various factors on the synthesis and degradation of extracellular components can be tested. They also can be used for morphological and ultrastructural evaluations.

In this study we have tested the effects of PST stimulation on the morphology and metabolism of *in vitro* human chondrocytes. These tests confirm the studies already carried out on *in vitro* experimental models: IL-1 β induces a decrease in the production of PG. When the cells were cultivated in the presence of IL-1 β and submitted to PST stimulation there was a restoration of PG concentration in the culture medium. The results concerning metabolic production are further confirmed by the morphological and ultrastructural evaluations obtained by TEM and SEM.

These data demonstrate the protective role played by PST which counteracts the IL-1 β induced effects on chondrocytes *in vitro*. These results confirm *in vitro* the data obtained in clinical studies on patients with osteoarthritis. Further studies are necessary in order to clarify the importance of this approach in for the treatment of cartilage disorders, such as osteoarthritis.