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# Biochemical and morphological study of human articular chondrocytes cultivated in the presence of pulsed signal therapy

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Pulsed signal therapy (PST) is an extension of pulsed electromagnetic fields (PEMF) therapy. PEMF have been used widely to treat non-healing fractures and related problems in bone healing since approval by the Food and Drug Administration in 1979.<sup>1,2</sup> Recently, PEMF therapy has been used to treat patients with osteoarthritis (OA) of the knee and cervical spine, with encouraging results.<sup>3,4</sup> In vitro studies have also shown a stimulating activity of PEMF on cartilaginous metabolism.<sup>5</sup>

PST, in contrast with PEMF, uses specific physiological changing rectangular pulses as stimuli, which are transmitted in a programmed alternating fashion that mimics the body's natural streaming potentials for one hour of treatment.

We studied the effects of PST on human OA chondrocytes cultivated in the presence and absence of a negative stimulus represented by interleukin 1 $\beta$  (IL1 $\beta$ ) and we evaluated the concentration of proteoglycans (PGs) in the culture medium and the morphology of the chondrocytes after exposure to PST.

Human OA chondrocytes were cultivated in alginate gel on Petri dishes for 72 hours with and without IL1 $\beta$  (5 ng/ml). Some dishes were exposed to PST for three hours a day.<sup>6</sup> The PST stimulation was administered by a device constructed for in vitro study. This apparatus has suitable dimensions for insertion in a CO<sub>2</sub> incubator. The device produces extremely low frequency (less than 30 Hz) varying pulsed electromagnetic fields averaging 10–20 G of magnetic energy at a coil current of up to 2 A drawn from a power source of 120 V AC. The pulse phase duration was 67 ms, including 15 micropulses with a pause duration of 0.1 s.

Control cultures were maintained under identical conditions but in the absence of PST. After the culture period the medium was removed and collected for determination of the PGs by an immunoenzymatic method on microplates for the quantitative measurement of human PGs.<sup>6</sup> Cells in alginate gel were immediately fixed for transmission electron microscopy (TEM) and for scanning electron microscopy (SEM). For each different experimental condition we observed 50 cells for TEM and 100 cells for SEM.

Table 1 shows the PG concentration in the culture medium at baseline, in the presence of IL1 $\beta$ , and with and without PST stimulation. The presence of IL1 $\beta$  produced a significant decrease ( $p < 0.05$ ) in PG levels, but when the cells were cultured in the presence of IL1 $\beta$  and given PST stimulation, PG

**Table 1** PG concentration (ng/ $\mu$ g DNA) in culture medium in various experimental conditions. The data are expressed as the mean (SD) of PG release into the culture medium per microgram of DNA in the eight tested cultures.

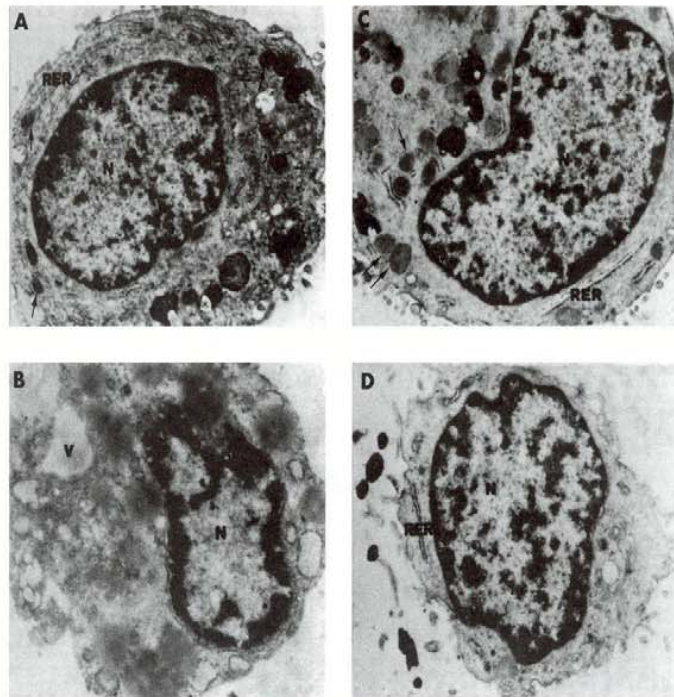
Basal	IL1	Basal PST	IL1 PST
435.9 (282.7)	208.9 (93.8)*	458.4 (259.3)	365 (210.7)*

Student's *t* test was used for statistical analysis.  
\* $p < 0.05$ , IL1 v basal and IL1 PST v IL1.

production was significantly restored ( $p < 0.05$ ). The results for metabolic production are further confirmed by the morphological findings obtained by TEM (fig 1) and SEM analysis (data not shown). TEM analysis showed that IL1 $\beta$  causes marked cellular damage, confirmed by the presence of several vacuoles in the cytoplasm, which is devoid of typical structures (fig 1B). When the cells were cultured in the presence of IL1 $\beta$  and given PST stimulation, the cell structures were clearly restored (fig 1D).

These experiments confirm the negative effect of IL1 $\beta$  on PG production.<sup>6,8</sup> Exposure of the chondrocytes to PST significantly restored the PG concentration in the culture medium. Such a resumption of anabolic activity was confirmed by morphological observations obtained by TEM and SEM analyses. The PST signals create a "streaming potential" of ionic flow similar to that generated in a moving joint, and thus they stimulate the anabolic activity of chondrocytes.

Our results are supported by numerous in vitro and in vivo observations, such as electric stimuli and PEMF enhanced cartilage repair processes. External oscillating electric fields increased the incorporation of [<sup>3</sup>H]thymidine into the DNA of chondrocytes isolated from embryonic chick epiphyses, and capacitatively coupled electric fields stimulated cell proliferation ([<sup>3</sup>H]thymidine incorporation) and glycosaminoglycan synthesis (<sup>35</sup>S<sub>4</sub> uptake) by isolated bovine growth plate chondrocytes.<sup>9,10</sup> One mechanism suggested for the actions of these electric and magnetic stimuli is an effect on charged transmembrane molecules, such as receptors.<sup>9,10</sup> Furthermore, electromagnetic fields cause a movement of calcium and other ions across cell membranes and stimulate DNA transcription, resulting in increased protein synthesis.<sup>9,10</sup>



**Figure 1** Transmission electron micrographs of chondrocytes cultured in vitro for 72 hours. (A) Basal conditions: the nucleus (N) appears euchromatic, the cytoplasm contains abundant rough endoplasmic reticulum (RER) and lipid droplets (arrows).  $\times 9000$ . (B) IL1 $\beta$  present: the cell shows a vacuolate cytoplasm devoid of the typical structure. N, nucleus; V, vacuole.  $\times 9000$ . (C) Basal conditions and given PST stimulation: the cell shows an euchromatic nucleus (N) and a cytoplasm rich in rough endoplasmic reticulum (RER) and lipid droplets (arrows).  $\times 10\,000$ . (D) IL1 $\beta$  present and given PST stimulation: the cell clearly recovers its good state of health. Rough endoplasmic reticulum (RER) is present and vacuoles are almost absent. N, nucleus.  $\times 9000$ .

Our study has confirmed, for the first time, the effect of PST on human chondrocytes cultured in alginate gel. The observed increased concentration of PGs in the culture medium, supported by ultrastructural and morphological analyses by TEM and SEM, confirmed the stimulating activity of this "non-pharmacological treatment" on chondrocytes. Further in vitro and in vivo studies are necessary to verify the effects and the mechanism of action of PST.

#### Authors' affiliations

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