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Chondroprotective Effects of Pulsed Electromagnetic Fields on Human Cartilage Explants

Alessia Ongaro,[1]* Agnese Pellati,[1] Federica Francesca Masieri,[1] Angelo Caruso,[1] Stefania Setti,[2] Ruggero Cadossi,[2] Roberto Biscione,[3] Leo Massari,[3] Milena Fini,[4] and Monica De Mattei[1]

[1]*Department of Morphology and Embryology, University of Ferrara, Ferrara, Italy*
[2]*Laboratory of Clinical Biophysics, IGEA, Carpi, Italy*
[3]*Department of Biomedical Sciences and Advanced Therapies, University of Ferrara, Ferrara, Italy*
[4]*Laboratory of Preclinical and Surgical Studies, Research Institute Codivilla Putti, Rizzoli Orthopaedic Institute, Bologna, Italy*

This study investigated the effects of pulsed electromagnetic fields (PEMFs) on proteoglycan (PG) metabolism of human articular cartilage explants from patients with osteoarthritis (OA). Human cartilage explants, recovered from lateral and medial femoral condyles, were classified according to the International Cartilage Repair Society (ICRS) and graded based on Outerbridge scores. Explants cultured in the absence and presence of IL-1β were treated with PEMF (1.5 mT, 75 Hz) or IGF-I alone or in combination for 1 and 7 days. PG synthesis and release were determined. Results showed that explants derived from lateral and medial condyles scored OA grades I and III, respectively. In OA grade I explants, after 7 days exposure, PEMF and IGF-I significantly increased ^{35}S-sulfate incorporation 49% and 53%, respectively, compared to control, and counteracted the inhibitory effect of IL-1β (0.01 ng/ml). The combined exposure to PEMF and IGF-I was additive in all conditions. Similar results were obtained in OA grade III cartilage explants. In conclusion, PEMF and IGF-I augment cartilage explant anabolic activities, increase PG synthesis, and counteract the catabolic activity of IL-1β in OA grades I and III. We hypothesize that both IGF-I and PEMF have chondroprotective effects on human articular cartilage, particularly in early stages of OA. Bioelectromagnetics 32:543–551, 2011.

Key words: proteoglycan metabolism; interleukin-1β; insulin-like growth factor-I; osteoarthritis

INTRODUCTION

Articular cartilage performs mechanical functions absorbing the different loads applied to a joint [Ulrich-Vinther et al., 2003]. In pathological conditions such as osteoarthritis (OA), alterations in the normal functional activities of chondrocytes contribute to the disruption of the balance between anabolic and catabolic processes of the cartilage. These processes are influenced by mechanical forces as well as abnormalities in autocrine, paracrine, and endocrine regulation at a cellular level, leading to alterations of the physiological tissue turnover within the joint. Further, alterations in genes encoding for extracellular matrix (ECM) components, inflammatory molecules, or matrix proteases and aging are involved in the molecular pathophysiological events leading to OA development [Herrero-Beaumont et al., 2009]. Interleukin-1β (IL-1β), the main catabolic cytokine in OA, exerts its activity by stimulating the increase of matrix metalloproteinase gene expression and by suppressing the synthesis of type II collagen and proteoglycans (PGs) [Daheshia and Yao, 2008], the main components of the articular cartilage ECM. On the other hand, the insulin-like growth factor I (IGF-I) plays an anabolic role in chondrocyte metabolism by stimulating chondrocyte proliferation and the synthesis of type II collagen and PGs [Bonassar et al., 2001].

Articular chondrocyte activities are also affected by physical forces, including mechanical stress and

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*Correspondence to: Alessia Ongaro, Department of Morphology and Embryology, University of Ferrara, Via Fossato di Mortara 64/B, 44121 Ferrara, Italy. E-mail: ngrlss@unife.it

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strain [Bonassar et al., 2001], low-intensity ultrasounds [Parvizi et al., 2002], and low-frequency, low-energy pulsed electromagnetic fields (PEMFs). In vitro studies have shown that PEMFs can stimulate human articular chondrocyte proliferation and increase the amount of cartilage ECM components in bovine articular explants and in pig chondrocytes cultured in collagen constructs [Pezzetti et al., 1999; De Mattei et al., 2001, 2003, 2004, 2007; Chang et al., 2010]. Furthermore, in vivo studies it has been reported that PEMFs stimulate PG synthesis in rats during endochondral ossification [Ciombor et al., 2002], and preserve the morphology of articular cartilage reducing lesion progression in the knee of OA guinea pigs [Ciombor et al., 2003; Fini et al., 2005b, 2008]. All of these data suggest that the exposure to PEMFs might be useful to preserve cartilage integrity and function. However, only a few studies investigated the effect of PEMFs on human cartilage cells [Pezzetti et al., 1999; De Mattei et al., 2001; Nicolin et al., 2007; Schmidt-Rohlfing et al., 2008]. PEMFs were able to increase cell proliferation, survival and the presence of collagen type II in human chondrocytes. In a study performed on human chondrocytes harvested from OA knee joints, PEMF exposure induced a slight, but not statistically significant enhancement of collagen II expression [Schmidt-Rohlfing et al., 2008]. Moreover, capacitively coupled electrical signals resulted in the upregulation of cartilage matrix protein expression and production, and the reduction of metalloproteinase expression in human OA cartilage explants [Brighton et al., 2008].

To our knowledge, there are no reports in the literature on the effect of PEMFs on cultured human articular cartilage explants. In this study, we investigated the effects of PEMF exposure on PG metabolism of human cartilage explants recovered from both femoral condyles of OA patients with cartilage degeneration more advanced in the medial condyle. The effect of PEMFs was also evaluated when IGF-I and IL-1β were added to the culture medium because they have profound effects on cartilage metabolism.

MATERIALS AND METHODS

Patients

Human cartilage samples from the femoral condyles were obtained from patients with various knee joint OA, undergoing total knee replacement ($n = 13$, 6 male and 7 female; mean ± SD age: 69 ± 3 years). The patients provided written informed consent to their participation in the study. OA severity was scored according to the criteria of the American College of Rheumatology Diagnostic Subcommittee on Osteoarthritis. Cartilage samples were recovered from the lateral condyle of all patients, while the severe cartilage degeneration of the medial condyle recovered enough cartilage for the experiments from four patients only. By gross examination, in agreement with other authors [Acebes et al., 2009], we have not included samples from peripheral regions of cartilage because osteophytes may be present in these areas as a result of the remodeling process. Areas where subchondral bone was visible (International Cartilage Repair Society (ICRS) Grade 4) were not considered. Cartilage explants from individual patients were subdivided into two groups; one group was used for the histological analysis and the second group for cultures and exposure to PEMF. Cartilage was scored according to the ICRS grading based on the Outerbridge score [Kleemann et al., 2005], ranging from healthy cartilage (ICRS Grade 0) to the absence of cartilage with exposed subchondral bone (ICRS Grade 4).

Cartilage Explant Cultures and Treatment Conditions

After surgery, cartilage was placed in Dulbecco's modified Eagle/Ham's F12 (DMEM/F12) medium (1:1) and antibiotics (penicillin 100 units/ml, streptomycin 0.1 mg/ml; Life Technologies, Paisley, UK). Full-thickness cartilage explants were obtained from the articular surface using a 4 mm dermal punch (Stiefel Laboratories, Milan, Italy) and cultured in multiwells (Nunc, Roskilde, Denmark; 6.6 × 6.6 cm^2, 1.6 cm diameter). Among the collected explants, we randomly subdivided them into two groups: untreated control group and treated group. Control explants were cultured in the base medium consisting of DMEM/F12 supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin 100 units/ml, streptomycin 0.1 mg/ml), in the absence of PEMF, IGF-I and IL-1β. Treated explants were cultured in base medium in the presence of IGF-I (50 ng/ml) or increasing doses (0.01, 0.1, 1, 10, and 50 ng/ml) of IL-1β. Parallel cultures were exposed to the PEMF (75 Hz, 1.5 mT) during the entire culture period (1 or 7 day). Medium was changed at the beginning of the exposure (time 0) and at the third day. PEMF unexposed cultures were placed inside the same incubator at a distance where no difference from background magnetic field was observed when the PEMF generator was turned on. In each experiment, controls and treatments were performed with triplicate wells, each well containing three explants.

Characteristics of PEMF and Exposure Conditions

The PEMF generator system was the same one used in previous studies [De Mattei et al., 2001, 2003, 2004, 2009; Varani et al., 2008]. It consisted of a pair of circular Helmoltz coils of copper wire, placed opposite to each other and in a signal generator (IGEA, Carpi, Italy). The multiwell plates were placed between this pair of Helmoltz coils so that the plane of the coils was perpendicular to the multiwell plates. The power generator produced a pulsed signal with the following parameters: pulse duration of 1.3 ms and frequency of 75 Hz, yielding a duty cycle of 0.10.

The intensity peak of the magnetic field was 1.5 mT and it was detected between two coils from one side to the other using the Hall probe (HTD61-0608-05-T, F.W. Bell, Sypris Solutions, Louisville, KY) of the gaussmeter (DG500, Laboratorio Elettrofisico, Milan, Italy), with a reading sensitivity of 0.2%. The induced electric field, as detected with a standard coil probe (50 turns, 0.5 cm internal diameter of the coil probe, 0.2 mm copper diameter), was 0.051 mV/cm. The shape of the induced electric field and its impulse length were kept constant. The intensity peak of the magnetic and induced electric field measured between the two coils had a maximum variation of 1% in the whole area in which the multiwell plate was placed. The specific PEMF characteristics were previously obtained from a dosimetry study [De Mattei et al., 2007].

Control cultures were maintained in the same incubator, placed at a distance from the coils where no difference from background magnetic field was observed when the PEMF generator was turned on. As in previous in vitro studies, we observed maximal PEMF effects at 24 h of stimulation [De Mattei et al., 2007]; cartilage explants were exposed to continuous PEMF for 1 or 7 days.

Quantification of PG Synthesis and Release

Proteoglycan synthesis was determined by radioactive sulfate incorporation into glycosaminoglycans (GAGs), which are biochemical components of PGs, during 1 or 7 days of culture, as previously reported [De Mattei et al., 2004]. Briefly, 5 μCi/ml of Na_2–$^{35}SO_4$ (2.2 mCi/ml; Amersham Pharmacia Biotech, Buckinghamshire, England) was added to the culture media of both unexposed and exposed explants at time 0 and when the medium was replaced (3rd day). After the radiolabeling, explants were rinsed and digested in 20 mM phosphate buffer (pH 6.8) containing 4 mg/ml papain (Sigma–Aldrich, Milan, Italy) at 60 °C for 12 h. The content of ^{35}S-labeled newly synthesized PGs (^{35}S-PGs) was measured following precipitation of the ^{35}S-PGs with cetylpyridinium chloride (Sigma–Aldrich) and filtration onto glass fiber filters (Whatman GF/C, Whatman International, Maidstone England). Filters were dried and radioactivity was quantitated by liquid scintillation counting.

Proteoglycan release into culture media was determined as total sulfated GAGs using the dimethylmethylene blue (DMMB) assay, with shark chondroitin sulfate (CS) as the standard [De Mattei et al., 2004]. Values obtained in media collected at the 3rd and 7th day were added. PG synthesis and PG release data were normalized to the explant DNA content, measured fluorometrically by binding to Hoescht 33258 dye [Kim et al., 1988].

Assay for Lactate

Lactate concentration, a marker of the general metabolic activity of chondrocytes [De Mattei et al., 2003], was measured in the culture media of human cartilage explants. Lactate production was determined by the lactate oxidase/peroxidase method using a kit supplied by Catachem (Bridgeport, CT).

Histological Analysis

Cartilage explants were fixed in a neutral buffered, isotonic formaline solution at 4 °C overnight, embedded into paraffin, and sliced in 5 μm serial slices using a hard-cutting microtome (RM2025, Leica Instruments, Nussloch, Germany). Slices were stained with haematoxylin and eosin for morphological observations and with Alcian blue (0.025 M $MgCl_2$) to assess for GAG content [Calvo et al., 2004].

Statistical Analysis

Analysis of data was done with Student's *t*-test because data was found to be parametric in configuration. When multiple comparisons were considered, Bonferroni correction was applied to determine statistical significance. Differences were considered significant at a value of $P \leq 0.05$. All data are reported as mean ± SD of at least three independent experiments.

RESULTS

Gross Examination and Histological Analysis of Cartilage

Cartilage degeneration and abrasion were evident in the specimens. In the lateral femoral condyles, cartilage degeneration was moderate, while

in the medial femoral condyles the cartilage degeneration was extremely severe. In relation to ICRS grading based on the Outerbridge score, cartilage from medial condyle was scored grade III, and cartilage from lateral condyle was scored grade I. Accordingly, the histological analysis showed that specimens from the lateral femoral condyle had discrete superficial irregularities and mild cracks (Fig. 1A). Specimens from the medial femoral condyle had deep cracks penetrating to the middle zone with evident vertical fissures, and small pieces of matrix were free from the surface (Fig. 1B). Cell density was reduced and isolated clones were localized in the superficial and deep zones of the tissue and sometimes were demarcated by vertical and horizontal clefts in the matrix. Finally, progressive reduction in matrix Alcian blue staining correlated with the severity of OA (Fig. 1C,D).

Effects of PEMF and IGF-I on PG Synthesis in Lateral Condyle Explants

The effects of PEMF exposure and IGF-I treatment alone or combined on PG synthesis, monitored as ^{35}S-sulfate incorporation, were evaluated at 1 and 7 days of culture (Fig. 2). At day 1, PEMF and IGF-I alone did not significantly modify ^{35}S-sulfate incorporation. When PEMF and IGF-I were used in combination, ^{35}S-sulfate incorporation significantly increased 38% compared to control (Fig. 2A). At 7 days, both PEMF exposure and IGF-I treatment alone significantly increased ^{35}S-sulfate incorporation by 49% and 53% over control, respectively. The increase in ^{35}S-sulfate incorporation induced by PEMF in combination with IGF-I was 94% with respect to control, suggesting an addictive effect of the two treatments (Fig. 2B). Because PEMF and IGF-I alone induced significant effects only after 7 days of culture, this experimental time was selected for all subsequent experiments.

Effects of PEMF and IGF-I on PG Synthesis in Lateral Condyle Explants Cultured in the Presence of IL-1β

Preliminary experiments were performed to investigate the effects of increasing doses of IL-1β (from 0.01 to 50 ng/ml) on PG synthesis. IL-1β significantly reduced PG synthesis in a dose-dependent manner, as shown in Figure 3. A plateau effect was

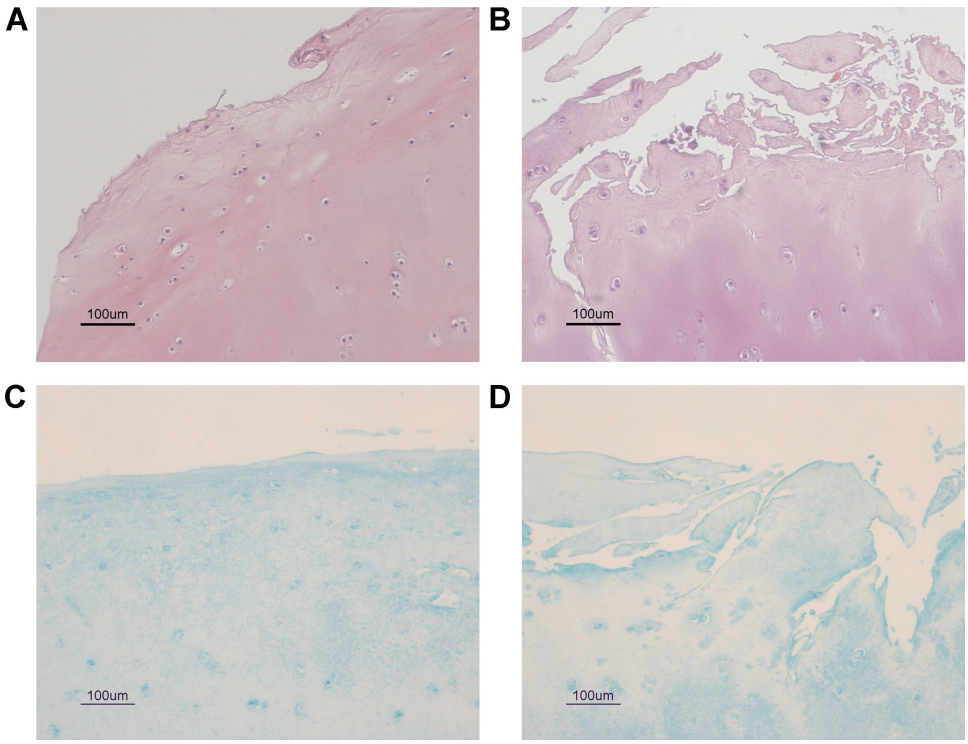

Fig. 1. Photomicrographs of human articular cartilage stained with hematoxylin and eosin (**A**,**B**) and Alcian blue (**C**,**D**). Cartilage from lateral condyle OA grade I shows moderate surface irregularities (A,C). Cartilage from medial condyle OA grade III includes deep cracks with vertical fissures and surface disorganization (B,D). Progressive reduction of matrix staining intensity was found in OA grade III (D) compared to grade I cartilage (C). Original magnification × 100. [The color figure for this article is available online at wileyonlinelibrary.com.]

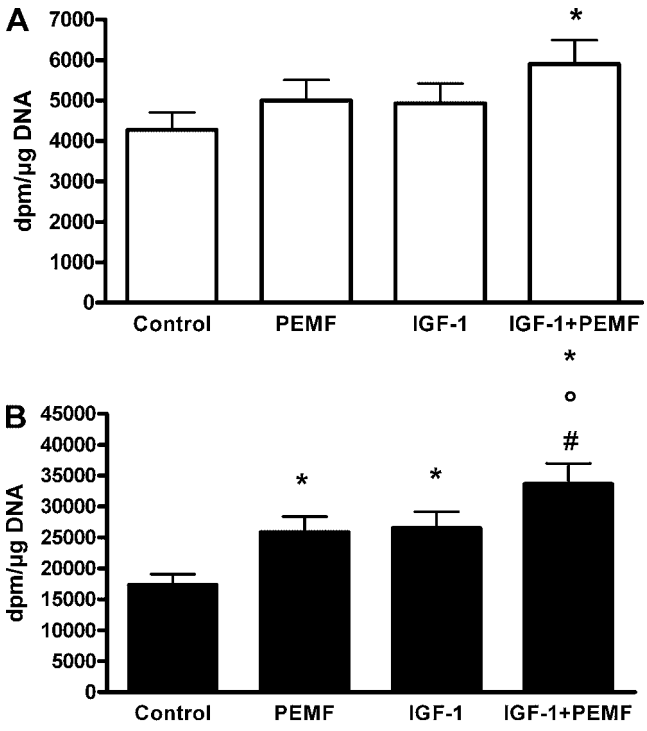

Fig. 2. Effects of PEMF and IGF-I on PG synthesis. Explants recovered from lateral condyles were treated for 1 (**A**) and 7 days (**B**). Each point was obtained from six independent experiments, each performed in triplicate. All data are expressed as means ± SD. *Statistically significant versus control; °statistically significant versus PEMF; #statistically significant versus IGF-I.

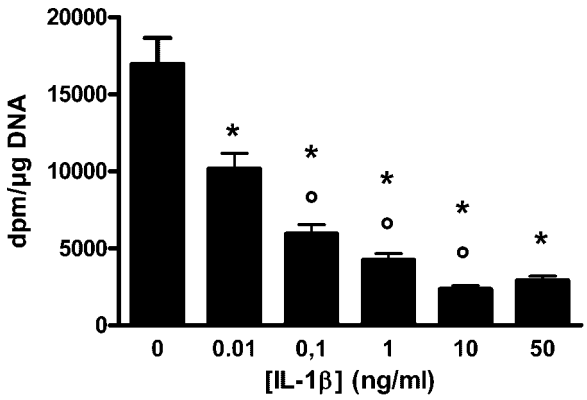

Fig. 3. Effect of increasing doses of IL-1β on PG synthesis in explants recovered from lateral condyles. IL-1β induces a dose-response reduction on PG synthesis after 7 days treatment. Each point was obtained from three independent experiments, each performed in triplicate. All data are expressed as means ± SD. *Statistically significant versus 0 ng/ml IL-1β dose; °statistically significant versus the previous IL-1β dose.

reached at the higher doses. Ten and fifty nanograms per milliliters of IL-1β reduced PG synthesis 87% and 83%, respectively. The analysis of lactate production in the culture media of explants at all IL-1β doses did not show any significant difference with respect to control (control lactate production = 303.64 µg lactate/µg DNA), suggesting that cells were metabolically active after IL-1β treatment (data not shown). In the subsequent experiments, two IL-1β concentrations were selected: the least (0.01 ng/ml) and most (10 ng/ml) efficient ones. Figure 4 shows the effect of PEMF and IGF-I on PG synthesis in explants cultured for 7 days in the presence of 0.01 or 10 ng/ml IL-1β. 0.01 ng/ml IL-1β reduced ^{35}S-sulfate incorporation 52% with respect to control explants. PEMF and IGF-I significantly limited the catabolic effect of 0.01 ng/ml IL-1β by increasing ^{35}S-sulfate incorporation 26% and 24%, respectively. The contemporary exposure to PEMF and IGF-I enhanced ^{35}S-sulfate incorporation 67% with respect to 0.01 ng/ml IL-1β treated cultures; in these experimental conditions, ^{35}S-sulfate incorporation was 80% of control cultures in which IL-1β was not added. When a concentration of 10 ng/ml IL-1β was used, no significant effect on ^{35}S-sulfate incorporation was induced by PEMF exposure or IGF-I treatment alone or in combination.

Effects of PEMF and IGF-I on PG Synthesis in Medial Condyle Explants

Explants could only be recovered from medial condyles in 4 of 13 patients. Because of the reduced amount of cartilage in these severely impaired cartilage explants, we limited our investigation to the effects of PEMF and IGF-I in basal conditions and

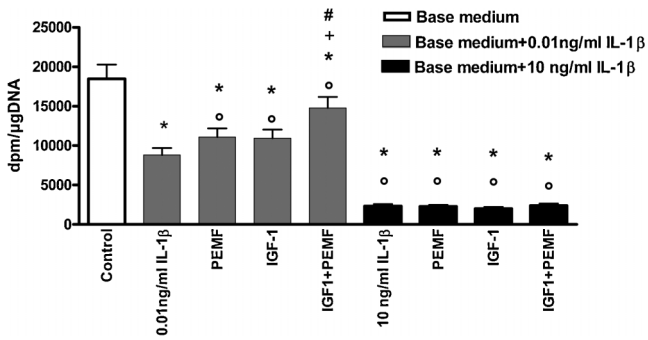

Fig. 4. Effects of PEMF and IGF-I on PG synthesis in explants recovered from lateral condyles treated with 0.01(gray) and 10 (black) ng/ml IL-1β in comparison to the control explants cultured in the absence of IL-1β (base medium; white). Each point was obtained from four independent experiments, each performed in triplicate. All data are expressed as means ± SD. *Statistically significant versus control; °statistically significant versus 0.01 ng/ml IL-1β; +statistically significant versus 0.01 ng/ml IL-1β in the presence of PEMF exposure; #statistically significant versus 0.01 ng/ml IL-1β in the presence of IGF-I.

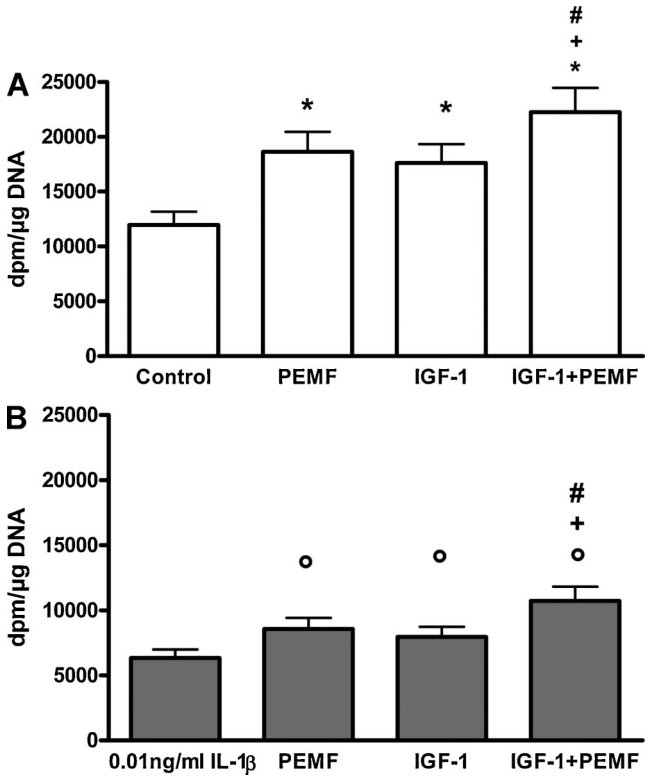

Fig. 5. Effects of PEMF and IGF-I on PG synthesis in explants recovered from medial condyles treated for 7 days. The cartilage explants were cultured in the absence (**A**) or presence (**B**) of 0.01 ng/ml IL-1β. Each point was obtained from four independent experiments, each performed in triplicate. All data are expressed as means ± SD. *Statistically significant versus control; °statistically significant versus 0.01 ng/ml IL-1β; +statistically significant versus PEMF; #statistically significant versus IGF-I.

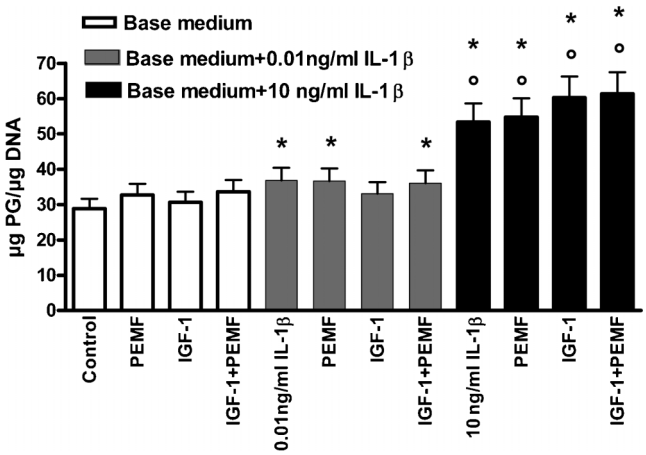

Fig. 6. Effects of PEMF and IGF-I on PG release. Explants recovered from lateral condyles were treated with 0 (white), 0.01(gray) and 10 (black) ng/ml IL-1β. Each point was obtained from three independent experiments, each performed in triplicate. All data are expressed as means ± SD. *Statistically significant versus control; °statistically significant versus 0.01 ng/ml IL-1β.

in the presence of 0.01 ng/ml IL-1β (Fig. 5). Basal ^{35}S-sulfate incorporation was 28% lower than in lateral condyle explants. PEMF exposure and IGF-I treatment increased ^{35}S-sulfate incorporation 56% and 47%, respectively, compared to control. The increase in ^{35}S-sulfate incorporation was 86% when the two stimuli were used in combination (Fig. 5A). IL-1β at 0.01 ng/ml concentration induced a 53% reduction in ^{35}S-sulfate incorporation with respect to explants cultured in the absence of IL-1β (Fig. 5B); this reduction was comparable to that observed in lateral condyle explants. PEMF and IGF-I limited the catabolic effect of IL-1β, increasing ^{35}S-sulfate incorporation by 35% and 25%, respectively. Also, in the presence of IL-1β when PEMF and IGF-I were used in combination, an addictive effect on ^{35}S-sulfate incorporation was observed, yielding a 68% increase with respect to 0.01 ng/ml IL-1β treated explants.

Effects of PEMF and IGF-I on Medium PG Release

Basal medium PG release in medial and lateral condyle cultures was not modified by PEMF and IGF-I alone or in combination. IL-1β induced a dose-dependent increase in PG release of 27% and 85% compared to control at 0.01 and 10 ng/ml IL-1β, respectively. In the presence of IL-1β, PEMF, and IGF-I alone or combined did not significantly modify PG release with respect to IL-1β. Figure 6 shows the data on PG release observed in lateral condyle cultures.

DISCUSSION

Preclinical research studies have shown that PEMFs favor the anabolic activity of chondrocytes and exert a significant chondroprotective effect on articular cartilage in animal models of spontaneous OA [Trock et al., 1994; Pezzetti et al., 1999; De Mattei et al., 2001, 2003, 2004, 2007; Jacobson et al., 2001; Ciombor et al., 2003; Fini et al., 2005b; Nicolin et al., 2007; Fini et al., 2005, 2008; Varani et al., 2008; Benazzo et al., 2008a]. In particular, in our previous studies performed on cartilage explants obtained from bovine joints, we showed that PEMF exposure influences the cartilage ECM metabolism by increasing PG synthesis [De Mattei et al., 2003, 2004]. Furthermore, we identified the optimal PEMF exposure conditions (exposure length, magnetic field amplitude, and pulse frequency) that could elicit the highest PG synthesis [De Mattei et al., 2007].

In the present study we investigated, for the first time, the effects of PEMF (1.5 mT, 75 Hz) on PG metabolism of human cartilage explants recovered from both femoral condyles of patients affected with knee OA, also in the presence of the anabolic growth factor IGF-I and the catabolic cytokine IL-1β. Cartilage explants with different OA severity were used, however, no difference in the response to PEMF and IGF-I stimulation was observed among explants when the PG synthesis and the catabolic effects of IL-1β were evaluated. PEMF significantly increased PG synthesis after 7 days treatment, while after 1 day of stimulation, no statistically significant effect was observed. Notably, the PEMF-induced increase in PG synthesis was similar to that induced by IGF-I, indicating that PEMF exposure has an effect of the same magnitude of that induced by the main cartilage anabolic factor [Bonassar et al., 2001]. Furthermore, when we analyzed the response to PEMF and IGF-I used in combination, we observed that their anabolic effects were additive, suggesting that PEMF can act in concert with IGF-I. PEMF and IGF-I could also limit the catabolic effect, evaluated as inhibition of PG synthesis induced by 0.01 ng/ml IL-1β. This effect was more evident when PEMF and IGF-I were used in combination, yielding a 67% increase in PG synthesis compared to IL-1β treated explants. At 10 ng/ml IL-1β, PEMF, and IGF-I alone or combined could not limit the decrease in PG synthesis induced by the cytokine, suggesting that this dosage is already too high, at least in our model, to observe effects. This is in agreement with previous studies showing a dose-dependent response of cartilage, evaluated as concentration of nitric oxide and prostaglandin E2 compared to the concentrations of both IL-1β and IGF-I [Peng et al., 2008]. Furthermore, the lack of an effect of IGF-I in the presence of 10 ng/ml IL-1β might be due to IGF-I desensitization, which is induced by high doses of IL-1β [Smeets et al., 2006]. Indeed, the dose of IL-1β (0.01 ng/ml) at which we found a significant effect of PEMF and IGF-I is in the range of IL-1β levels evaluated in the synovial fluid of OA patients [Hussein et al., 2008]. Finally, these observations suggest that the ability of PEMF to limit the catabolic effect of IL-1β on articular cartilage may be relevant, particularly in the early stages of OA, to prevent or delay cartilage degeneration.

Some studies address the possible connection between pro-inflammatory cytokines and apoptosis in OA cartilage [Kim et al., 2009; Weng et al., 2009]. Although we did not aim to evaluate chondrocyte apoptosis specifically in our study, we found that lactate production, a marker of the general metabolic activity of chondrocytes, was not modified at the higher IL-1β doses (10 and 50 ng/ml) in comparison to control, suggesting that at these IL-1β doses, cells were still metabolically active and viable, in accordance with the report by Kim et al. [2009] in both normal and OA chondrocytes.

Overall, data of this study indicate that PEMFs stimulate anabolic activity of human cartilage explants. Nevertheless, the exposure length required to stimulate OA explants is significantly longer than that used in bovine explants [De Mattei et al., 2003, 2004]. This difference can be ascribed to differences in the cellularity and metabolism in OA cartilage.

In cartilage explants treated with PEMF and/or IGF-I, we also measured that the release of PG into the medium did not differ from control cartilage explants, both in OA grades I and III. Further, in the presence of 0.01 and 10 ng/ml IL-1β, PEMF, and IGF-I alone or in combination did not significantly modify the PG release induced by the cytokine. Our results confirm that both PEMF exposure and IGF-I treatment do not induce PG loss and/or the activation of catabolic events in human cartilage.

Previous studies concerning the mechanisms by which PEMF exposure can modulate cellular behavior suggest that the PEMF effects we have observed in chondrocytes may be mediated by an upregulation of the gene expression of members of the TGF-β superfamily [Fini et al., 2005a] or genes of the ECM components [Fassina et al., 2006; Sollazzo et al., 2010], and/or the increase in specific cell membrane receptors, such as adenosine receptors, previously involved in GAG metabolism [Varani et al., 2008]. Further, recent evidence shows that the signal transduction networks induced by PEMF include phosphorilation of the insulin receptor substrate-1 (IRS-1), which mediates IGF signaling [Schnoke and Midura, 2007]. In this light, a potential mechanism to explain our results concerning the analogous effect of PEMF and IGF-I on PG synthesis might include the IRS-1 phosphorylation.

To our knowledge, this is the first study that analyses the effect of PEMF exposure in human cartilage explants showing that human chondrocytes are biologically responsive to PEMF action. Furthermore, the observation that PEMFs influence the chondrocyte activity in different OA grades suggests that human chondrocytes maintain susceptibility to PEMF during OA progression. To date, there are limited clinical studies examining the effects of PEMFs in humans, especially in patients affected by OA. However, the use of PEMFs after surgical procedures of the knee has been shown to shorten the recovery time, reduce the use of non-steroidal anti-inflammatory drugs and limit joint inflammatory

reaction, finally protecting cartilage from the catabolic effect of joint inflammation [Zorzi et al., 2007; Benazzo et al., 2008b]. The anti-inflammatory effect of PEMF through the activity of the $A2_A$ adenosine receptor agonist has been advocated to explain the chondroprotection observed in animal models of OA [Varani et al., 2008; De Mattei et al., 2009]. In conclusion, although the results of this study should be confirmed in a larger number of samples, we have shown that chondrocytes in human cartilage explants are sensitive to PEMF stimulation by increasing PG synthesis and limiting the catabolic effect of IL-1β. These results provide the scientific rationale to investigate whether PEMFs may play a role in preventing the progression of OA in patients, finally resulting in a chondroprotective activity.

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