CONCISE REPORT

Effect of pulsed electromagnetic fields on proteoglycan biosynthesis of articular cartilage is age dependent

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Objective: To investigate the effects of a pulsed electromagnetic field (EMF) on articular cartilage matrix biosynthesis with regard to age and cartilage damage using a matrix depleted cartilage explant model.

Methods: Cartilage explants were obtained from metacarpophalangeal joints of calves and adult cows. After depletion of the extracellular matrix by trypsin digestion, samples were maintained in serum-free basal medium with and without the addition of interleukin 1 β (IL1 β). Half the samples were subjected to an EMF for 24 minutes daily; the other half were left untreated. Undigested and untreated explants served as negative controls. After 7 days, biosynthesis of matrix macromolecules was assessed by [³⁵S]sulphate incorporation and values were normalised to hydroxyproline content.

Results: The EMF increased matrix macromolecule synthesis in undigested, untreated explants ($p < 0.009$). In matrix depleted samples the EMF had no stimulatory effect on proteoglycan biosynthesis. IL1 β significantly decreased the de novo synthesis of matrix macromolecules ($p < 0.00004$) in young and adult samples, but an EMF partly counteracted this inhibitory effect in cartilage samples from young, but not old animals.

Conclusion: EMF promoted matrix macromolecule biosynthesis in intact tissue explants but had no stimulatory effect on damaged articular cartilage. The suppressive effects of IL1 β were partially counteracted by EMF exposure, exclusively in cartilage derived from young animals. An EMF has age dependent chondroprotective but not structure modifying properties when cartilage integrity is compromised.

Articular cartilage is a connective tissue with a unique structure that provides smooth and frictionless joint movement as well as mechanical resistance to compression and shear forces. These mechanical strains induce mechanical-electrical transduction mechanisms caused by movement of fluid and electrolytes^{1,2} and may be involved in cartilage remodelling, altering the tissue to adapt to the changing demands placed upon it.

External biophysical forces, such as a pulsed electromagnetic field (EMF), may lead to cellular stimulation via “streaming potentials”.³ The stimulatory effect of an EMF on chondrocyte metabolism has been demonstrated in several in vitro studies.^{4,5} Moreover, in vivo studies showed that an EMF had a beneficial effect on either structure modification or clinical outcome measures in osteoarthritis (OA).^{6,7} Thus, the concept of EMF stimulation has attracted much attention in the treatment of OA, because only a few structure modifying modalities are known.

Although OA is crucially linked to patients’ age,⁸ the influence of age on the effect of an EMF on articular cartilage metabolism has not yet been examined. We used bovine

articular cartilage explants derived from animals of different ages in order to investigate possible age related differences on proteoglycan biosynthesis under EMF stimulation. Additionally, we set up a matrix depleted cartilage explant model to study the effects of EMF on tissue damage, as seen in trauma or OA.

MATERIALS AND METHODS

Cartilage explant cultures and EMF exposure conditions

Cartilage samples from the metacarpophalangeal joints of three 3 month old calves and three 18–20 month old adult cows were obtained aseptically after slaughter and were washed twice in phosphate buffered saline (PBS; GibcoBRL, Life Technologies, Paisley, Scotland). Thereafter, tissue explants were distributed in 24 well plates at 100–150 mg cartilage per well and cultured in a serum-free basal medium (BM). Half of the explant cultures were digested using 5 μ g/ml trypsin (Sigma Chemical Co, St Louis, MO, USA) over a period of 16 hours at 37°C as previously described,⁹ while the other half of the samples were left undigested. Digested samples were washed twice with PBS to remove the trypsin. Afterwards the tissue samples were divided into an EMF test group and a non-EMF control group and were incubated with and without the addition of interleukin 1 β (IL1 β ; 10 ng/ml). IL1 β and BM were replaced every second day. Undigested and unstimulated explants served as negative controls. Cultures were maintained at 37°C in humidified air and 5% CO₂.

The EMF system used for the experiments was a commercially available machine for home use (MRS 2000+; Vita Life, Lind, Austria). The EMF was generated by one pair of coils, the magnetic flux density was 40 μ T at a pulse rate of 200 Hz. The EMF test group was exposed for 24 minutes a day for a period of 7 days.

Biosynthesis of macromolecules

For [³⁵S]sulphate incorporation assays, the explants were washed three times with PBS. The cultures were then labelled in identical aliquots of 1 ml BM containing 20 μ Ci/ml of [³⁵S]sulphate (carrier-free, Amersham, Buckinghamshire, UK) for 3 hours at 37°C. Thereafter, the medium was discarded and explants were washed three times with ice cold buffer (10 nM EDTA, 0.1 M sodium phosphate, pH 6.5) followed by an overnight digestion in 1 ml sodium phosphate wash buffer containing proteinase K (1 mg/ml) at 80°C. Unincorporated isotope was removed using Sephadex G-25 (PD-10 columns; Pharmacia Biotech, Piscataway, NJ, USA) gel chromatography. Values were obtained by liquid scintillation counting (1410 liquid scintillation counter; Wallac Oy, Turku, Finland) of aliquots from void volume fractions and

Abbreviations: BM, bone marrow; EMF, electromagnetic field; IL1 β , interleukin 1 β ; OA, osteoarthritis; PBS, phosphate buffered saline

Figure 1 Cartilage explants were derived from 3 month old calves, digested using trypsin and cultured in serum-free basal medium (BM) with and without the addition of interleukin 1β (IL1β; 10 ng/ml). Undigested, untreated cartilage samples served as negative controls. The cultures were divided into a non-electro magnetic field (EMF) group (white columns) and an EMF group (grey columns) that was exposed to an EMF over a period of 7 days. Thereafter explant cultures were labelled with [³⁵S]sulphate for 5 hours. The radiolabel incorporated into newly synthesised matrix proteoglycans was then measured, normalised to hydroxyproline content, and given as counts per minute/μg hydroxyproline. Values are the mean (SEM). *Significant increase v non-EMF controls: undigested BM (EMF) v undigested BM (non-EMF) $p < 0.009$ and IL1β (EMF) v IL1β (non-EMF) $p < 0.00002$.

normalised to hydroxyproline content using a previously described method.¹⁰

Statistical analysis

Statistical analysis was performed using Student's *t* test. Significance was defined as a $p < 0.05$.

RESULTS

Effect of EMF on the synthesis of matrix macromolecules in cartilage explant cultures derived from joints of 3 month old calves

The loss of proteoglycans from articular cartilage represents an early event in OA.¹¹ We used a well established matrix depleted cartilage explant model⁹ to study the effects of an EMF on cartilage damage, reminiscent of structural changes in OA.

In cartilage explant cultures from juvenile animals, the overnight trypsin digestion significantly stimulated the biosynthetic activity of the resident chondrocytes. We observed a more than threefold increase in isotope uptake into newly synthesised matrix macromolecules compared with undigested cultures (BM 4597.3 (817.4) (mean (SEM) cpm/μg hydroxyproline); undigested BM control 1368.6 (233.8); $p < 0.0002$). In the undigested control samples exposure to the EMF significantly increased isotope uptake twofold (non-EMF 1368.6 (233.8), EMF 2820.4 (457.6.1); $p < 0.009$). Unlike in undigested explants, the EMF had no further stimulatory effect on matrix macromolecule synthesis in the matrix depleted, trypsinised samples when compared with non-EMF cultures (non-EMF 4597.3 (817.4); EMF 5663 (794.1); $p = 0.29$) (fig 1).

In matrix depleted samples that were incubated with IL1β, an expected decrease in proteoglycan biosynthesis was

Figure 2 Matrix depleted bovine cartilage explants were derived from adult cows and incubated in serum-free basal medium (BM) with and without the addition of interleukin 1β (IL1β) at a final concentration of 10 ng/ml. Undigested, untreated cartilage samples served as negative controls. Grey columns represent EMF stimulated cultures, while white columns represent non-EMF controls. After a stimulation period of 7 days, the [³⁵S]sulphate incorporation rate was measured and values were normalised to hydroxyproline content (counts per minute/μg hydroxyproline). Values are the mean (SEM). *Significant increase v non-EMF controls: undigested BM (EMF) v undigested BM (non-EMF) $p < 0.009$.

observed (BM 4597.3 (817.4), IL1β 178.5 (233.8); $p < 0.00004$). This catabolic effect was partly antagonised when the explants were exposed to the EMF, which increased matrix macromolecule synthesis by fivefold, when values were compared with non-EMF samples (IL1β (non-EMF) 178.5 (38.8); IL1β (EMF) 932.7 (98.5); $p < 0.00002$) (fig 1).

Effect of EMF on biosynthetic activity of bovine articular cartilage explants derived from adult animals

Trypsin treatment of adult cartilage did not stimulate matrix synthesis as seen in the cartilage samples derived from young animals. Exposure to the EMF slightly increased proteoglycan synthesis rate in the undigested control group (untreated BM control (non-EMF) 1234.3 (110.7), untreated BM control (EMF) 1673.2 (126.6); $p < 0.009$), but had no effect on matrix depleted explants (fig 2).

When samples were incubated with IL1β, proteoglycan biosynthesis decreased (BM 1223.1 (144.7), IL1β 324 (47.1); $p < 0.00002$). In contrast with the results obtained in young animals, EMF did not counteract the IL1β effect in old ones (IL1β (non-EMF) 324 (47.1); IL1β (EMF) 227.7 (23.4); $p = 0.1$) (fig 2).

DISCUSSION

Pulsed EMF has been shown to have anabolic effects on chondrocytes with respect to cell differentiation,⁴ cell proliferation,¹² and matrix synthesis.^{4,5} This has led to an examination of the potential therapeutic effects of EMF in OA. However, OA is mostly a disease of older age, and even in younger patients with OA, chondrocytes behave as if they were aged.¹³ In the present study we show that EMF has no effect on damaged cartilage and has differential effects on chondrocytes from adult compared with younger animals.

Firstly, although we confirmed previous reports^{4,5} of a significant EMF-induced increase in matrix macromolecule synthesis in the cultures of undamaged cartilage, in the

trypsin-treated, damaged explants exposure to an EMF yielded no increase beyond that induced by the damage itself, either in young or in adult tissue. These data support the notion of potential anabolic effects of the EMF on cartilage metabolism, but in damaged tissue the EMF does not increase the matrix biosynthesis rate and may therefore be insufficient for augmenting cartilage repair in vivo. It is of note, that these experiments were conducted with EMF intensity and pulse rate defined by the manufacturer and the possibility cannot be ruled out that different settings might have differentially influenced cartilage explants. This might be the case with the study of Liu *et al*, who reported an EMF-induced decrease in proteoglycan synthesis in embryonic chick sternal cartilage explants.¹⁴ However, our data on undamaged cartilage are paralleled by previous reports, indicating the reliability of the findings.

Importantly, chondrocytes from adult tissue did not respond to the stimulus induced by cartilage digestion as they did in young animals. This additional finding suggests that responsiveness to anabolic stimuli decreases with increasing age, as was reported for signals triggered by different growth factors,¹⁵ and is in line with the failure of adult chondrocytes to become activated upon exposure to EMF. Consequently, the physiological aging process may be partly responsible for impaired cartilage homeostasis at an older age.

IL1 β is thought to have a major role in cartilage destruction.¹⁶ Although in undamaged cartilage explants derived from adult cows the EMF potentially counteracted the IL1 β triggered proteoglycan loss,⁵ in our culture settings, the EMF could partly compensate for the IL1 β dependent decrease in matrix macromolecule synthesis, but only in cartilage samples derived from young animals. Our data indicate the chondroprotective properties of the EMF, but the effects on matrix macromolecule synthesis seem to be limited to younger subjects. Ciombor *et al* showed that the number of IL1 β positive chondrocytes and cartilage degradation were reduced after treatment with an EMF.⁶ One limitation of our study design is that no analysis of the culture supernatant was carried out, but the above observations suggest that an EMF has chondroprotective properties. Nevertheless, the effects on matrix macromolecule synthesis seemed to be limited to younger subjects.

In conclusion, the EMF applied here had the potential to stimulate matrix biosynthesis in undamaged young and adult articular cartilage. On the other hand, in the matrix depleted cartilage explant model that resembled cartilage damage, no stimulatory effect could be seen. The suppressive effects of IL1 β were partly counteracted by the EMF, but this effect was restricted to cartilage derived from young animals. Our conclusions are based on the measurement of total proteoglycan synthesis, thus we cannot rule out the presence of different proteoglycans or even type II collagen. However, our results suggest that an EMF has chondroprotective properties

in the young, rather than structure modifying properties in conditions of compromised cartilage integrity. Thus, EMF may not be useful as a therapeutic measure for OA, although it may have chondroprotective properties in young subjects.

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REFERENCES

- 1 Frank E, Grodzinsky A. Cartilage electromechanics. I. Electrokinetic transduction and the effects of electrolyte pH and ionic strength. *J Biomech* 1987;**20**:615-27.
- 2 Frank E, Grodzinsky A. Cartilage electromechanics. II. A continuum model of cartilage electrokinetics and correlation with experiments. *J Biomech* 1987;**20**:629-39.
- 3 Guzelsu N, Walsh W. Streaming potential in intact wet bone. *J Biomech* 1990;**23**:673-85.
- 4 Ciombor D, Lester G, Aaron R, Neame P, Caterson B. Low frequency EMF regulates chondrocyte differentiation and expression of matrix proteins. *J Orthop Res* 2002;**20**:40-50.
- 5 De Mattei M, Pasello M, Pellati A, Stabellini G, Massari L, Gemmati D, *et al*. Effects of electromagnetic fields on proteoglycan metabolism of bovine articular cartilage explants. *Connect Tissue Res* 2003;**44**:154-9.
- 6 Ciombor D, Aaron R, Wang S, Simon B. Modification of osteoarthritis by pulsed electromagnetic field—a morphological study. *Osteoarthritis Cartilage* 2003;**11**:455-62.
- 7 Track D, Bollet A, Markoll R. The effect of pulsed electromagnetic fields in the treatment of osteoarthritis of the knee and cervical spine. Report of randomized, double blind, placebo controlled trials. *J Rheumatol* 1994;**21**:1903-11.
- 8 Creamer P, Hochberg MC. Osteoarthritis. *Lancet* 1997;**350**:503-8.
- 9 Erlacher L, Ng CK, Ullrich R, Krieger S, Luyten FP. Presence of cartilage-derived morphogenetic proteins in articular cartilage and enhancement of matrix replacement in vitro. *Arthritis Rheum* 1998;**41**:263-73.
- 10 Schafer SJ, Luyten FP, Yanagishita M, Reddi AH. Proteoglycan metabolism is age related and modulated by isoforms of platelet-derived growth factor in bovine articular cartilage explant cultures. *Arch Biochem Biophys* 1993;**302**:431-8.
- 11 Doherty M, Hutton C, Bayliss M. Osteoarthritis. In: Maddison P, Isenberg D, Woo P, Glass D, eds. *Oxford Textbook of Rheumatology*. Oxford: Oxford University Press, 1993:959-83.
- 12 De Mattei M, Caruso A, Pezzetti F, Pellati A, Stabellini G, Sollazzo V, *et al*. Effects of pulsed electromagnetic fields on human articular chondrocyte proliferation. *Connect Tissue Res* 2001;**42**:269-79.
- 13 Bobacz K, Erlacher L, Smolen J, Soleiman A, Graninger W. Chondrocyte number and proteoglycan synthesis in the aging and osteoarthritic human articular cartilage. *Ann Rheum Dis* 2004;**63**:1618-22.
- 14 Liu H, Abbott J, Bee J. Pulsed electromagnetic fields influence hyaline cartilage extracellular matrix composition without affecting molecular structure. *Osteoarthritis Cartilage* 1996;**4**:63-76.
- 15 Guerne P, Blanco F, Kaelin A, Desgeorges A, Lotz M. Growth factor responsiveness of human articular chondrocytes in aging and development. *Arthritis Rheum* 1995;**38**:960-8.
- 16 Van den Berg W, Joosten L, Kollias G, van de Loo F. Role of tumor necrosis factor α in experimental arthritis: Separate activity of interleukin 1 β in chronicity and cartilage destruction. *Ann Rheum Dis* 1999;**58**:140-8.