THE STRUCTURAL EFFECTS OF CONTINUOUS AND PULSED ELECTROMAGNETIC FIELD THERAPY ON HUMAN CARTILAGE EXPLANTS

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Summary

Pulsed electromagnetic field therapy (PEMF) is a promising treatment method of osteoarthritis, fulfilling many of the characteristics of a disease modifier in this disorder. The efficiency of the method is still under evaluation, aiming to find the optimal frequency, intensity and treatment duration. Fewer studies have followed the effects of PEMF on human articular explants, although the cartilage matrix could play a role in the biologic effects of these therapy by the means of type II collagen piezoelectric properties. The present study aims to describe structural modifications induced by continuous and pulsed magnetic field on human articular explants resulting from hip osteoarthritis patients that benefitted from total hip arthroplasty. Ten osteoarthritis cartilages originating from the femoral heads were obtained from patients who benefitted from total hip arthroplasty. The joint cartilage from the non-portant region of the femoral head, with best microscopic aspect was detached by a cut tangential to the bone surface. The piece was then divided into 4 equal parts and transferred into 4 sterile containers filled with 10ml DMEM (Dulbecco’s Modified Eagle Medium) (Lonza), Penicillin 100UI/ml and Streptomycin 100µg/ml (Lonza). Thus, four groups resulted. The fragments in the first group (initial group) were histologically and histochemically analised by the safranin-orange coloration in less then 36 hours from the operation and quantified according to osteoarthritis modified Mankin score. The fragments in the second group (control group) were incubated at 37°C for 4 days, the fragments in the third group were incubated at 37°C and treated with continuous magnetic field (CMF), with an intensity of 0,1mT, three hours a day, for 4 days, and the fragments in fourth group were incubated in 37°C and treated with PEMF with an intensity of 30mT, a frequency of 1.5Hz, three hours a day for 4 days. At the end of the study, the fragments coming from the control, CMF, PEMF were also histologically and histochemically analysed by the safranin orange coloration and quantified according to osteoarthritis modified Mankin score. The modified Mankin score in the control group showed a worsening tendency versus the initial group (9.5 vs 8.95). In the CMF group the score had lower values compared to the control group (9.05 vs 9.5) and in the PEMF group, the score had even lower values than in the initial group (8.7). Still, the differences between the groups were not statistically significant, the lowest p was attained when comparing the PEMF group to the control group (p=0.27). The safranin orange coloration subscore in the control group had a worsening tendency when compared to the initial group (2.95 vs 2.5). In the CMF group the subscore had lower values than in the control group (2.65 vs 2.95) and in the PEMF group the subscore was lower even than in the initial group (2.45). The differences in the safranin orange coloration subscore were not statistically significant, the lowest p was found when comparing the control and the PEMF group (p=0.14) and when
Comparing the initial to the control group (p=0.17). Exposing human articular explants in low intensity CMF of 0.1 mT and in low frequency (1.5 Hz) medium intensity (30 mT) PEMF for three hours a day during four days did not lead to significant changes in the modified Mankin score. Nevertheless, there was a tendency of improvement of the modified Mankin score in the PEMF group versus the control group resulting from the increase in intensity of the safranin orange coloration in the pericellular and territorial matrix of the joint cartilages (p=0.14). Further similar studies conducted for a longer duration are necessary in order to follow the structural effects of PEMF or CMF on joint cartilages.

**Keywords:** pulsed electromagnetic field, continuous magnetic field, osteoarthritis, human articular explants

**Introduction**

The joint cartilage (JC) is a hyaline cartilage that is different from those found in the pavilion of the ear, epiglottis or larynx, as it lacks the perichondrium. This is the reason why articular cartilage derives its nutrition from the articular fluid and healing of the posttraumatic or degenerative lesions is a difficult process.

Arthritis is the most frequent musculoskeletal condition, with a prevalence of 25% after the age of 50 (Duncan et al., 2011), when the diagnosis is established exclusively according to radiologic criteria. In the setting of the increasing life expectancy and population aging, the World Health Organisation estimates that arthritis will become the fourth cause of disability by the year 2020 (Woolf et al., 2003).

Despite the progresses achieved in the last years, current arthritis treatment is unsatisfactory and new cost-effective therapies are needed.

Pulsed electromagnetic field therapy (PEMF) has been approved since 1979 by the United States Federal Drug Administration (FDA) for the treatment of unconsolidated fractures and it is currently under evaluation for the treatment of osteoarthritis (Trock et al., 2000).

Several in vitro experimental studies on chondrocyte cultures and ex vivo on animal models have shown the disease-modifying profile of this therapy: it inhibits chondrocyte apoptosis (Li et al., 2011), stimulates chondrocyte proliferation (Sakai et al., 1991; Pezzetti et al., 1999; De Mattei et al., 2001), stimulates the extracellular matrix synthesis (Liu et al., 1997; Bobacz et al., 2006; De Mattei et al., 2007), inhibits the 1β interleukin (Bobacz et al., 2006; Ongaro et al., 2011) and stimulates tumor growth factor β (Ciombor et al., 2003, Benazzo et al., 2008).

Few studies have described the effects of PEMF on human articular explants, although the cartilage matrix could play a role in the biologic effects of the therapy by the piezoelectric properties of the type II collagen (Trock et al., 2000).

The present trial aims to study the structural changes after exposure to continuous and pulsed electromagnetic field in the human articular explants obtained from hip osteoarthritis patients who had benefited from total hip replacement.

**Material and methods**

**Cartilage sampling**

Ten femoral head joint cartilage fragments were obtained from patients who had benefited from total hip arthroplasty in the Orthopedic settings of the Cluj-Napoca Traumatology and Orthopedics Clinic and in the Traumatology and Orthopedics Department of the Alba-Iulia County Emergency Hospital during the 1st of June 2011 and the 1st of March 2012. The average age of the patients was 62 ± 3 years and the gender ratio was of 8/2 females/males.

The joint cartilage taken from the non portant area of the femoral head, with the best macroscopic appearance was detached from the bone by tangential
section to the bone surface. Then the cartilage fragment was splitted into 4 equal pieces and transferred to 4 sterile containers containing 10 mL of Dulbecco’s Modified Eagle Medium (DMEM) (Lonza), 100 UI/mL Penicillin and 100 µg/ml Streptomycin (Lonza). One fragment was sent in maximum 36 hours to the Pathology Laboratory of the Railwail Clinical Hospital in Cluj-Napoca, where safranin orange stain was performed. The other 3 fragments were incubated in less than 6 hours from prelevation at 37°C for 4 days.

**Cartilage treatment**

The second fragment was used as control, the third fragment was treated with continuous electromagnetic field (CEMF) with a 0.1 mT intensity for 3 hours a day and the fourth one was exposed to 1.5 Hz and 30 mT PEMF. After 4 days, the three fragments were also histochemically analysed using the safranin orange stain.

The apparatus consisted in the 5000 series BTL magnetotherapy using 13/13 cm flat coils that ensure a magnetic field penetrance of up to 30 cm.

**Tissue processing**

Tissues were processed in paraffin and sectioned. Sections were deparaffinized and washed with distilled water. The first stain applied was Weigert’s ferric hematoxylin for 10 minutes, followed by washing with current water and treated with 0.001% Fast Green solution for 5 minutes, followed by an 1% acetic acid for 10-15 seconds and 1% safranin orange stain for 5 minutes. The pieces were then dehydrated with 90º alcohol for 2 minutes and absolute alcohol for 2 minutes, clarified with xylene and then paraffin mounting was performed.

**Examining procedure**

Hystologic and histochemical analysis of the cartilages was performed by two independent examiners by means of the modified Mankin score (Ostergaard et al., 1997). In this grading system, 0 to 6 points are attributed to the extracellular matrix lesions, 0-3 points to cartilage cells anomalies, 0-4 points to the progressive reduction of the safranin orange stain intensity and 0-1 points to the tidemark integrity. Since the sections were tangent to the bone surface, the tidemark was not available in the pieces and it was registered as 0 in all cartilages. Thus, the highest score, of 13, corresponded to a severe damage of the articular cartilage.

**Statistics**

Normal distribution of the quantitative variables was tested using the D’Agostino-Pearson test, the Mann-Whitney test was used to assess differences between nonparametric variables and the Student test was used for the parametric ones. Statistical significance threshold chosen was p≤0.05. The Medcalc 2012 statistical software was used to perform the statistical analysis.

**Results**

The results of the modified Mankin score in the four cartilage sets are presented in Table I.

<table>
<thead>
<tr>
<th>Set</th>
<th>Structure average±standard deviation/median(25-75percentiles)</th>
<th>Cells</th>
<th>SO discolouration</th>
<th>Total Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>3.5(3.5-4)</td>
<td>3(3-3)</td>
<td>2.5±0.75</td>
<td>8.95±1.34</td>
</tr>
<tr>
<td>Control</td>
<td>3.75±0.86</td>
<td>3(3-3)</td>
<td>2.95±0.69</td>
<td>9.5±1.53</td>
</tr>
<tr>
<td>CMF</td>
<td>3.7±0.95</td>
<td>2.7±0.48</td>
<td>2.65±0.63</td>
<td>9.05±1.55</td>
</tr>
<tr>
<td>PEMF</td>
<td>3.75±0.89</td>
<td>2.5(2-3)</td>
<td>2.45±0.76</td>
<td>8.7±1.62</td>
</tr>
</tbody>
</table>

Table I Results of the modified Mankin score and its subdivisions in the 4 cartilage sets

Legend: CMF=continuous electromagnetic field, PEMF=pulsed electromagnetic field, SO=safranin orange
As shown in Figure 1, the modified Mankin score in the control group was aggravated compared to the initial set (9.5 vs 8.95). In the CMF subset, the score was 9.05, lower than in the control subset. The PEMF treated cartilage had a modified Mankin score that was even lower than that of the initial subset (8.7). Nevertheless, the differences found were not statistically significant, the lowest p value (0.27) was found for the comparison between the control and the PEMF subset.

Fig. 1 Evolution of the modified Mankin score (0-13) in the four sets averages and 95% confidence intervals
Legend: CMF=continuous electromagnetic field, PEMF=pulsed electromagnetic field

As shown in figure 2, the safranin orange subscore in the control group was aggravated compared to the initial set (2.95 vs 2.5). The safranin orange score in the CEMF set was was lower than in the control set (2.65). In the PEMF set, it was even lower than in the initial set (2.45). The differences were not statistically significant; the lowest p values were associated with the comparison between the control set and the PEMF set (p=0.14) and with the comparison between the initial and the control sets (p=0.17).

Fig. 2 Evolution of the Safranin orange subscore (0-4) in the 4 sets, Averages and 95% confidence intervals.
Legend: CMF=continuous electromagnetic field, PEMF=pulsed electromagnetic field

The decreasing trend of the intensity of the safranin orange staining between the cartilage fragments in the initial and in the control group is shown in pictures 3a and 3b. Subsequently, the increasing trend in the safranin orange staining of the pericellular matrix intensity in the CMF and PEMF sets is shown in pictures 3c and 3d.

Fig. 3a – initial set
Fig. 3b – control set
Fig. 3. Safranin orange staining, 200x magnification of 4 fragments taken from the same cartilage: 3a -initial set, 3b – control group, incubated for 4 days in antibiotic enriched DMEM media, 3c – CMF set, incubated for 4 days in antibiotic enriched DMEM media and treated with CMF for 3 hours a day, 3d – PEMF set, and treated with PEMF for 3 hours a day, for 4 days

Discussion

An initial abnormality in the natural history of arthritis is the advent of small fisures in the surface of the extracellular matrix that subsequently expand to the transitional zone and to the radial zone (Vincent et al., 2010). The injury of the superficial zone of the matrix leads to exposure of the cytokines synthetized by the cartilage cells (especially 1 and 6 interleukines) and of the molecules resulting from the extracellular matrix degradation leading to an inflammatory and immunologic response from the immune system of the synovia (Ungur et al., 2008). This mechanism explains the pain in the initial stages of the illness that affects the cartilage, an avascular and aneural tissue.

In order to repair the defects in the extracellular matrix, the chondrocytes (the only types of cells in the cartilage) respond by accelerated cell division but later on their number decreases, due to apoptosis, an important phenomenon in the cartilage damaging, present from the initial stages of the illness (Thomson et al., 2011, Kim et al., 2003).

In the moderate stages of the illness, extracellular matrix fisures expand to the calcified region of the cartilage and to the subchondral bone (Buckwalter et al., 2005), resulting in local hyperemia and advent of bone edema that can be appreciated by IRM (Yusuf et al., 2011) and in osteoblast activation, with marginal osteophytes and subchondral sclerosis.

The ten cartilages used in the current study were taken from patients that had had a hip arthroplasty. Although the fragments were prelevated from the region with the best microscopic appearance, most of them had lesions in the radial zone and all of them had low cellularity. Most of the samples showed moderate or severe reduction in the safranin orange staining intensity resulting in an average modified Mankin score of 8.95. In the control set, a trend for aggravation in the modified Mankin score was found on the account of the reduction of the safranin orange staining intensity. The reduction in the safranin orange staining in the control set could be the result of diffusion of proteoglycans in the culture media.

In the PEMF subset of cartilages there was an improvement tendency (p=0.14) in the modified Mankin score on the account of the improvement in the safranin orange staining subscore. The increase in intensity of the pericellular and territorial matrix staining was seen in the majority of the cartilage fragments in this group. This could be a consequence of a more intense proteoglycan synthesis by the chondrocytes exposed to PEMF.
The cellularity subscore of the modified Mankin score did not significantly change in the four sets of cartilage pieces. In our experience, replication of cartilage cells isolated from the extracellular matrix begins after the fourth incubation day.

The reduction in the modified Mankin score after low frequency PEMF (1.5 Hz, EBI protocol) was described in guinea pigs with age induced osteoarthritis after 6 months of treatment (Ciombor et al., 2003). The same frequency was proven to have analgetic effects in early and moderate knee arthritis (Kellgren score 1 and 2) patients after 10 days of treatment (Moldovan et al., 2012).

A recent analysis of all the randomized placebo-controlled studies on the effects of the PEMF showed a significant improvement in the algofunctional parameters after 4 weeks of treatment (Ryang et al., 2012).

Two possible action mechanisms were proposed to explain the beneficial effects of PEMF on articular cartilage. PEMF stimulates the synthesis of the Ca^{2+}-calmodulin complex, leading to the increased release of nitric oxide and decreased synthesis of interleukin Ibeta (Rohde et al., 2012), that exerts a catabolic effect on the articular cartilage. On the other hand, PEMF therapy stimulates the synthesis of tumor growth factor β (Ciombor et al., 2003, Benazzo et al., 2008), which has an anabolic effect on the joint cartilage.

In the final stages of the osteoarthritis condition, due to the severe alterations in the articular biomechanics, the injuries expand to the ligaments, tendons and muscles surrounding the articulation (Buckwalter et al., 2005). Chronic neuropathic pain in these stages is attributed to the sensitivisation of the afferent spinal ganglion of the affected joint. The recently discovered pain mediators at this level are nerve growth factor (NGF) and valinoid receptor 1 (TPRV 1) (Sofat et al., 2011). Neuropathic pain in osteoarthritis involves also changes of the central nervous system that can be assessed by the functional MRI (Baliki et al., 2008).

The complexity of these changes, initiated in the joint cartilage and ended in the central nervous system explain the difficulties encountered in the treatment of osteoarthritis.

**Conclusions**

The exposure of human explants to low intensity (0.1mT) continuous magnetic field and to low frequency (1.5 Hz) medium intensity (30mT) pulsed electromagnetic field for 3 hours a day for 4 consecutive days was not associated with significant changes in the modified Mankin score.

There was, however, a tendency of improvement of the score in the PEMF treated pieces of cartilage compared to control, on the account of the increase in the safranin orange staining intensity in the pericellular and territorial matrix of the joint cartilage (p=0.14).

Additional similar studies, conducted on a longer period of time are necessary to clarify the structural effects of PEMF and CEMF on articular cartilages.

**References**


Ciombor D.M., Aaron R.K., Wang S., Simon B. Modification of osteoarthritis by pulsed