

RESEARCH ARTICLE

Effect of Pulsed Electromagnetic Field on MMP-9 and TIMP-1 Levels in Chondrosarcoma Cells Stimulated with IL-1 β

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Abstract

Chondrosarcoma, the second most common type of bone malignancy, is characterized by distant metastasis and local invasion. Previous studies have shown that treatment by pulsed electromagnetic field (PEMF) has beneficial effects on various cancer cells. In this study, we investigated the effects of PEMF applied for 3 and 7 days on the matrix metalloproteinase (MMP) levels in chondrosarcoma SW1353 cells stimulated with two different doses of IL-1 β . SW1353 cells were treated with (0.5 and 5 ng/ml) IL-1 β and PEMF exposure was applied either 3 or 7 days. MMP-9 and TIMP-1 levels were measured in conditioned media by enzyme-linked immunosorbent assay. The results were relative to protein levels. Statistical analyses were performed using one-way analysis of variance (ANOVA). $P < 0.05$ was considered significant. PEMF treatment significantly decreased MMP-9 protein levels in human chondrosarcoma cells stimulated with 0.5 ng/ml IL-1 β at day 7, whereas it did not show any effect on cells stimulated with 5 ng/ml IL-1 β . There was no significant change in TIMP-1 protein levels either by IL-1 β stimulation or by PEMF treatment. The results of this study showed that PEMF treatment suppressed IL-1 β -mediated upregulation of MMP-9 protein levels in a dual effect manner. This finding may offer new perspectives in the therapy of bone cancer.

Keywords: Pulsed electromagnetic field - bone cancer - chondrocytes - matrix metalloproteinase

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Introduction

Chondrosarcoma is one of the tumours of bone and soft tissue known as sarcomas and it is composed of cells derived from transformed cells that produce cartilage. It has a poor response to current therapeutic approaches such as chemotherapeutic drugs and radiotherapy (Tang and Tsai 2012). Therefore in the field of oncology new innocuous approaches are needed such as pulsed electromagnetic field (PEMF) exposure which may decrease the chondrosarcoma cells' evolution and metastasize ability.

Cancer cell invasion and metastasis processes involve degradation of extracellular matrix (ECM) and the breakdown of ECM by proteinases (Roomi et al., 2013a; Shang et al., 2014). One family of proteases which take a role in tumour invasion and metastasis is matrix metalloproteinases (MMPs) (Wang et al., 2014). Matrix metalloproteinases (MMPs) are a multigenomic endopeptidase family composed of collagenases (MMP-1, -8, -13), gelatinases (MMP-2, -9), stromelysins (MMP-3, -7, -10, -11) and membrane type MMP (MMP-1, -2, -3, -4) (Daniel et al., 2001). Proteolytic activities of MMPs are inhibited by tissue inhibitors of matrix metalloproteinases (TIMPs). It is known that the balance between MMP and TIMP levels is a critical determinant of proteolytic

degradation. Numerous clinical and experimental studies have demonstrated that elevated levels of MMPs are associated with tumour growth, cancer progression and metastasis (Nelson et al., 2000; Benassi et al., 2001; Wang et al., 2014; Yadav et al., 2014; Zhu et al., 2014). Also MMPs play important roles in pathological diseases such as arthritis and vascular diseases (Zitka et al., 2010). In cancer the production of MMP is stimulated by inflammatory mediators and the activity of MMP under different stimulators has been reported (Pei et al., 2006; Lim et al., 2011; Lee et al., 2012).

In recent years the beneficial effects of PEMF on various cancer cells has been demonstrated in several studies (Kaszuba-Zwoinska et al., 2010; Vincenzi et al., 2012; Crocetti et al., 2013). On the other hand, there also has been found by several authors that PEMF has no significant effects on cancer cells (Zhao et al., 2008; Zhang et al., 2011). Despite recent successes of PEMF the results are still not conclusive and even conflicting.

It is known that biophysical forces like PEMF lead to cellular induction and modify some physiological parameters such as proliferation (Chang et al., 2010) and the ECM components' synthesis (De Mattei et al., 2001; Ciombor et al., 2002). Furthermore, various studies reported the effect of PEMF on MMP activity in osteosarcoma cells (Zhang et al., 2011), and THP-1 cells

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(Patruno et al., 2012). To our knowledge, there are no reports in the literature on the effect of PEMF on MMP-9 and TIMP-1 levels for chondrosarcoma cell lines.

In this study, we investigated the effects of PEMF applied for 3 or 7 days on the MMP-9 and tissue inhibitor of metalloproteinases-1 (TIMP-1) production in chondrosarcoma cells stimulated with low and high doses of IL-1 β .

Materials and Methods

Cell culture

The SW1353 human chondrosarcoma cell line was grown in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Carlsbad, CA) supplemented with %10 fetal bovine serum (FBS, Biological Industries, USA), 2 mM L-Glutamine (Biological Industries, USA) and %0.1 Penicillin Streptomycin (Biological Industries, USA) and incubated with %5 CO₂ at 37°C. Chondrocytes were seeded at 2 x 10⁵ cells/well in two 24-well tissue culture plates (Costar, Cambridge, MA, USA) named PEMF group and Control group. Recombinant human IL-1 β (Millipore, Billerica, MA) was used in two different doses (0,5 ng/ml and 5 ng/ml) to stimulate SW1353 cells. The negative control wells were neither stimulated with IL-1 β nor treated with PEMF.

PEMF exposure

Following 24 hours culturing period, PEMF group was exposed to PEMF for 30 minutes in north-south direction each day, for either 3 or 7 days. During this period the control plate was maintained at identical conditions except PEMF exposure. At the end of the culture period culture media in wells were harvested and stored at -20°C for enzyme-linked immunosorbent assays (ELISA).

PEMF exposure conditions

A pair of Helmholtz coil placed opposite to each other was used to generate PEMF and powered by a pulse generator (Igea, Carpi, Italy). The pulse duration of the signal was 1.3 ms and the frequency 75 Hz, yielding duty of cycle of 1/10. The intensity of the magnetic field was 2.3 mT and the induced electrical field was 2 mV. The maximum electric field was estimated to be 0.02 mV/cm. Intensities of magnetic fields were measured by using a digital Gauss/Teslameter (Model 7030, F.W. BELL, Syprus, Orlanda, USA).

Analysis of MMP-9 and TIMP-1 production in IL-1 β -stimulated SW1353 Cells

MMP-9 and TIMP-1 levels in culture media were measured by using highly sensitive enzyme-linked immunosorbent assay kits (for MMP-9 detection, RayBiotech Inc. Norcross, GA, USA. inter-assay: CV<10%, intra-assay: CV<12%, sensitivity: 10 pg/ml; for TIMP-1 detection Bender MedSystems GmbH, Vienna Austria, inter-assay: CV<3,9%, intra-assay: CV<4,9%, sensitivity: 10 pg/ml) according to manufacturer's instructions. MMP-9 and TIMP-1 concentrations in media were normalized to the total protein content and expressed as pg/mg protein.

Statistical analysis

Data were expressed as Means \pm SEM. Statistical analyses were performed using one-way analysis of variance (ANOVA) with post hoc Tukey tests, cross-comparing all study groups. $p<0.05$ was considered significant.

Results

In order to investigate the effect of PEMF on MMP-9 and TIMP-1 protein levels produced by human chondrocytes, medium was harvested and subjected to ELISA. IL-1 β caused a significant increase in MMP-9 levels starting from day 3. This increase was further emphasised at day 7 (Figure 1). At day 3, PEMF caused a decrease in MMP-9 levels in cultures treated with low dose of IL-1 β but not with the higher dose of 5ng/ml. No statistically significant change was observed for MMP-9 levels in both IL-1 β treated and control groups at day 3 following PEMF application. At day 7, MMP-9 levels were significantly up-regulated both for low and high doses of IL-1 β ($p<0,01$). A significant decrease was observed with PEMF treatment in low dose IL-1 β group (0,5 ng/ml; $p<0,01$). On the other hand when higher dose of IL-1 β was given (5ng/ml) PEMF caused an increase

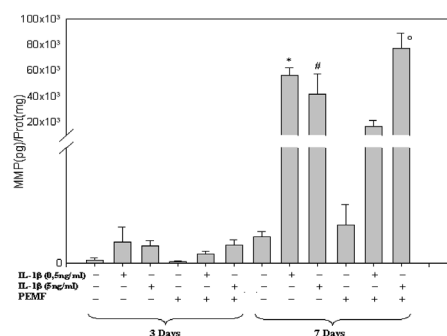


Figure 1. Effects of PEMF on MMP-9 Protein Levels Produced by Human Chondrosarcoma Cells Treated for 3 and 7 days. SW1353 cells were also treated with (0,5 and 5 ng/ml) IL-1 β . All data were expressed as mean \pm SEM of four independent experiments. *Statistically significant vs IL-1 β (0,5 ng/ml) treatment for 3 days; #statistically significant vs IL-1 β (5 ng/ml) treatment for 3 days; °statistically significant vs IL-1 β (5 ng/ml) treatment for 7 days ($p<0.01$)

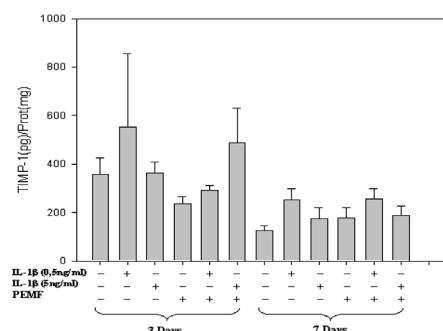


Figure 2. Effects of PEMF on TIMP-1 Protein Levels Produced by Human Chondrosarcoma Cells Treated for 3 and 7 Days. SW1353 cells were also treated with (0,5 and 5 ng/ml) IL-1 β . All data were expressed as mean \pm SEM

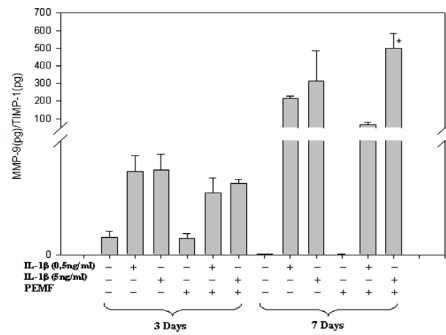


Figure 3. Effects of PEMF on MMP-9/TIMP-1 Rate for 3 and 7 Days. SW1353 cells were treated with (0.5 and 5 ng/ml) IL-1 β and PEMF exposure. All data were expressed as mean \pm SEM of four independent experiments. *Statistically significant vs control with PEMF treatment ($p < 0.01$)

in MMP-9 levels rather than an inhibition (Figure 1). Interestingly, there was also a slight increase in MMP-9 levels in control group with PEMF treatment. The effect of PEMF on MMP-9 production seemed to be dependent on not only the IL-1 β concentration applied but also the duration of the proinflammatory stimulus (Figure 1).

Interestingly, there was no significant change in TIMP-1 levels either with IL-1 β stimulation or by PEMF treatment (Figure 2). The slight increase in TIMP-1 levels with low dose IL-1 β was not consistent and at day 7, although not statistically significant, the inhibitory effect of IL-1 β (both low and high doses) became more prominent. PEMF treatment caused a slight increase in TIMP-1 protein level in cells treated with low dose IL-1 β (0.5 ng/ml), however this effect was reversed at longer culture period (Figure 2). There was no significant difference observed between any treatment groups.

MMP-9/TIMP-1 ratio also gave important acquisitions about the effects of PEMF on cells treated with IL-1 β . In cell cultures treated with IL-1 β MMP-9/TIMP-1 ratio slightly decreased following PEMF exposure compared to non-PEMF group during third day (Figure 3). In IL-1 β treated groups (both at low and high doses) due to the high levels of MMP-9, there was a significant increase in MMP-9/TIMP-1 ratio, at day 7. At this time point of cultures, MMP-9/TIMP-1 ratio decreased in cells treated with 0.5 ng/ml IL-1 β following PEMF exposure compared to the non-PEMF group (Figure 3). On the other hand when a higher dose of IL-1 β was given (5 ng/ml) an increase was observed in MMP-9/TIMP-1 ratio in PEMF treatment group compared to non-PEMF controls (Figure 3) due to the increased levels of MMP-9 in IL-1 β and PEMF treated group (Figure 2).

Discussion

In this study, we investigated for the first time, the effects of PEMF on the MMP-9 and TIMP-1 production in chondrosarcoma cells stimulated with low and high doses of IL-1 β . The principle finding of this study is that PEMF treatment led to a dual effect on MMP-9 production stimulated with low and high doses of IL-1 β at different time points.

Matrix metalloproteinases, especially MMP-2 and MMP-9 can degrade type IV collagen, a major component

of extracellular matrix and thus helps tumour invasion and metastasis. It has been reported that increased levels of MMPs and low levels of TIMPs are associated with the aggressive behaviour of sarcomas (Benassi et al., 2001; Roebuck et al., 2005). Therefore to investigate the alteration of MMP levels for cancer tumour cells under different conditions has a great interest. Development of new therapies capable of reducing or inhibiting the activities of MMPs could potentially lead to an inhibition in tumour invasion and metastasis.

The effects of mechanical stimulations on MMP-9 gene expression and protein levels have been already investigated by the application of ultrasound on prostate cancer cells (Wei et al., 2014) and shear stress on breast cancer cells (Zhao et al., 2014). To our knowledge, this is the first study showing the effects of PEMF treatment on MMP-9 activity in SW1353 human chondrosarcoma cell line.

In recent years low frequency PEMF has been shown to have anabolic effects on chondrocytes with respect to cell differentiation (Ciombor et al., 2002), cell proliferation (De Mattei et al., 2001) and matrix synthesis (De Mattei et al., 2003). Furthermore the effects of PEMF on MMP activities were investigated for many different cells and organ systems. Patruno, et al (2011) reported that electromagnetic field exposure on THP-1 cancer cells caused a weak increase in MMP-2 and -9 activities. We investigated the effects of PEMF in MMP-9 production in human chondrosarcoma cells. Our results indicated a significant inhibitory effect of PEMF exposure on MMP-9 levels for chondrosarcoma cells stimulated with low dose IL-1 β . However Zhang et al. (2011) indicated that PEMF exposure had no effect on MMP-9 activity of osteosarcoma cells. One reason for these different results may be the different magnetic field intensities, duration and amplitude values and different types of cells exposed to PEMF in these studies. To our knowledge this study is the first investigation for the effects of PEMF on MMP-9 production in chondrosarcoma cells.

It has been shown that cytokines such that IL-1 β not only down-regulate the synthesis of major ECM components by inhibiting anabolic activities of chondrocytes (Shi et al., 2004) but also stimulates chondrocytes to release several proteolytic enzymes, among which are matrix metalloproteinases: MMP-1 (Gebauer et al., 2005), MMP-3 (Baker et al., 2012), MMP-9 (Lu et al., 2011), MMP-13 (Klatt et al., 2006). In this study stimulation with IL-1 β was performed to increase the level of MMP-9 since IL-1 β is considered as a key catabolic stimulant for the production of MMP. The effects of IL-1 β with different doses have been described in the scientific literature for cartilage and chondrocytes, respectively. Lu, et al (2011) and Roomi, et al (2013b) reported that the expression of MMP-9 increases with respect to the increasing dose of IL-1 β . Ongaro, et al (2011b) also reported that IL-1 β induced a dose-response reduction on proteoglycan synthesis after 7 days treatment. In our study we showed a dose-dependent change in MMP-9 levels when stimulated by IL-1 β . Our findings are in agreement with the studies which show an increase in MMP-9 and MMP-13 levels when stimulated with 5 ng/

ml IL-1 β (Akhtar et al., 2011) and with 0,5 ng/ml IL-1 β (Pei et al., 2006).

It was reported that PEMF in vitro was able to counterbalance efficiently the cartilage degradation induced by the catabolic cytokine IL-1 β (Fioravanti et al., 2002). In our study we observed a significant decrease in MMP-9 levels stimulated by low dose IL-1 β when exposed to PEMF for 7 days. On the other hand when a higher dose of IL-1 β was given (5ng/ml) rather than an inhibition an increase was observed in MMP-9 levels compared to non-PEMF group suggesting a dual effect by PEMF on chondrocyte IL-1 β -stimulated MMP-9 production. Not only the importance of physical properties of PEMF and the exposure time, but the dose of IL-1 β seem to play an important role on MMP-9 production.

MMP-9 and TIMP-1 protein levels have been investigated initially as a function of the duration. Our data showed no statistically significant difference for MMP-9 levels in both PEMF and control groups at day 3. In our study, greater effects of PEMF associated with longer exposure duration. PEMF caused a slight increase in MMP-9 levels while inhibiting TIMP-1 production in a more prominent way at day 7 (See figures). Our results confirm previous observations showing that alterations in measured parameters are significant for PEMF exposure for 7 day (Ciombor et al., 2002; Bobacz et al., 2006). Sadoghi, et al (2013) also reported the limitations of 4 days PEMF exposure on human osteoarthritic chondrocytes.

Previous studies have shown that mechanical stimulation such as dynamic compression and hydrostatic pressure have no effect on TIMP-1 levels of human chondrocytes (Trindade et al., 2004; Bevill et al., 2009). It was also reported that electromagnetic field exposure increased the TIMP-1 protein level in rat cerebral cortex (Qui et al., 2011). To our knowledge this is the first study that analysed the effect of PEMF on TIMP-1 protein levels of human chondrosarcoma cells stimulated with two different doses of IL-1 β . It was previously reported that the levels of TIMP-1 in human chondrocytes increased when stimulated with 5ng/ml IL-1 β (Dunn et al., 2013). In our study however, there was a general decrease in TIMP-1 levels at day 7, we did not detect any significant effect of PEMF on TIMP-1 production in IL-1 β -stimulated SW1353 cells at either 3 or 7 days.

We acknowledge several limitations in this study. The experiment was performed using a human chondrosarcoma cell line (SW1353), whereas the use of a primary human chondrocyte cell culture might better replicate what happens in humans in vivo. We chose the SW1353 cell line since these cells show a phenotype similar to that of normal human chondrocytes. They provide a reproducible experimental model to study the role of MMP in cartilage destruction and are suitable for the microarray analyses (Vinceti and Brinckerhoff, 2001).

In conclusion, our study confirmed, for the first time, the effect of PEMF on MMP-9 and TIMP-1 levels in human chondrosarcoma cells. Further studies are planned to be performed to verify the effects and to enlighten the mechanism of PEMF effects. The PEMF stimulation might be a promising therapeutic approach for cancer because of its action on chondrocyte metabolism.

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