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Original Research

Pulsed electromagnetic field ameliorates cartilage degeneration by inhibiting mitogen-activated protein kinases in a rat model of osteoarthritis

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ABSTRACT

Objectives: We assessed the effects of pulsed electromagnetic field (PEMF) on cartilage degeneration, and expression of mitogen-activated protein kinases (MAPKs) and matrix metalloproteinases (MMPs), in an experimental rat model of osteoarthritis induced by anterior cruciate ligament transection (ACLT).
Design: Experimental.
Setting: University animal laboratory.
Participants: 30 male Sprague–Dawley rats.
Main Outcome Measures: We performed histological examination, enzyme-linked immunosorbent assay, quantitative real-time polymerase chain reaction, to assess cartilage degeneration, urine *C*-terminal cross-linking telopeptide of type II collagen (CTX-II), and mRNA expression of extracellular signal-regulated kinase (ERK), c-Jun *N*-terminal kinase (c-Jun), p38, and MMPs.
Results: Urinary CTX-II in the PEMF group was significantly lower than in the ACLT group at 9 and 13 weeks. Mankin scores in the PEMF group significantly lower than that in the ACLT group ($P < 0.01$). mRNA expression of ERK1, c-Jun, p38, MMP-13 and MMP-3 was significantly higher in the ACLT group than in the Sham group, while that with the sole exception of MMP-3 in the PEMF group was significantly lower than in the ACLT group.
Conclusions: PEMF may regulate the catabolic factor, MMP13, and inhibit cartilage destruction, at least partially, by inhibiting MAPKs signaling pathway.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease, characterized by progressive loss of articular cartilage, synovial inflammation, subchondral bone lesions, and osteophyte formation (A. Adatia, Rainsford, & Kean, 2012; P. Suri, Morgenroth, & Hunter, 2012; P. M. van der Kraan, 2012). Osteoarthritis is a leading cause of disability in the elderly (David T Felson, 2006) and is a major public health problem. The aging of the Chinese population will significantly increase the occurrence of osteoarthritis-related disability and socio-economic burden. Though there is a need in the literature for high-quality studies to support an increased relationship between sports and osteoarthritis (S. A. Richmond et al., 2013; G. Tran et al., 2016). Exposure to team sports, including soccer was shown to be a risk factor for osteoarthritis (J. A. Kettunen, Kujala, Kaprio, Koskenvuo, & Sarna, 2001; K. B. Klunder, Rud, & Hansen, 1980). High exposure to sport activity increases the odds of hip osteoarthritis compared to low exposure (E. Vingard, Alfredsson, Goldie, & Hogstedt, 1993). Exposure to gymnastics was found to be an increased risk of knee osteoarthritis in Hong Kong Chinese (E. C. Lau et al., 2000). Anterior cruciate ligament transection induced knee joint damage resembles cartilage degeneration observed in post-traumatic osteoarthritis (S. Kamekura et al., 2005). Treatment options for osteoarthritis include analgesics and anti-inflammatory drugs, glucosamine, physical exercises, and, surgical intervention as a last resort (Kim L Bennell, Hunter, & Hinman, 2012). Noninvasive therapeutic modalities such as physiotherapy including pulsed

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electromagnetic field (PEMF) have shown positive effects on osteoarthritis. Although a systematic review suggested that pulsed electromagnetic field treatment offers no clinical benefit in reducing the pain of knee osteoarthritis (C. J. McCarthy, Callaghan, & Oldham, 2006), there is indeed evidence that pulsed electromagnetic field provides meaningful benefits in these patients with respect to pain, stiffness, and disability (G. L. Bagnato, Miceli, Marino, Sciortino, & Bagnato, 2016; A. Gobbi, Lad, Petrera, & Karnatzikos, 2014; T. Iannitti, Fistetto, Esposito, Rottigni, & Palmieri, 2013; F. R. Nelson, Zvirbulis, & Pilla, 2013; P. Nicolakis et al., 2002; S. T. Sutbeyaz, Sezer, & Koseoglu, 2006; G. Thamsborg et al., 2005; H. Wuschech, von Hehn, Mikus, & Funk, 2015). In a clinical study, PEMF therapy reduced the total Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) global scores in osteoarthritis patients (H. Wuschech, von Hehn, Mikus & Funk, 2015). Another study documented significant improvement in symptoms, knee function, and activity at 1-year follow-up. Although the results appeared to have deteriorated at 2 years, outcomes were still superior to pretreatment levels (A. Gobbi et al., 2014). *In vivo* studies have shown that pulsed electromagnetic field preserves the morphology of articular cartilage and slows the progression of osteoarthritis lesions in the knee of aged osteoarthritic guinea pigs (M. Fini et al., 2005), protects cartilage by inhibiting serum tumor necrosis factor-α(TNF-α) levels in anterior cruciate ligament transection (ACLT) rabbits (H. Guo et al., 2011), prevents ovariectomy-induced cartilage degeneration through up-regulation of X-linked inhibitor of apoptosis protein mRNA expression and down-regulation of Bax mRNA expression in rats (S. Li et al., 2011), and inhibit chondrocyte apoptosis and downregulate metalloproteinase-13 expression of knee joint cartilage in ovariectomized rats (Q. Luo et al., 2009). Our previous study also demonstrated the therapeutic effect of pulsed electromagnetic field on experimental osteoarthritis mediated via inhibition of apoptosis in chondrocytes (W. Xie, Zhou, Luo, Liu, & He, 2014). In an *in vitro* study, PEMF promoted chondrogenic differentiation of rat bone marrow-derived mesenchymal stem cells (rBMSCs) (F. Qiu et al., 2012). However, the underlying mechanisms by which PEMF inhibits cartilage degeneration are not fully understood.

Mitogen-activated protein kinases (MAPKs) play an important role in regulating cell growth, proliferation, differentiation, and apoptosis (K. K. Brown et al., 2008; Surena Namdari, Wei, Moore, & Chen, 2008, Indira Prasadam et al., 2010). MAPKs such as, extracellular signal-regulated kinase (ERK), c-Jun *N*-terminal kinase (c-Jun), and p38, play important roles in cartilage degenerative processes. The involvement of mitogen-activated protein kinases in the chondrogenic differentiation and cartilage formation and maturation has been demonstrated (Brent E Bobick & Kulyk, 2008). Mitogen-activated protein kinases have been demonstrated to be involved in murine collagen-induced arthritis (M. A. Rosillo et al., 2015). Moreover, inhibitors of mitogen-activated protein kinases have been shown to protect against articular cartilage erosion (Christelle Boileau et al., 2006, I. A. Schepetkin et al., 2015; Z-H Wen et al., 2010). Matrix metalloproteinases (MMPs) include a family of zinc-dependent enzymes that degrade extracellular matrix components. Cartilage degradation is associated with elevated expression of MMPs (A. Knapinska & Fields, 2012). In addition, mitogen-activated protein kinases signaling plays an important role in the matrix metalloproteinases-derived catabolic response of chondrocytes (Mohammed El Mabrouk, Sylvester & Zafarullah, 2007, Abdelhamid Liacini et al., 2003, B-C Sondergaard et al., 2010; SW Yoon, Chun, Sung, Kim, & Poo, 2008). The objective of the present study was to investigate the effects of pulsed electromagnetic field on urinary *C*-terminal cross-linking telopeptide of type II collagen (CTX-II), and cartilage morphology in ACLT-induced osteoarthritis rats. Further, we examined the effects of pulsed electromagnetic field on the expression of mitogen-activated protein kinases and metalloproteinases.

2. Methods

2.1. Study design and surgical technique for induction of osteoarthritis

All surgical and therapeutic procedures were approved by the ethics committee at the First Affiliated Hospital of University of South China and performed in accordance with national guidelines (The Ministry of Science and Technology of the People's Republic of China. Guidance Suggestions for the Care and Use of Laboratory Animals. 2006-09-30) equivalent to those of the National Institutes of Health “Guide for the Care and Use of Laboratory Animals”. Three-month old male Sprague–Dawley rats (N = 30) were obtained from the experimental animal center at the University of South China. All rats were housed in cages at a relative humidity of 55 ± 5% and a room temperature 24 ± 2 °C under a 12/12 h light/dark cycle. Access to water and food was unrestricted. The rats were randomly assigned to the following groups (N = 10): sham operation plus placebo pulsed electromagnetic field (Sham group); anterior cruciate ligament transection (ACLT) plus placebo pulsed electromagnetic field (ACLT group); and ACLT plus pulsed electromagnetic field treatment (PEMF group). ACLT procedures were performed as described in our previous study (Y. Liao, Li, Li, & Zhou, 2016). Briefly, rats were anesthetized with an intraperitoneal injection (300 mg/kg) of chloral hydrate. The right knees were shaved and disinfected with povidone iodine. A medial parapatellar incision in the skin followed by medial arthrotomy was performed. Once the anterior cruciate ligament was visible, it was cut through the mid-substance. For the sham operation, the same incision was performed in the right knee joint capsule without transection of the anterior cruciate ligament. All procedures were performed under sterile conditions. Post-operatively, all rats were allowed unrestricted activity in cage.

2.2. Pulsed electromagnetic field treatment

One week after ACLT, rats in the PEMF group received pulsed electromagnetic field exposure as described in our previous study (W. Xie et al., 2014). PEMF was generated by the pulsed electromagnetic field stimulation apparatus (Hunan Forever Elegance Technology Co., Ltd., China). PEMF parameters were: 8 mT magnetic field intensity and 20 Hz frequency for 40 min/day, five days/week, for 12 weeks. Rats in the Sham and ACLT groups were exposed to placebo PEMF (identical instrument in switched off mode).

2.3. Urinary C-terminal cross-linking telopeptide of type II collagen

On the first day of week 0, 1, 5, 9, and 13 after operation, animals were housed in metabolic cages; 24-h urine samples were centrifuged at 2000 g for 20 min and stored at −20 °C. Urinary *C*-terminal cross-linking telopeptide of type II collagen concentrations were detected using a commercial enzyme-linked immunosorbent assay (ELISA) system, according to the manufacturer's protocol (Beijing Bioss Biological Technology Co., Ltd., China).

2.4. Histopathology

After 12 weeks of pulsed electromagnetic field treatment, the right knee joints were dissected 0.5 cm above and below the joint line. The cartilage in the tibial plateau was fixed by immersion in buffered formalin for 72 h, then decalcified in 10% ethylene

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