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ARAŞTIRMA / RESEARCH

Pulsed magnetic field action on antioxidant system in λ-carrageenan induced acute paw edema

Pulslu manyetik alanın λ-carrageenan'ın indüklediği akut pençe ödeminde antioksidan sistem üzerine etkisi

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Abstract

Purpose: λ-Carrageenan-induced (λ-Carr) paw edema is a commonly used test for determining the acute phase of inflammation. In the present study, our aim was to investigate whether efficacy of pulsed magnetic field (PMF) on antioxidant enzymes levels is λ-Carr-induced rat paw edema.
Material and Methods: The rats were whole-body exposed to PMF (1.5 mT intensity and 1, 10, 20, 40 Hz consecutive frequencies between Helmholtz coils) at the same hour in an hour in a day throughout 4 days at 3 hour later from injection of λ-Carr. Paw edema was determined at the end of fourth day by killing the rats, removed the paws at the ankle joint and paw mass (g) and paw thickness (mm) were was determined the right and left paw of rats. Then, the paw tissues were collected for the measurement of lipid peroxidation (Malondialdehyde; MDA) and antioxidant enzymatic activity levels (Catalase; CAT).
Results: Carrageenan-induced right paw masses and paw thicknesses increased in compare to left paw of rats. While CAT levels were significantly decreased, MDA levels were increased in the carrageenan-induced rat right hind paw compare to control rat left paw.After exposed PMF to λ-Carr-induced right paw, while the CAT enzyme level decreased, the MDA enzyme level increased and there was no significant change in the weight and thickness of the right paw compare to unexposed groups.
Conclusion: PMF increased edema in the rat paw and negatively affected antioxidant enzyme levels.
Key words: Pulsed magnetic field, carreganan, acute inflammation model, antioxidants.

Öz

Amaç: λ-Carrageenan ile indüklenen (λ-Carr) pençe ödemi, inflamasyonun akut evresinin belirlenmesinde yaygın olarak kullanılan bir testtir. Bu çalışmanın amacı, λ-Carr'ın indüklediği sıçan pençe ödeminde pulslu manyetik alanın (PMF) antioksidan enzim düzeylerine etkinliğinin olup olmadığını araştırmaktı.
Gereç ve Yöntem: Sıçanlar, λ-Carr injeksiyonundan 3 saat sonra 4 gün boyunca her gün günde 1 saat 1.5 mT şiddetinde ardışık 1, 10, 20, 40 Hz frekansları veren Helmholtz bobinlerinden oluşan pulslu manyetik alan etkisine maruz bırakıldılar. Dördüncü gün sıçanlar dekapite edildi ve pençelerindeki ödem değerlendirildi; Önce ayak bileği kesilerek pençe çıkarıldı, sağ ve sol pençe kütleleri (g) ve pençe kalınlıkları (mm) ölçüldü. Sonra pençelerde bulunan dokular lipit peroksidasyon (katalaz (CAT)) ve antioksidan enzim (malondialdehid (MDA)) aktivitelerini belirlemek için ayrıldı.
Bulgular: Çalışmamızda sıçanların sol pençeleri , λ-Carr ile indüklenen sağ pençeler ile karşılaştırıldığında λ-Carr ile indüklenen sağ pençelerde kütlelerinin, pençe kalınlıklarının, CAT ve MDA enzim seviyelerinin arttığı görüldü. PMA uygulamasından sonra sağ pençelerin ağırlığı ve kalınlıklarında anlamlı değişiklik olmazken CAT enzimi azaldı, MDA enzim seviyesi ise arttı.
Sonuç: PMF sıçan pençesinde ödemi arttırdı, antioksidan enzim seviyelerini de negatif olarak etkiledi.

Anahtar kelimeler: λ-Carrageenan, akut inflamasyon modeli, pulslu manyetik alan, antioksidanlar.

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INTRODUCTION

Using the pulsed magnetic field in alternative therapy such as soft tissue injuries is also used for the treatment of acute and chronic inflammatory conditions. The Carrageenan (CAR) induced hindpaw inflammation model has been extensively used as an experimental inflammatory pain animal model[1,2].

This model allows for investigate the anti-inflammatory effects of potential therapeutic choices. CAR induced inflammation is related to neutrophil infiltration, the production of free radicals and the release of inflammatory mediators[3,4]. Oxidative stress, therefore, has been suggested as a major contributor to the inflammatory pain process[5]. Previous studies have been reported an increase in the production of reactive oxygen species and a decrease in the antioxidant defense system in CAR induced inflammation[6,7]. The acute phase process of inflammation is associated with the symptoms (pain, heat, swelling and redness) and reactive oxygen species (ROS). ROS play an important role in the pathogenesis of many diseases, such as rheumatoid arthritis, local or systemic inflammatory disorders and neurodegenerative diseases[8-10]. For this reason, living organisms have enzymatic and nonenzymatic antioxidant defense systems. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) are the key antioxidant enzymes and malondialdehyde (MDA) is one of the end products of lipid peroxidation.

Traikov et al. indicated that static magnetic field (SMF) with 25 mT for 30 min diminished the inflammatory process and decreased the levels of inflammatory markers and stress markers into the λ-Carr-induced acute inflammation model[11]. In the other study, a magnetic field of 5 Hz x4 mT, 90 min was found to be optimal in lowering the paw edema volume and decreasing the activity of lysosomal enzymes. Also, soft tissue swelling was shown to be reduced as evidenced by radiology. Histological studies confirmed reduction in inflammatory cells infiltration, hyperplasia, and hypertrophy of cells lining synovial membrane[12-14]. Therefore, the aim of the present study was to investigate whether efficacy of pulsed magnetic field (PMF) on antioxidant enzymes levels of paw tissue in λ-Carr-induced acute inflammation model.

MATERIALS AND METHOD

Animals

A total of 20 female Wistar rats (200-250g) (female rats are less aggressive than adult male rats), were used for this study. All procedures were approved by the Cukurova University Animal Care and Use Committee and followed the guidelines for the treatment of animals of the International Association for the Study of Pain15. The rats were divided into two groups. The first group was evaluated as pulsed magnetic field (PMF) and the second group as control group. Rats were maintained for one week in the laboratory for adaptation. The rats in each group were allowed to roam freely in the cage irrespective of exposure to the PMF. They were fed a standard rat chow and tap water ad libitum. The temperature and humidity are monitored continuously throughout the experimental period that kept in an environmentally controlled room at 21-23°C, relative humidity 40-60%, with a light/dark cycle of 12/12 h.

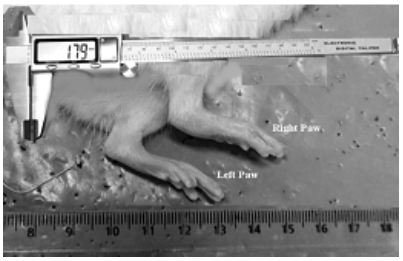

Figure 1. Left and right paw thicknesses of rats in λ-Carr-induced inflammation were measured with digital calliper.

Acute inflammation model

λ-Carr-induced ((lambda carrageenan, Sigma, Germany) hind paw edema model was used for determination of acute inflammation model16-19. λ-Carr intraplantarly injected into the rat hind paws under light anesthesia (sevofluorane (1-2 % in oxygen)), edema was induced by injecting 200 μL of 2% (w/v) λ-Carr suspension in the right hind paw of rats. The left hind paw of each rat served as the control and received an injection of saline (0.9% w/v, NaCl, 200 μl). In all animals injected λ-Carr in the paw we looked for, but did not observe signs of discomfort, reduction in mobility or changed gait during the experimental periods.

Paw edema was determined at the end of fourth day by killing the rats, removed the paws at the ankle joint and paw mass (g) was measured by balance, paw thickness (mm) was determined using calipers in the right and left paw of rats (fig 1.). MDA and CAT biochemical analysis in Paw Edema

Measurement of paw mass, paw thickness and biochemical analysis were done at 78 h after the CAR injection. After the sensory tests were performed, rats were sacrificed by decapitation under light sevofluorane inhalation. Paw edema was determined at the end of experiments by removing the paws at the ankle joint and measuring paw mass (g). Subsequent to this inflamed paw tissue was removed from the plantar surface by sharp dissection and processed for biochemical analysis. CAT, MDA in the rat paw tissues were determined. After washing the tissues with physiological saline (0.9% NaCl), samples were taken and kept at −80 ◦C until analysis. For obtaining tissues supernatants; the tissues were homogenized for 5 min in 50mM ice-cold KH_2PO4 buffer solution (pH 7.0) (1:10, w/v) using a glass-porcelain homogenizer (Ika Ultra- Turrax Homogenizer) and then centrifuged at 10000 rpm for 15 min. All biochemical assays were performed in homogenate. Homogenates were filtered and centrifuged using a refrigerated centrifuge at 4 ◦C. All assays were carried out at room temperature in triplicate. Lipid peroxidation levels in paw edema tissues were determined by estimating MDA using the thiobarbituric acid test. The level of MDA was determined by measuring the colorintensity of the complex formed between MDA and thiobarbituric acid at 532 nm (Thermo Electron Corporation Spectrometer) according to the method of Ohkawa et al.[20] CAT activity was assayed by the method of Aebi[21]. Hydrogen peroxide decomposition by CAT was monitored spectrophotometrically by following the decrease in absorbance at 240 nm.

Pulsed Magnetic Field Exposure System

PMF was treated to rats by using a system with Helmholtz coils 60 cm in diameter, placed 30 cm apart as previously described detailed[22]. These coils in faraday cage connected to a signal generator (ILFA Electronic, Adana, Turkey) produced a magnetic field peak amplitude of 1.5 mT (1.49–1.51 mT, F.W. Bell Model 6010, Sypris, Orlando, FL, USA). The time varying magnetic field consisted of a quasitriangular waveform, with a rise time of 0.5 ms and a fall time of 9.5 ms. The induced electric field was a unipolar rectangular waveform having peak electric fields of 0.6 V/m (0.59–0.61 V/m) between the coils (fig. 2). The waveforms of magnetic field were determined using a search coil probe and there were no clear differences in the shape and magnitude of the waveforms.

Rats were placed in a plexiglass cage (30 cm long, 20 cm wide, and 15 cm high) located between the coils. The distribution of the magnetic density was measured using a gauss meter. The density was homogeneous within 5% in the exposure area..Our PMF (trained; t-PMF) application was presented in consecutive three phases. Each phase included for four extremely low frequencies: 1, 10, 20 and 40 Hz. Pulse width was 500 μs for all frequency. The duration of each frequency train was 4 minutes; a 1 min interval occurred between each frequency train and a digital timing device that controlled the timing of trains. Also, sham exposure (SPMF) to animals was performed under the same environmental conditions, using another apparatus in a Faraday cage outfitted with only the Helmholtz coils.

All t-PMF treatments were carried out at the room temperature (23-25°C) and, any temperature changes were not observed in the cage during the t-PMF applications. t-PMF treatments began the after 3 hours from λ-Carr injection or saline injections and was applied for 1 hour each day and carried out 4 times throughout the experiments at the same time period each day (12:30-13:30). The rats were treated with whole body exposure to t-PMF along the 4 days. Each animal was placed in an all-plastic restrainer located in the homogeneity region of the magnetic field between the coils (fig 2.).

Statistical analysis

Data of enzyme activities and thickness and weights of paw in carrageenan-induced inflammation and control groups were subjected to one-way ANOVA, with and without the exposure of PMF, using SPSS 11.5 software. Differences between means were carried out differences among groups were considered significant at levels of $p<0.05$. All results were expressed as mean ± standard error of the mean (S.E.M.).

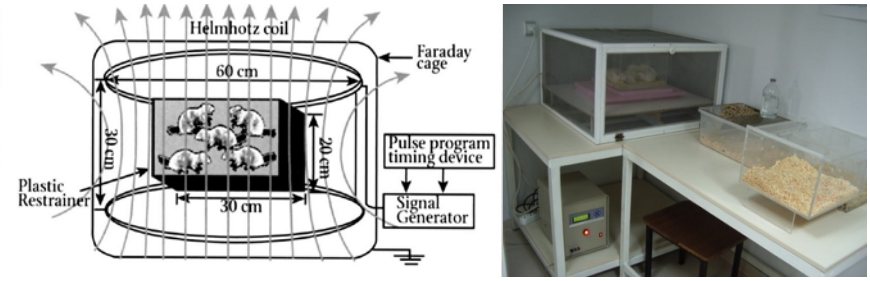

Figure 2. Pulsed magnetic field (PMF) stimulation and pulsed program timing device (right-below), animals were placed in plastic restrainer located between Helmholtz coils (left and right).

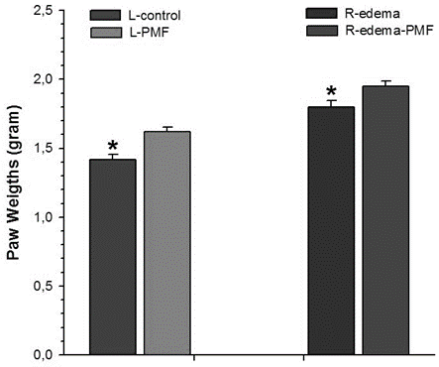

Figure 3. Paw weights of rats were measured with balance in exposed-PMF and unexposed-PMF groups.

Groups are left paw control (L-control), λ-Carr-induced right paw (R-edema), control exposed-PMF (L-PMF) and R-edema exposed-PMF (R-edema-PMF) (mean± SEM).

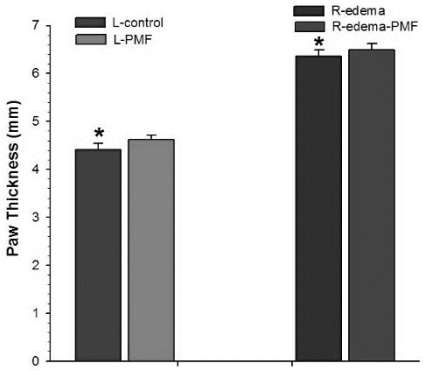

Figure 4. Paw thicknesses of rats were measured with calliper in exposed-PMF and unexposed-PMF groups.

Groups are left paw control (L-control), λ-Carr-induced right paw (R-edema), control exposed-PMF (L-PMF) and R-edema exposed-PMF (R-edema-PMF) (mean± SEM).

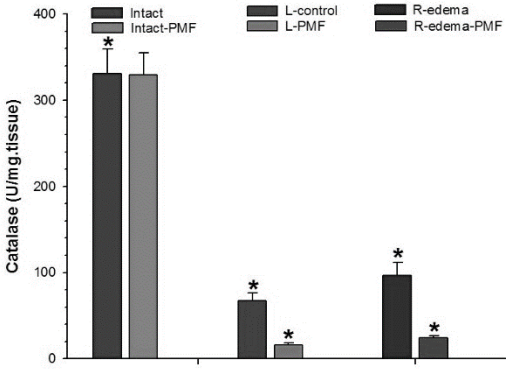

Figure 5. Catalase enzyme levels of rat paws were measured in exposed-PMF and unexposed-PMF groups.

Groups are CAT levels in paw tissue of untreated rats (Intact), Intact-exposed-PMF (Intact-PMF), control left paw (L-control), λ-Carr-induced right paw (R-edema), control exposed-PMF (L-PMF) and R-edema exposed-PMF (R-edema-PMF) (mean± SEM).

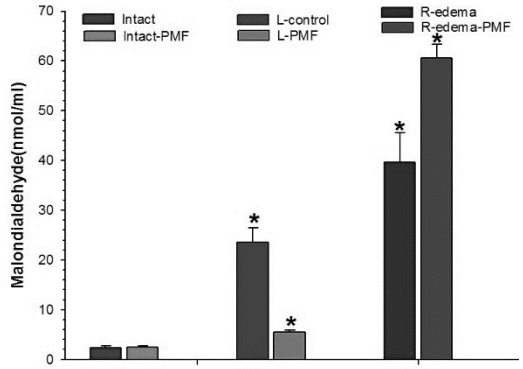

Figure 6. Malondialdehyde enzyme levels of rat paws were measured in exposed-PMF and unexposed-PMF groups.

Groups are malondialdehyde levels in paw tissue of untreated rats (Intact), Intact-exposed-PMF (Intact-PMF), control left paw (L-control), λ-Carr-induced right paw (R-edema), control exposed-PMF (L-PMF) and R-edema exposed-PMF (R-edema-PMF) (mean± SEM).

RESULTS

The right paw weights of rats in λ-Carr-induced inflammation group (R-edema) significantly increased to compare with left paw weights of rats in control left group (L-control) ($p<0.05$). After exposed-PMF, the weights of the left and right paw of rats increased in both of the L-PMF and R-edema-PMF groups to compare with L-control and R-edema groups. (fig 3). The right paw thicknesses of rats in λ-Carr-induced inflammation group (R-edema) significantly increased to compare with left paw thicknesses of rats in L-control ($p<0.05$). After exposed-PMF, left and right paw thickness of rats in both of the groups increased but results was insignificant statistically (fig 4.) ($p>0.05$).

Antioxidant enzyme CAT significantly decreased both of the left and λ-Carr-induced right paws compare with intact group ($p<0.05$). However, in R-edema group, decreasing CAT level in right paw tissue of rats in R-edema group was less than L-control group. After exposed-PMF, decreasing in CAT levels continued in both of the groups ($p<0.05$) (fig 5.)Lipid peroxidation activity level (MDA) significantly increased in both of the L-control and R-edema groups to compare with paw tissues of rats in Intact-group. After exposed-PMF, while MDA level in right paw tissue of rats in R-edema group was significantly continued to increase, in L-control group, MDA level in left paw tissue of rats in L-control was significantly decreased *($p<0.05$)* (fig 6.).

DISCUSSION

Magnetotherapy is a non-invasive physical therapy based on magnetic field interaction. It is used in the field of health, diagnosis and treatment of many diseases[23-27]. Warnke et al. claimed that the magnetic field resonates with organisms, organs, tissues, cells, or even molecules and affects pH balances, and were shown that low frequency magnetic fields affect the cell membrane. Also Warnke et al. were explained the effects of low intensity pulsatile magnetic field on pain, active enlargement of the diameter of the secondary blood vessels, increase in partial oxygen pressure in terminal tissues, and changes in capillary blood flow velocity[24]. Although magnetic field therapy is tried to be explained through different mechanisms in literature, therapeutic effects of many cases of antiinflammatory, antiemetics and analgesics, especially locomotor system of pulsatile magnetic field, have been shown in clinical trials. It has also been advocated to activate calcium channels in cell membranes, which regulate healing, spasmolytic, vegetative nervous system, perfusion, hormonal and enzymatic processes[24-31].

The λ-Carr-induced acute inflammation is highly sensitive to non-steroidal antiinflammatory drugs, and has been accepted as a useful PMF therapy for investigating new alternative therapies. We have evaluated the whether there is an antinflammatory effect of PMF on λ-Carr-induced acute inflammation model. The acute phase process of inflammation is associated with the symptoms (pain, heat, swelling and redness). We observed all the symptoms of acute inflammation. The signs of swelling in paw inflammation are increase in the thicknesses. The thicknesses and weights of right paws of rats in R-edema group were significantly increased by λ-Carr-induced acute inflammation. Also, weights and thicknesses of left paws of rats in L-control group increased. Although we were created the acute inflammation in right paw of rats, sign of inflammation is swelling that was observed in the left paws of rats. The inflammation on right paw of rats in λ-Carr-induced acute inflammation could be reflected left paw or acute inflammation, affected the system that acute inflammation might be created systemic effects and these effects might be reflect in the left paw of rats as inflammation had have made effects in right paw of rats.

After exposed-PMF, weights and thicknesses of rat paws in both of the L-control and R-edema groups continued to increase. PMF might have to water retention in the body of rats as a stress factor or used methods to measure in the weight and thickness of paws of the rats was not suitable. During normal body conditions, a balance exists between free radicals and the natural scavengers of the body, but in a acute or chronic inflammatory state, the balance diminishes and reactive oxygen metabolites increase dramatically in number[32-34]. Carrageenan is a strong chemical for the release of inflammatory and pro inflammatory mediators. The Carr-induced inflammatory response has been linked to macrophages and neutrophils which secrete a number of mediators (eicosinoids, oxidants, cytokine and lytic enzymes) responsible for the initiation, progression and persistence of the acute or chronic inflammation state[35].

Our results showed that CAT activity was decreased

by λ-Carr injection to compare with paw tissue of rats in intact or control group. However, when decreasing CAT level in right paw tissue of rats in R-edema group was less than left paw tissue of rats in L-control group. The causing formation of the λ-Carr-induced acute inflammation process might be an augmentation of CAT activity[36]. After exposed-PMF, CAT level decreased in both of the L-control and R-edema groups. According to the present findings the levels of antioxidant system enzymes were adversely affected by edema induction. PMF can alleviate this effect of edema on these enzymes. The antiinflammatory properties of PMF could be related to its positive effects on the antioxidant system in rats with paw edema. There is a growing body of experimental evidence that primary afferents become sensitized under inflammatory conditions[37,38].

In CAR induced inflammation model, sensitization of primary afferent nociceptor by inflammatory mediators released by cell lysis, inflammatory cells, and nerve endings also produce the inflammatory pain indices. PMF might be induced λ-Carr-induced edema. Lipid peroxide activity or MDA in R-edema group increased more than L-control group. Under the effects of the PMF, while MDA level in the R-edema-PMF group was continued to increase, MDA level in the L-PMF group decrased. 1.5 mT pulsed magnetic field might be induce the effect of increasing MDA level in carrageenan-induced paw edema. This indicates that the tissues were subjected to increased lipid peroxide activity or MDA level in the R-edema-PMF group while a statistically significant (p<0.05) reduction of CAT level was observed in the both of the L-PMF and R-edema-PMF groups. Although we only induced by λ-Carr to right paw of rats, we observed to the differences as decreasing of CAT activity and increasing of MDA level.in both of the right and left paw of rats Therefore, left paw accepted as control paw is not suitable in rats that λ-Carr induced right paw of rats.

These results indicate that an anti-inflammatory effect of PMF on λ-Carr-induced acute inflammation model is not clear. These results were showed that PMF was increased paw edema and affected antioxidant enzymes levels negatively in acute inflammation model.

Our results demonstrated that PMF treatment to CAR injected rats ameliorates inflammation, decreased oxidative stress in inflamed paw. These results can at least suggest that PMF may be used as a therapeutic strategy for inflammatory pain conditions in order to reduce drug dependencies, invasive procedures and side effects. This study is keep going, by increasing the number of data, set up a longer-lasting model of inflammation and investigate with different methods of analysis.

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