Research Paper
Effect of Three Therapeutic Methods of Exercise, Ozone, and Stem Cells on the MEF2C Expression and Myostatin Levels in Femoral Muscle Tissue of the Osteoarthritis Rats

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ABSTRACT

**Aims**: Myostatin and Myocyte Enhancer Factor 2C (MEF2C) are involved in muscle changes associated with bone problems. The aim of the present study was to determine the effect of three therapeutic methods of exercise, ozone, and stem cells on MEF2C gene expression and myostatin levels of femoral muscle tissue in osteoarthritis rats.

**Methods & Materials**: This experimental study was done on 63 male rats (mean age of 8-12 weeks and weight of 250-300 g). They were randomly divided into nine groups: the healthy control and osteoarthritis group and seven intervention groups of osteoarthritis rats, including saline, exercise, ozone therapy, MSCs, MSCs+ozone therapy, exercise+ozone therapy, and exercise+MSCs. Osteoarthritis was induced in rats by surgery. The training program consisted of 30 min of running on a non-slip treadmill at a speed of 16 m/min. Rats in the MSCs group received 1×10⁶ cells/kg. The ozone was injected into the articular line of the knee tibiofemoral at a concentration of 20 μg/mL. Tissue levels of myostatin were measured by enzyme-linked immunosorbent assay kits and MEF2C gene expression was measured by real-time polymerase chain reaction method.

**Finding**: Cell+exercise, exercise+ozone, and cell+ozone groups showed a significant increase in MEF2C gene expression and a significant decrease in myostatin levels compared with the cell, exercise, and ozone groups (P<0.05). In the exercise+cell group, these changes were greater.

**Conclusion**: The results showed that the combination of exercise and MSCs with an increase in MEF2C gene expression and a decrease in myostatin levels, can possibly have beneficial effects on the stimulatory and inhibitory factors of interactions between muscle and bone in the osteoarthritis rats, and ultimately reduce the risks of muscle weakness due to osteoarthritis complications.
Extended Abstract

1. Introduction

Osteoarthritis is the most common joint disease that may occur due to several causes, including age, sex, genetics, bone density, endocrine disorders, major joint injuries, excessive pressure on joints caused by extreme jobs or workout routines, congenital or developmental disorders, previous defects of the joints, and inflammatory diseases of the joint [1].

The main limitation in the management of patients with osteoarthritis, which slows down the patient’s recovery, is the lack of an appropriate treatment method. Methods, such as exercising and weight loss improve the symptoms, but to date, no factors have been found to affect the disease progression [2, 3]. This is also true about non-steroidal anti-inflammatory drugs, intra-articular hyaluronic injections, dietary supplements, anterior cruciate ligament repair surgery, and meniscus. Therefore, the lack of intervention to target the course of the disease has led to a significant increase in knee replacement surgery [2, 4].

The safe, effective, and cheaper treatment that can change the course of the disease will have a major impact on the quality of life and health care costs in the future. Due to the poor repairing ability of chondrocytes, cartilage damages caused by osteoarthritis and trauma have posed major challenges in clinical management. In the meantime, stem cell therapy and cartilage tissue engineering open up new ways for treating damaged cartilages [5]. Mesenchymal Stem cells (MSCs) have been considered as a biological therapeutic agent for the treatment of inflammatory diseases and tissue repair. The intra-articular injection of MSCs is effective in treating osteoarthritis [5, 6]. Stem cells are derived from various tissues and have the potential to become different tissues [7]. In 2016, Lee et al. showed that MSCs treatment method can repair damaged cartilages in mice [5]. Also in 2012, Bowell et al. found that arthritic mice had more ability to put weight on the affected foot after mesenchymal therapy [8].

Another treatment recommended for osteoarthritis is ozone therapy. This method is used for therapeutic purposes in various body structures, especially in chronic diseases, such as rheumatism and osteoarthritis [9, 10]. Several studies have been conducted on the use of intra-articular ozone in the treatment of osteoarthritis [9, 11-13]. Because osteoarthritis is a progressive and chronic destructive disease and causes many disabilities in a person, regular exercise is an integral part of treating knee osteoarthritis [14]. Regular exercise at an appropriate intensity is useful to prevent muscle loss and resistance to daily activities. It also leads to pain management and the prevention of a limited range of motions in the joint [15]. However, water exercises are preferred for osteoarthritis patients because of their unique characteristics, such as floating [16].

Numerous studies have examined the effect of exercise on knee osteoarthritis [17-19]. On the other hand, studies have reported that in some patients, the knee pain is associated with joint instability, muscular dystrophy, and the inability of the patient [20]. Another study reported that the degree of disability in a patient with osteoarthritis may be associated with weakness, erosion, or muscle damage in involved parts [21]. Therefore, considering that one of the goals of treatment of these patients is to reduce pain, maintain joint mobility, and minimize disability, examining strategies for strengthening, growing, rebuilding, and the mechanisms involved in repairing damaged or atrophied muscles in knee osteoarthritis can help for better understanding of the problem. Cellular studies have reported that factors, such as myostatin and myocyte enhancer factor 2C (MEF2C) play a role in muscle changes associated with bone diseases [22].

Myostatin has been reported to negatively regulate muscle mass, and MEF2C is an important factor that interacts with myogenic regulatory factors. Mice without MEF2C in osteocytes showed a decrease in sclerostin levels [22]. Therefore, given that MSCs and ozone therapy are relatively new treatment methods that appear to be effective in the interactions between muscle tissue and bone metabolism, no research has yet been done on the effect of these methods on the regulatory and inhibitory factors affecting the interactions between muscle tissue and bone metabolism in osteoarthritis patients with regular aerobic exercise. Thus, in this study, we investigated the effect of three treatment methods of exercise, ozone, and stem cells on myostatin levels and MEF2C gene expression in an arthritic mouse model to find a useful and appropriate solution to improve the physical performance of people with osteoarthritis.

2. Materials and Methods

The present experimental study was conducted on male Wistar rats aged 8-12 weeks with an average weight of 250-300 g. The sample size was determined 63 rats using the software G*Power V. 3.1.9.2, the effect size of 0.55, alpha of 0.05, and power of 0.8. The rats were randomly divided into nine groups of 7, including two healthy control and unhealthy osteoarthritis groups, and seven intervention groups of osteoarthritis rats, including saline (saline injection to control the possible effects of injection in the research), exercise, ozone therapy, MSCs, MSCs+ozone therapy, exercise+ozone...
therapy, and exercise+MSCs. The subjects were kept in an environment with an ambient temperature of 22.2°C and a humidity of 55.5% and were freely fed and watered.

Research protocol

How osteoarthritis was induced

According to Malfait et al. surgery method (2015), osteoarthritis was induced in the rats [23]. The rats were anesthetized with ketamine and xylene. After modifying the right knee, a one-centimeter incision was made to make the knee joint appear and then, immediately opened with lateral displacement of the patella and patellar ligaments. A longitudinal cut was made by cutting the medial parapatellar. The lateral transfer of patella and patellar ligaments was done by forceps and then, an incomplete incision was made in the internal cruciate ligament without damage to the articular cartilage and other ligaments. Finally, the joint capsule with 6 absorbable stitches and the skin with 6 silk stitches were closed.

Exercise program

One month after the surgery, a week was spent getting acquainted with the research environment and the conveyor belt. For this purpose, the rats were active on the treadmill for 3 days a week, 10 min each session at a speed of 16 m/min, VO2max of about 60%–70%, and a slope of 0%. By observing the overload principle, the main training program in the first week was changed into 50 min training in the eighth week. Also, 5 min of running at a speed of 8 m/min was included before and after the training for warming up and cooling down the animals. During the exercise, the control group only stood on the treadmill and the device was not turned on.

Stem cells preparation and injection

MSCs were extracted from the bone marrow of healthy Wistar male rats after being anesthetized with ketamine and xylazine. Isolated MSCs were incubated for 24 h in Dulbecco’s Modified Eagle’s Medium (DMEM) environment with 20% Fetal Bovine Serum (FBS) for the cell adhesion process. Every 3 days, the cultivations were changed from the flask environment to separate the non-stick cells and MSCs reached 90% purity after 3–4 times passaging and were selected for injection. Rats in the MSCs group received 1×106 cells/kg by intra-articular injection of MSCs injected into the right knee joint of them.

Ozone therapy

Ozone was made from grade B medical oxygen by the OZOMED 01 device. It was produced by a low-intensity electric discharge, and its concentration was measured using ultraviolet light at 254 nm. Ozone was injected once a week for 3 weeks, 21 days after osteoarthritis was first induced in the rats, in the knee’s tibiofemoral joint line, at a concentration of 20 µg/mL.

Sampling and measuring research variables

At the end of the research procedure, all animals with completely similar conditions and following 12-14 h of fasting and 48 h after the last training session and injections (to eliminate the acute effects of exercise and other interventions) were anesthetized by intramuscular injection of ketamine and xylazine and then, were killed. After opening the abdominal cavity, the muscle tissue was carefully separated and frozen after rinsing with distilled water. All frozen muscle tissues were homogenized in protease buffer in liquid nitrogen after being completely grinding followed by centrifugation for 20 min at 12000 rpm and 4°C. The solution was frozen at -80°C to be used for chemical analysis.

The measurement of the myostatin tissue levels of the thigh muscle was done with an Enzyme-Linked Immunosorbent Assay (ELISA) kit made by Lsbio Co. according to the manufacturer’s instructions. Gene expression analysis in the muscle tissue was measured by real-time Polymerase Chain Reaction (PCR) method and after quantifying, gene expression values were analyzed using 2-ΔΔCt formula. Initially, the muscle samples were homogenized with a homogenizer in the phosphate buffer (pH 7.0) at 4°C. All RNAs were extracted from the muscle tissue of all rats according to the manufacturer’s protocol (Qiagen, Germany).

The spectrophotometric method with light absorption property at 260 nm was used to estimate the quantity and quality of the extracted RNAs. After extracting RNA with purity and high concentration from all animals, the steps of cDNA synthesis were performed according to the manufacturer’s protocol (Fermentas, USA). Then, the synthesized cDNA was used to perform the reverse transcription reaction. First, all designed primers for the MEF2C gene were examined, and the expression of genes was investigated using real-time quantitative PCR. The expression of the desired genes was performed by real-time PCR method and by RT-qPCR kits (Ampliqon, Denmark) in 40 cycles. Table 1 presents the sequence of primers used.

Data were expressed using Mean±Standard Deviation, and the Shapiro-Wilk test was used to determine whether
the data distribution was normal. Also, to investigate the significant changes in variables between different groups, One-way Analysis of Variance (ANOVA) was used and when a significant statistical difference was observed, Tukey’s range test was used. All statistical analyses were performed using GraphPad prism V. 8 software at a significance level of P<0.05.

3. Results

The results of one-way ANOVA showed that there was a difference between different expression levels of the MEF2C gene in femoral muscle between different groups (P<0.0001; F=56.11) (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
<th>F</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control</td>
<td>0.08148±0.0104</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unhealthy osteoarthritis</td>
<td>0.002774±0.000981</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.002368±0.0007969</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozone therapy</td>
<td>0.006176±0.0005154</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEF2C gene expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSCs</td>
<td>0.007976±0.001037</td>
<td>56.11</td>
<td>8.36</td>
<td>&gt;0.0001</td>
</tr>
<tr>
<td>Exercise</td>
<td>0.00843±0.000927</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSCs+ozone therapy</td>
<td>0.02492±0.01217</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Exercise+ozone therapy</td>
<td>0.03284±0.01424</td>
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<td></td>
<td></td>
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<tr>
<td>Exercise+MSCs</td>
<td>0.05371±0.01179</td>
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</tr>
</tbody>
</table>

The results of the Tukey post-hoc test also showed that there was a significant difference between the mean expression of the MEF2C gene in femoral muscle in rats with osteoarthritis compared with MSCs+exercise, exercise+ozone, and MSCs+ozone groups (P<0.05) (Figure 1). The results of the Tukey test also showed that there was a significant difference between the mean muscle-building effect of myostatin in rats with osteoarthritis compared with other groups (P<0.05) (Figure 2). The MSCs + Exercise, exercise+ozone, and MSCs+ozone groups showed a significant reduction in myostatin levels of femoral muscle compared with the MSCs, exercise, and ozone groups (P<0.05). Significantly, the reduction in myostatin levels of femoral muscle was higher in treated rats with a combination of MSCs+exercise than in other groups (P<0.05) (Figure 2).

Table 1. Specifications of the primers used to match the Myocyte Enhancer Factor 2C (MEF2C) gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Primer 5´-3´</th>
<th>Reverse Primer 5´-3´</th>
<th>Annealing Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEF2C</td>
<td>ATCTCTCCCTGCTTCTACTC</td>
<td>CTCCCATCGTAGAACTGC</td>
<td>60°C</td>
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</table>

MSCs: Mesenchymal Stem Cells
In this study, we evaluated the effectiveness of three therapeutic methods, including exercise, ozone, and stem cells, and their combination on myostatin levels and the expression of the MEF2C gene in the femoral muscle of arthritic rats. Our results showed that the expression of the MEF2C gene in the femoral muscle of the arthritic rat model decreased, whereas the levels of muscle myostatin increased.

MEF2C is an important factor that interacts with myogenic regulatory factors, such as MyoD and Myf5. This interaction synergistically activates muscle-specific genes and biogenic differentiation. Decreased MEF2C in mice is associated with decreased sclerostin levels in osteocytes. Sclerostin is a blood factor produced by osteocytes and acts as an inhibitor of the Wnt signaling pathway that increases bone formation.

These findings suggest that the MEF2C-Sclerostin signaling pathway regulates the interaction between muscle and bone through Sclerostin [22]. In contrast, one of the mechanisms that can regulate muscle volume and strength is myostatin messaging. Myostatin is a member of the Transforming growth factor-beta (Tgf-β) family, specifically expressed in skeletal muscle. Cellular effects of myostatin by autocrine and paracrine methods are the main regulators of skeletal muscle growth; therefore, its activation leads to the inactivation of the hypertrophic pathway and increasing its expression resulting in muscle atrophy.

Myostatin inhibits the proliferation and differentiation of myeloblasts as well as the Akt/mTOR pathway, which regulates muscle protein synthesis. Myostatin is a negative regulator of muscle growth, which acts by reducing the regulation of Akt/mTOR messaging pathways and phosphorylation of P70S6K2, rps63, Akt, and 4E-binding protein in the interruption of muscle hypertrophy [24]. Myostatin also regulates the fiber-type composition of skeletal muscle by regulating the expression of the MEF2 gene [25].

On the other hand, no use of the arthritic joint leads to muscle weakness, and since the muscles play a significant role in protecting the cartilage, it is very important to strengthen the muscles around the joint. It has shown that when the muscles and limbs do not move much due to pain, they become weak and the muscle volume decreases by about 5% per day. Muscle strength is enhanced by the force produced inside it and exercise is one of the easiest ways to strengthen the muscle, by which the muscle can repeatedly contract and expand [26, 27].

Figure 1. Comparison of the mean mRNA expression levels of the Myocyte Enhancer Factor 2C (MEF2C) gene in muscle identified by real-time polymerase chain reaction (PCR) in the healthy control, unhealthy osteoarthritis, saline, ozone therapy, Mesenchymal Stem Cells (MSCs), exercise, MSCs + Ozone, ozone+exercise, MSCs + Exercise groups.

There was a significant difference in the mean mRNA levels for the MEF-2C gene expression in the groups marked with (****P<0.0001; ***P=0.0001; **P>0.0001; *P<0.05; ns: not significant).

4. Discussion

In this study, we evaluated the effectiveness of three therapeutic methods, including exercise, ozone, and stem cells, and their combination on myostatin levels and the expression of the MEF2C gene in the femoral muscle of arthritic rats. Our results showed that the expression of the MEF2C gene in the femoral muscle of the arthritic rat model decreased, whereas the levels of muscle myostatin increased. MEF2C is an important factor that interacts with myogenic regulatory factors, such as MyoD and Myf5. This interaction synergistically activates muscle-specific genes and biogenic differentiation. Decreased MEF2C in mice is associated with decreased sclerostin levels in osteocytes. Sclerostin is a blood factor produced by osteocytes and acts as an inhibitor of the Wnt signaling pathway that increases bone formation.

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The results of the present study showed that moderate-intensity exercise on the treadmill increased the expression of the MEF2C gene and reduced myostatin muscle levels. Carvalho et al. (2010) stated that inactivity or strenuous exercise worsens the osteoarthritis. In contrast, regular exercise programs at the right intensity help in preventing decreased muscle strength and resistance to daily activities [15]. Other meta-regulatory studies reported the reduced miRNA and myostatin protein levels after 6-12 weeks of exercise after a hypertrophic protocol.

Co-administration of myostatin showed an increase in serum Follicle-Regulating Gene (FLRG) and a decrease in activin Ib receptor. In addition, inhibition of endogenous myostatin in mice significantly reduces muscle damage and increases muscle strength and mass [28]. In a review article, Marlene Franzen et al. (2017) examined the effect of exercise on knee osteoarthritis using 44 experiments. They showed that exercise significantly reduced pain and improved physical performance immediately after the treatment. By a review of 13 studies, they showed that exercise significantly improved quality of life immediately after treatment; however, it was not significant. Moreover, 12 studies showed a significant reduction in knee pain 2-6 months after exercise [29].

An increase in MEF2 has also been shown after exercise [30]. Elimination of MEF2C in mice can reduce the ratio of type I fibers, and MEF2C can respond to several calcium-regulated signals and regulate the fiber-type composition of skeletal muscle. MEF2C also uses FOXJ3 as a downstream target gene, which can reduce type I fiber ratios by activating transcription by FOXJ3 [31].

In contrast, in the Anderson study, it was shown that MEF2C is required for the overall growth of the body in skeletal muscle; however, in assessing the ability of control mice and mice without MEF2C to perform running for one week on a training treadmill, no significant difference was observed [32]. As mentioned earlier, in addition to exercise therapy, other methods, such as cell therapy and ozone therapy are also used to treat patients with osteoarthritis.

In the present study, the effect of bone marrow-derived stem cell injection, as well as ozone therapy on arthritic rats, was investigated. The results of our study showed that both cell therapy and ozone therapy increased the expression of the MEF2C gene and reduced myostatin levels in the femoral muscle. However, the combination of these interventions showed significant results, so that MSCs+exercise, exercise+ozone, and MSCs+ozone caused a significant reduction in myostatin levels and a significant increase in femoral muscle MEF-2C gene expression compared with other groups. These changes were significantly higher in treated mice with a combination of MSCs+exercise than in other groups.

In line with our findings, Gibbs et al. (2015) examined the combined effect of the exercise rehabilitation program and intra-articular injection of stromal bone marrow transplantation and Platelet-Rich Plasma (PRP) in patients with knee osteoarthritis. Their findings showed an improvement in people with osteoarthritis following this combined treatment [33].

In 2016, Lee et al. also showed that treatment with MSCs led to the repair of damaged cartilage in mice [5]. Also in 2012, Bevel and et al. studied the distribution of pain in arthritic mice after injecting MSCs into the affected knee and observed that the mice were more able to weigh on the affected leg after mesenchymal therapy [8]. The effect of stem cells on the treatment of atrophy by inhibiting myostatin has also been shown [34].

The use of stem cells, such as mesenchymal cells not only affects the structure of the damaged joint but also affects the anti-inflammatory and modulating aspects of the immune system. Stem cells have two important characteristics that distinguish them from other types of cells. These cells are non-specialized cells, which means that they do not act as heart muscle cells. They have the power to revival and regenerate themselves through cell division and can become specialized-directed cells under laboratory conditions. Young et al. showed that MSC uses the anti-inflammatory mechanism in the clinical treatment of knee injury that improves cell differentiation [35].

On the other hand, ozone therapy also produces ozone in the tissues and regenerates damaged tissues and weak tissues around the joints. Ozone therapy stops pain and inflammation, improves blood flow, increases nutrients for tissues, and delivers oxygen to damaged tissues. This method also improves movement and reduces joint pain.

Kamelia et al. reported the following beneficial effects of ozone: increased supply of oxygen to the tissues affected by inflammation and pain, increased blood flow, removing metabolic wastes in the affected joints, eliminating the blockage of substances that maintains inflammation and pain, strengthening the immune system, stimulation of the mechanisms that improve health in the body resulting in better joint mobility, and better functioning of the body system [36].
In general, the possible mechanisms of action of ozone therapy are its analgesic, anti-inflammatory, antioxidant effects (by activating cellular metabolism, reducing prostaglandin synthesis) as well as improving the quality of oxygen delivery to tissues (through anaerobic action, vasodilation, and stimulation of angiogenesis) [9, 10]. It has shown that ozone therapy can significantly reduce the pain by moderating osteoarthritis and improving functional status of the patients. The mechanism, by which ozone can raise the pain threshold is likely the stimulation of the analgesic system mediated by endogenous serotonin and opioids [37].

Therefore, our findings showed that the combination therapy with bone marrow-derived stem cell injections and moderate-intensity exercise, ozone therapy, and exercise improved MEF2C gene expression and muscle myostatin levels and also the effective interaction between muscle and bone in rats with osteoarthritis. The results showed that this method is more appropriate than other treatments, such as cell, ozone, and exercise therapies alone. It should be noted that the combined treatment using bone marrow-derived stem cell injections and moderate-intensity exercise has been more effective than the combination of ozone therapy and exercise. Although stem cells have restorative, anti-inflammatory, and immune-modulating properties, their combination with exercise therapy can be more effective in reducing disorders caused by osteoarthritis.

5. Conclusion

Overall, our results showed that a combination of exercise and MSCs by a significant increase in MEF2C gene expression and a significant decrease in myostatin levels in the femoral muscle could have beneficial effects on the stimulation and inhibition of the interactions between muscle and bone in the arthritic rat model. Therefore, it reduces the risk of muscle wasting and weakness due to osteoarthritis.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Animal Care Committee at the Islamic Azad University, Sari Branch (Approval No.: IR.IAU.REC.1398.33).

Funding

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مقدمه

استثناییتی شیوع بیماران مفصلی است که همیل مکملی در اینجا این تکنیک طراحی شده است. سن، جنس، وزن، تراکم استخوان، اختلالات آندوکرینی، ضربه‌های مفصلی عمده، فشار بیش از حد به مفاصل ناشی از نوع فشاری و ریزبی ناشی از سنگینی، و زخمی کننده ساکت، محدودیت اصلی [1] محدودیت اصلی در مدیریت بیماران مبتلا به استثناییتی، قطعی یک روی درمان مبنی‌تر است که منجر به کند شدن روی درمان بیماران مبتلا به استثناییتی، قطعی یک روی شد.

استثناییتی...
مطالعات سالیوی کِروز همین‌طور که فاکتورهای، مانند Myostatin در تغییرات عضلانی مربوط به شکل‌سازی استخوانی و ثروت‌آور‌ترهای یکی از مهم‌ترین راه‌هایی است که در این مدل موش‌های بنیادی استخوان در بیماران استئوآرتریت با یک روش ساده و مناسبی برای بهبود عملکرد عضله و متابولیسم استخوان، در سال‌های اخیر، به دست آمده است. Myostatin از یک روش ساده و مناسبی برای بهبود عملکرد عضله و متابولیسم استخوان در مدل موش‌های استئوآرتریت استفاده می‌شود.

در بخش پیشین و در مدل موش‌های استئوآرتریت، بررسی نشان داد که Myostatin از یک روش ساده و مناسبی برای بهبود عملکرد عضله و متابولیسم استخوان در مدل موش‌های استئوآرتریت استفاده می‌شود.

یکی از اهداف درمان این بیماران از جمله کاهش درد، حفظ تحرک مفصل و به حداقل رساندن ناتوانی است. این حال تمریناتی که در آب انجام می‌شود، به‌دلیل ویژگی‌های فردی می‌شود، فعالیت ورزشی منظم جزء اصلی در درمان استئوآرتریت و شناخته می‌شود. این تمرینات به دلیل ویژگی‌های فردی می‌شود، فعالیت ورزشی منظم جزء اصلی در درمان استئوآرتریت و شناخته می‌شود.

با توجه به اینکه یکی از اهداف درمان استئوآرتریت، اُزن تراپی است. این اُزن تراپی در درمان بیماری استئوآرتریت مؤثر دانسته می‌شود.

3. Myocyte enhancer factor -2C

2. Mesenchymal Stem Cells
از طریق برش میانی پاراپتالار ایجاد شد. جهت جابجایی جابجایی پاراپتالار، با استفاده از شیمنی و پس از نفوذ، هورمون‌های سطحی یا خونی می‌شود. 

3- شماره 26 دوره 1399 تابستان

از طریق برش میانی پاراپتالار ایجاد شد. جهت جابجایی پاراپتالار، با استفاده از شیمنی و پس از نفوذ، هورمون‌های سطحی یا خونی می‌شود.

برنامه تمرینی

یک ماه بعد از عمل جراحی، یک هفته صرف آشنایی و سازگاری با محیط پژوهش و نوار گردان شد. بدین منظور موش‌ها متر در دقیقه 16 سه روز در هفته به مدت ده دقیقه با سرعت Vo2max درصد 70 تا 60 تشدید ترمیل فعالیت داشتند. برنامه تمرین اصلی شامل سی دقیقه متر در دقیقه در 16 دویدن روی ترمیل بدون شیب و با سرعت دقیقه 50 هفته اول با رعایت اصل اضافه بار به صورت پیشرونده به 20 درصد اضافه بار در هفته هشتم رسید. همچنین پنج دقیقه با سرعت برای گرم و سرد کردن حیوانات اختصاص یافت. گروه کنترل در طول اجرای تمرین فقط روی تردمیل ایستاده بود و دستگاه روشن نمی‌شد.

نحوه تهیه و تزریق سلول‌های بنیادی

از مغر استخوان موش‌های نر نژاد ویستار سالم پس MSCs جداسازی شده. از بیهوشی با کتامین و زایلازین استخراج شد در طول یک شبانه روز 5% FBS درصد 20 با 4% DMEM در محیطی برای انتخاب سلول‌های چسبان انکوبه شدند. کشت‌ها از محیط فلasks هر سه روز تعویض شدند تا سلول‌هایی که نچسبیده اند 90< با پاساژ شدن به MSCs جداسازی و خلوص رسیدند و به هدف تزریق انتخاب شدند. موش‌های گروه مصرف و سیس تزریق شدند. در مفصل زانوی گروه MSCs دریافت کردند. MSCs دریافت شده موش‌ها تزریق شد. اوُزن تراپی OZOMED توسط دستگاه 2 ازن از اکسیژن پزشکی درجه ساخته شد. ازن توسط یک تخلیه الکتریکی با شدت کم 0.14. Dulbecco's Modified Eagle's Medium 5. Fetal Bovine Serum

نحوه تهیه و تزریق سلول‌های پهلوایی

از متر استخوان موش‌های از ترو تا ویستار سلم پس MSCs از بیهوشی کاملاً مشابه و به ساعت پس از آخرین 48 دنبال دوازده تا چهارده ساعت ناشتایی و جلسه تمرینی و تزریقات (جهت حذف اثرات حاد تمرین و دیگر پیمان‌های کاری) با تزریق داخلی صفاقی کتامین و زایلازین هورمونی عضله رانی با کیت الایزای ساخت شرکت Myostatin با کیت الایزای ساخت شرکت Fermentas, USA (شرکت سازنده) جهت انجام واکنش رونویسی معکوس مورد استفاده قرار گرفت.

برای پیش‌مرور اثربخشی سلول‌های MSCs در مدل موش‌های آرتروزی، سطوح MEF-2C مشخصات پرایمرهای مورد استفاده جهت همبسته‌سازی تن در مدل موش‌های آرتروزی به عنوان ژن‌های استفاده گردید.

<table>
<thead>
<tr>
<th>جدول 1: مشخصات پرایمرهای مورد استفاده جهت همبسته‌سازی تن</th>
<th>Annealing Temperature</th>
<th>Reverse primer</th>
<th>Forward primer</th>
<th>Lanes</th>
</tr>
</thead>
<tbody>
<tr>
<td>60℃</td>
<td>ATCTCCTGCTGCTTACTC</td>
<td>ATCTCCTGCTGCTTACTC</td>
<td>MEF-2C</td>
<td></td>
</tr>
</tbody>
</table>

6. Real time polymerase chain reaction

านا سهیلی ۱۳۹۹ شماره ۳
پژوهش‌کنندگان از دانشگاه علوم پزشکی مشهد، از رویت‌های آزمایشگاهی، تحقیق در مورد اثرات روش ترکیبی MSCs و Myostatin بر عضله رانی در موش‌های تحت درمان استفاده کردند. در این طرح، گروه‌هایی از موش‌های استئوآرتریت (بررسی بالینی) در دو گروه به صورت کنترل و ترکیبی MSCs و Myostatin دریافت کرده بودند. نتایج آزمون تحلیل واریانس یک داده بر میزان بیان ژن Myostatin نشان داد که در گروه ترکیبی MSCs و Myostatin، میزان بیان ژن Myostatin کاهش یافته بود. این نتایج نشان می‌دهد که استفاده از روش ترکیبی MSCs و Myostatin می‌تواند بهبودی در عضله رانی موش‌های استئوآرتریت را به‌دست آورد.

<table>
<thead>
<tr>
<th>شماره</th>
<th>فصل</th>
<th>تعداد</th>
<th>میزان بیان ژن Myostatin (fg/pg مایکروسال)</th>
<th>میزان بیان ژن Myostatin (fg/g مایکروسال)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>0/5</td>
<td>1 46 38 28 32 43 28 32 43 28 32 43 28</td>
<td>1 46 38 28 32 43 28 32 43 28</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0/5</td>
<td>1 46 38 28 32 43 28 32 43 28</td>
<td>1 46 38 28 32 43 28 32 43 28</td>
</tr>
</tbody>
</table>

نتایج آزمون تحلیل واریانس یک داده بر میزان بیان ژن Myostatin نشان داد که در گروه ترکیبی MSCs و Myostatin، میزان بیان ژن Myostatin کاهش یافته بود. این نتایج نشان می‌دهد که استفاده از روش ترکیبی MSCs و Myostatin می‌تواند بهبودی در عضله رانی موش‌های استئوآرتریت را به‌دست آورد.
عنوان فاکتورهای رشدی است که به طور خاص در عضله اسکلتی می‌یابد. قدرت عضله با نیروی تولید شده در آن تقویت می‌شود که تقویت عضلات اطراف مفصل بسیار مهم است. تحقیقات نشان داده که عضلات نقش قابل توجهی در حفاظت از غضروف به عهده‌دارند، استفاده از مفصل آرتروزی، منجر به ضعف عضلانی شده و از آنجا، گروه 4E Myostatin و پروتئین اتصالی TGF منجر به افزایش بیان آن به آتروفی عضلانی منجر می‌شود. 

یک فاکتور مهارکننده مسیر سیگنالینگ Myostatin در موش‌ها با کاهش سطوح اسکلروستین در استئوسیت‌ها نشان داده شده است. اسکلروستین یک فاکتور عضله رانی مدل موش‌های آرتروزی کاهش می‌یابد و در مقابل، در عضله رانی مدل موش‌های آرتروزی را کاهش می‌دهد.

در این پژوهش، ما اثرات مثبت سه روش درمانی، تمرین، ازن و سلول‌های بنیادی بر بیان ژن MEF-2C که به وسیله MEF-2C، می‌تواند در سطوح میانگین میزان مهارکننده مسیر سیگنالینگ Myostatin با ترکیبی از تمرین + ازن + سلول‌های بنیادی عمل کرده و سبب افزایش میزان میزان این ژن می‌گردد.

امنیت سلول‌های بنیادی در سطوح میانگین میزان میزان این ژن می‌گردد.
نتایج تحقیق حاضر نشان داده تختیب می‌تواند ویژه‌ای باشد و منابع سیستمی می‌شود.

کلبه طبیعی Myostatin می‌تواند که بتواند مثیلی با ویژه‌های بهبود می‌شود و افزایش عرضه اکسیژن در بافت‌های تحت تأثیر قلبی ازون را بهبود می‌دهد. این می‌تواند بهبودگری‌های درمانی باعث توقف درد و التهاب بافت‌ها و بازسازی بافت‌های آسیب‌زده و بافت‌های ضعیف در مورد دیگر، این درمانی نیز می‌تواند بهبود ماده و گاز ازون در درمان بالینی آسیب‌زده، یک مکانیسم ضد التهابیباشد.

در تحقیقاتی که توسط سلول‌های با کارگردان تخصصی مقدار زیادی از MEF-2C و ac-FLG را مشاهده نموده‌اند، نشان داده شده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای سلول‌های استوانه‌ای همراه با تمرین ورزشی در مدل موش‌های آرتروزی درمان نشان داده که سلول‌های استوانه‌ای SMCs در روز تولید شده و موجب افزایش قدرت و توده عضلانی می‌شود.

MEF-2C و ac-FLG در عضله رانی شد. با این حال، زمانی که از مغز استخوان و نیز اثر اُزن تراپی روی موش‌های مدل آرتروزی همان طور که قبلاً بیان شد علاوه بر تمرین درمانی از روش‌ها استفاده می‌کند، که می‌تواند نسبت الیاف را بهبود بخشید. علاوه بر این، اصول عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضله، عضلا
بافت عضله رانی در مدل موش های آرتروزی Myostatin و سطوح MEF-2C

زهرا حدادپور و همکاران. اثر سه روش درمانی، تمرین، ازن و سلول های بنیادی بر بیان ژن Myostatin و سطوح MEF-2C

نتایج گیری

به طور کلی، نتایج محققند که ترکیبی از تمرین و سلول با پیشین در افزایش مقدار ژن Myostatin و سطوح MEF-2C انرژسیون و مهاری تعاملات بین عضله و استخوان در موش های آرتروزی خسته کرده و افزایش می‌دهد.

ملاحظات اخلاقی

پژوهش از اصول اخلاق پژوهشگری این پژوهش تبسته کیفیت محیط‌های حیوانات در آزمایشگاه‌های تحقیقاتی و استفاده از آن در دانشگاه آزاد اسلامی واحد ساری در هر جهتی در کار عادی تأیید شده است.

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