Effect of Autologous Bone Marrow Derived Mesenchymal Stem Cells in Treatment of Rheumatoid Arthritis

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Abstract

Introduction: Chronic inflammation causes articular bone and cartilage degeneration in people with rheumatoid arthritis (RA). Despite recent advancements in the management of RA, adverse side effects and ineffective treatments remain a problem. Access to treatment is hampered by financial restrictions. As a result, medications that reduce inflammation and bone resorption that are both safe and less expensive are required. Mesenchymal stem cells (MSCs) have recently been identified as a potential therapy for RA.

Aim of study: The aim of this study was to examine the anti-arthritic effect of rat bone marrow-MSCs, oligosaccharides and human placental extract, individually and combined on RA model, using Complete Freund's adjuvant (CFA) induced arthritis in rats.

Materials and methods: In female rats, RA was induced by injecting CFA in the paw of hind limb. Rat bone marrow-MSCs, oligosaccharides, and human placental extract (HPE) were given individually and in combination via intra-peritoneal route. Complete blood picture (CBC), erythrocyte sedimentation rate (ESR), serum cortisol, urea, uric acid and other biochemical parameters were measured to determine the safety and efficacy of the different treatments. Histopathological analysis of bone sections was carried out.

Result: Combining oligosaccharides and HPE with rat-bone marrow MSCs had a favorable anti-arthritic and anti-inflammatory effect in CFA-induced arthritis in rats, with no negative effects on CBC, renal functions, serum cortisol, ESR, and liver enzymes. Histopathological analysis showed healing and remodeling of osteoporotic lesions in arthritic rats.

Conclusion: Combination of rat MSCs, oligosaccharides and HPE has potential to be an effective treatment for rheumatoid arthritis.

Introduction

Rheumatoid arthritis (RA) is a systemic chronic inflammatory disease characterized by joint discomfort and swelling ¹. Rheumatoid arthritis develops when the proinflammatory and anti-inflammatory states are out of balance, resulting in synovial membrane inflammation and joint destruction. One of the methods through which RA promotes joint injury is increased expression of cytokines such as interleukin-6 (IL-6), tumor necrosis factor alpha (TNF α), IL-1b, and IL-1².

Four classes of medications are used for treatment of RA: steroidal hormones, biological agents, immunosuppressants and anti-arthritic drugs. Use of anti-arthritic drugs is limited because of high cost, side effects and low specificity. Antibodies like anti-TNF, anti-IL-1, and anti-IL-6 target and neutralize inflammatory cytokines. However, they are not always effective and have short-term effects; 50 percent of patients who are prescribed these treatments stop using them within two years due to a decrease in symptomatic relief or the absence of symptom alleviation ³. Treatment of RA with mesenchymal stem

cells (MSCs) is a multifaceted therapy. Administration of MSCs results in reduced levels of TNF- α in the circulation and can reduce the degree of arthritic inflammation up to 30% in most RA experimental models ⁴.

Other novel treatments for RA include human placental extract (HPE) and oligosaccharides (Os). HPE has an anti-inflammatory effect and may act by increasing growth of chondrocytes and repressing cartilage damage. Experiments performed on arthritic animal model showed that local injection of HPE reduced the symptoms of arthritis and the expression of inflammatory cytokines ⁵. Oligosaccharide (Os) therapy for RA proved to disrupt the glycosylation on the Fc domain of IgG, which is elevated in the serum and synovial fluid of rheumatoid patients ⁶.

The aim of this study was to examine the anti-arthritic effect of rat bone marrow-MSCs (rBM-MSCs), Os and HPE, individually and combined in an RA model, using Complete Freund's adjuvant (CFA).

Materials And Methods

Animals and experimental design

This study was carried out according to regulations and approval of Ethics Committee of Faculty of Medicine for Girls, Al-Azhar University, Nasr City, Cairo, Egypt, Registered at Central Administration of Research & Development; Egyptian Ministry of Health: Reg No. RHBIRB2018122001. The study is reported in accordance with ARRIVE guidelines (<https://arriveguidelines.org>).

Eighty female Sprague Dawley (SD) rats weighing 150–200 g were housed in standard animal house conditions under relative humidity (30–70%) and temperature 20–26°C according to Animal Research Committee, 2021 (ARC) temperature and humidity standards. Rats were allowed free access to water and standard pellet diet. An adaptation period of one week was allowed before beginning the experiment. Experimental animals were kept and used in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Rats were divided into eight groups of ten rats each as follows:

Group I: Negative control group.

Group II: Positive control group, rats were injected intradermally with a single dose of 0.1 mL CFA into right hind paw for induction of arthritis.

Group III: CFA induced arthritic rats treated with rBM-MSCs at the first day of the experiment intravenously (IV) (2M cells/rat/ once /month).

Group IV: CFA induced arthritic rats treated with Os I.P (intraperitoneal) (0.2 mL/rat) twice weekly for one month.

Group V: CFA induced arthritic rats treated with HPE intraperitoneal (I.P) (0.4 mL/rat) once weekly for one month.

Group VI: CFA induced arthritic rats treated with rBM-MSCs (2M cells/rat) once + Os (0.2 mL/rat) twice weekly for one month.

Group VII: CFA induced arthritic rats treated with rBM-MSCs (2M cells/rat) once + HPE (0.4 mL/rat) once weekly for one month

Group VIII: CFA induced arthritic rats treated with rBM-MSCs (2M cells/rat) once + Os (0.2 mL/rat) twice weekly + HPE (0.4 mL/rat) once weekly for one month.

Drugs and chemicals

Sprague Dawley (SD) rats aged 8–12 weeks were used to isolate mesenchymal stem cells. Cells were extracted from the tibias and femurs and separated at room temperature using an 80 percent Percoll gradient centrifuge at 1300 rpm for 10 minutes. Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 mg/mL streptomycin sulphate was used to wash cells in the low-density fraction (HyClone, USA). The cells were then seeded onto 10 cm culture dishes (SPL, South Korea) containing a control medium and grown as adherent cells for 3–4 weeks in a humidified atmosphere at 5% CO₂ and 37°C. Every three days, the media was replaced. Cells were subcultured at a ratio of 1: 4 for up to four passes after achieving a confluence of 70–80%. For the characterization of rBM-MSCs from SD rats, anti-CD34 monoclonal antibody was employed as a negative control and anti-CD29 and anti-CD44 as positive markers. Only positive markers stain rBM-MSCs ⁷.

Oligosaccharides (3'-Sialyllactose, Product no: A8681), CFA (product no F5881) were purchased from Sigma Aldrich Chemical Co USA, and HPE (Laennec) from Japan Bio Products. ELISA kits for estimating inflammatory cytokines and diagnostic kits for measuring liver enzymes and kidney functions were procured from Abcam Company, United Kingdom.

Measurement of biochemical parameters and inflammatory cytokines

At the appropriate time after 4 weeks and after 24 hours of the last dose, rats of all studied groups were anesthetized by light halothane inhalation. Blood samples were collected for analysis of CBC, serum cortisol⁸, erythrocyte sedimentation rate (ESR)⁹, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), urea, uric acid, inflammatory cytokines TNF- α , IL-6 and IL-10 according to manufacturer instructions. Then, all animals were sacrificed by decapitation under anesthesia according to the Ethics Committee recommendations of Al-Azhar University.

Histopathologic examination

Specimen preparation: the head of each femur was carefully dissected out and processed for histological studies. Each femur head was cleaned from attached muscle, trimmed or dissected as appropriate, and immediately fixed in 10% formal saline solution for 7 days¹⁰. The specimens were washed by tap water for half an hour then they were decalcified in the chelating agent disodium EDTA. Each femur head was then immediately fixed in glutaraldehyde solution and prepared for scanning electron microscopy. Decalcified femur heads were cut longitudinally in a coronal plane along the central portion and specimens were processed to form paraffin blocks. Serial sections 5 micrometer thick were cut and stained. Hematoxylin and Eosin (H&E): was used to demonstrate the general histological structures of femur heads in the experimental groups ¹¹.

Statistical analysis:

Data were processed using the Statistical Package for Social Science (SPSS), version 20. Results are expressed as mean $\pm$ SD. One-way analysis of variance, ANOVA, followed by least significant difference (LSD) post hoc test for multiple comparisons. A p value less than 0.05 was considered statistically significant.

Results

Biochemical parameters in the studied groups

Induction of arthritis with CFA resulted in reduction in hemoglobin concentration, mean corpuscular hemoglobin concentration (MCHC) and WBCs count and in an elevation in mean corpuscular volume and platelet count. All these parameters were improved by treatment, the best was by combination therapy with rBM-MSCs, Os and HPE. (Supplementary table 1).

Liver enzymes (ALT and AST), urea and uric acids were significantly elevated in CFA induced arthritic group compared to negative control group. Combination therapy of rBM-MSCs, OS and HPE reduced levels of all parameters more than other groups (Supplementary table 2).

Erythrocyte sedimentation rate and cortisol markers were significantly higher in CFA induced arthritic group compared to negative control group. Combination therapy of rBM-MSCs, Os and HPE improved these parameters more than other groups (Supplementary table 3).

Inflammatory cytokines were greatly increased in arthritic rats, and were markedly improved upon treatment with a combination of rBM-MSCs, Os, and HPE more than other groups (Supplementary table 4).

Histopathological analysis of the studied groups

a: Photomicrograph of bone tissue of negative control group (Fig.1)

Photomicrograph of bone tissue showed normal cortical structures (blue arrow), normal osteocytes (black arrow) and normal haversian canals (yellow arrow). 400 X H&E stain

b: Photomicrograph of bone tissue of CFA induced arthritic group (Fig. 2)

Photomicrograph of bony tissue showed early osteodystrophic changes with characteristic endo-osteoblastic proliferation (orange arrow) and osteoporotic reactions (yellow arrow) in the form of osteocytes apoptosis (green arrows) and osteodegenerative areas with increased activity of the osteoclasts (red arrows) especially around the degenerated areas (green arrows). Bone matrix showed hypocellularity with deep bluish coloration denoting demineralization (blue arrow). 400 X H&E stain

c: Photomicrograph of bone tissue of group treated with combination therapy of rBM-MSCs, Os and HPE (Fig.3)

Photomicrograph of bone tissue showed healing and remodeling processes of previous osteoporotic lesions with presence of a few reactive osteoclastic change (black arrow) and focal osteo-fibroblastic dysplasia and endosteoblastic hyperplasia (blue and brown arrows). A few bone cells still suffered osteodegenerative changes although the chondroplasty compensatory process is remarkable (red arrows). The haversian canals appear moderately dilated with hyperemic sinusoids (green arrows). 200, 400 H&E X stain

Discussion

Disease-modifying antirheumatic medications (DMARDs) and non-steroidal anti-inflammatory drugs (NSAIDs) are two of the few therapy options for RA, and they both focus on symptom control rather than preventing disease progression¹². Anti-TNF α , anti-IL-1, and anti-IL-6 biological antibodies are another RA therapeutic option. These drugs target and neutralize inflammatory cytokines. They don't, however, function in every circumstance. As a result, identifying effective treatments for patients who do not respond to currently available drugs is critical³

Our findings showed that adding treatments such as Os and HPE to rBM-MSCs in a CFA-induced arthritic rat model resulted in a favorable anti-arthritic inflammatory effect, with complete safety on blood picture, renal functions, cortisol, ESR, and liver enzymes. CFA-induced arthritic rats had increased levels of TNF α , IL-6¹³ and IL-10¹⁴. Treatment with rBM-MSCs lowered levels of inflammatory cytokines. This agrees with the findings reported by **Zhang et al**,¹⁵ where intravenous injection of MSCs into the body reduced systemic and joint inflammation. Treatment with oligosaccharides had a significant anti-inflammatory effect. These results agree with those of Rademacher et al⁶, who treated immune complexes serum or synovial fluid by exposing them to an oligosaccharide with a structural component that inhibits or disrupts the degree of occupancy of the Fc carbohydrate binding site on IgG. This antiarthritic effect was

superior to that observed with treatment with rBM-MSCs alone. The strongest antiarthritic effect was observed with combination therapy with rBM-MSCs + OS + HPE. Arthritic rats treated with HPE showed an improvement in inflammatory cytokines when compared to groups IV, III^{16,17}. This differs from the data reported by **Park et al**¹⁸, where the HPE had no anti-inflammatory effect on RA.

All treated groups had improvements in CBC, kidney and liver functions, as well as in ESR and cortisol, indicating that the treatments do not have toxic side effects. Furthermore, histopathological analysis of bone tissue sections from rats treated with rBM-MSCs, OS, and HPE showed the best improvement in osteotrophic changes with combination therapy rather than with monotherapy.

In conclusion, combination of rBM-MSCs, OS, and HPE had a powerful healing effect on CFA-induced arthritis in animal models, with no adverse effect on blood, liver enzymes or renal functions.

Future clinical trials will be needed to better understand the mechanism of action of this combination in arthritis, with the goal of producing medications with higher therapeutic efficacy and fewer side effects than current therapies.

Declarations

Data sharing statement:

I confirm hereby that the manuscript has not been submitted or is not simultaneously being submitted elsewhere, is not at the time of submission under consideration by another journal or other publication, and that no portion of the data has been or will be published elsewhere while the manuscript is under review by the journal, unless rejected or withdrawn by the author.

Data availability statement: Data generated and analyzed during this study are included in the published article.

Authors' contributions: All authors contributed in the design of the study. All authors participated in data collection and analysis, data interpretation and manuscript writing.

Codes for contribution:

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AE , AM, DA, SB, and **NM** suggested the protocol and developed the codes, supervised the research.

YE, AR, AM, and **SB** performed the experiments.

AE, NM, AR, DA, SB, and **AM** wrote and revised the manuscript.

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Competing interests: The author confirms that there are no any potential conflicts of interest.

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I confirm also that there are not any financial support or other benefits from commercial sources for the work reported on in the manuscript, or any other financial interests that I may have, which could create a potential conflict of interest or the appearance of a conflict of interest with regard to the work.

Ethical approval information:

This study was carried out according to regulations and approval of Ethics Committee of Faculty of Medicine for Girls, Al-Azhar University, Nasr City, Cairo, Egypt, Registered at Central Administration of Research & Development; Egyptian Ministry of Health: Reg No. RHBIRB2018122001

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Figures

Figure 1

Photomicrograph of bone tissue of negative control group

Figure 2

Photomicrograph of bone tissue of CFA induced arthritic group

Figure 3

Photomicrograph of bone tissue of group treated with combination therapy of rBM-MSCs, Os and HPE

Supplementary Files

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