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Effect of *in vivo* low-level laser therapy on bone marrow-derived mesenchymal stem cells in ovariectomy-induced osteoporosis of rats☆

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ABSTRACT

Postmenopausal osteoporosis (PMOP) is considered by decreased bone strength that escalates the threat of fractures. Positive effects of photobiomodulation (PBM) with pulse wave have been demonstrated in cell culture and animal models. The aim of this study was to assess the *in vivo* effects of PBM on viability and calcium ion release of ovariectomy induced osteoporosis (OVX) - bone marrow derived mesenchymal stem cells (BMMSCs).

Material and Methods: 18 female rats were distributed into the following groups: 1) control healthy, 2) LASER-healthy (890 nm, 80 Hz, 1.5 J/cm^2, three days weekly, 60 days), 3) control OVX, 4) LASER-OVX, 5) Alendronate (Alen.)-OVX [0.5 mg/kg, 5 days per week, 60 days], and 6) Alen. + LASER-OVX. Ovariectomy was done on rats of groups 3, 4, 5 and 6. After that all rats were euthanized and their MSC harvested and cultured in complete osteogenic medium. In all groups, BMMSC viability, and calcium colorimetric assay were performed.

Results: We observed a significant increase in optical density (OD) of BMMSCs viability in LASER healthy group compared to control-OVX, Alen.-OVX, LASER-OVX, LASER + Alen.-OVX, groups. LASER + Alen.-OVX group displayed a significant escalation in OD of BMMSCs viability compared to LASER-OVX, Alen.-OVX, and control-OVX groups. There were a significant increase in calcium ion release of LASER-healthy group compared to control healthy, control-OVX, Alen.-OVX, LASER-OVX, and LASER + Alen.-OVX groups. LASER + Alen.-OVX group displayed a significant escalation in calcium ion release compared to LASER-OVX, Alen.-OVX, and control-OVX groups.

Conclusion: Pulse wave (PW) PBM significantly stimulated viability and cell proliferation of healthy BMMSCs compared to those of control-OVX, OVX-alendronate, OVX-LASER, and LASER + alendronate-OVX. In addition stimulatory effect of LASER + alendronate on viability and cell proliferation of OVX-BMMSCs compared to those of control-OVX, alendronate-OVX, and LASER-OVX groups were found.

1. Introduction

Postmenopausal osteoporosis (PMOP) is a skeletal condition characterized by decreased bone strength that escalates the danger of fracture (10. Fractures are related to significant injury, mortality and health care costs [1]. PMOP is associated with a reduced capability of bone to heal due to estrogen absence which causes significant morbidity [2]. Osteopenia and OP could also take place in women of reproductive age having hypoestrogenism due to an illness named hypothalamic amenorrhea (HA) [3]. It has been reported that there were more than two million fractures at a charge of $17 billion occurred in 2005 at the US. Female accounted for 71% of fractures and 75% of costs [4]. The current therapeutic protocols do not adequately address this problem [5].

In PM women bone mass initiates to decrease as resorption outpaces formation which leads to increased fracture threat [6]. The opposing actions of osteoblasts and osteoclasts are coupled by molecular connections between them that are to be influenced by the activities of the pioneer cells of the osteoblast lineage, mesenchymal stem cells (MSCs)

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[6]. MSCs have an important role in controlling normal skeletal homeostasis [6]. They also have essential role in fracture repair [6]. In OP, increased susceptibility to fractures results from insufficient osteoblastogenesis and increased osteoclastogenesis [7]. Ovariectomy induced OP (OVX) is associated by the decrease in both the number of MSCs in the bone marrow (BM) and their growth [7]. Osteoporotic-MSC demonstrates impaired engraftment to fracture and osteoinduction as well disordered bone repair [8]. And the capacity of MSCs-derived from osteoporotic PM women to produce and preserve type I collagen-rich extracellular matrix is reduced, favoring their adipogenic differentiation [9]. Moreover in OP increased BM adipocyte production is compensated by diminished production of osteogenic cells [10].

Xu et al. have demonstrated positive effects of photobiomodulation (PBM) with pulse wave low-level laser therapy (PWLLLT) on osteoblasts *in vitro* [11]. *In vivo* studies have suggested that PBM with both PW and continuous wave (CW) improve some biomechanical properties of tibia and vertebra compared to healthy bones in rats [12–14]. Consistency, it has been reported that PBM not only made alterations in the depth of the epiphyseal cartilage but also improved the number of the chondrocytes [15]. A review article have revealed positive effects of PBM on the *in vitro* proliferation of MSCs [16]. MSCs have the ability to generate cells of connective tissue lineages, including bone [17]. MSCs have made a great deal of interest due to their potential use in tissue engineering and regenerative medicine [17]. Researchers have shown that PBM with red and infra red (IR) and different energy densities stimulates the growth, proliferation and differentiation of SC [18–23]. Tuby et al. reported beneficial effects of PBM (IR, 810 nm, 1–4 J/cm^2) on the improvement of proliferation of MSCs *in vitro* [18]. Liao et al. reported Helium-Neon laser with 2 J/cm^2 improved the proliferation and migration of human epidermal SC *in vitro* [19]. Soleimani et al. reported that CW PBM (810 nm, 2–4 J/cm^2, 50 mW, ~167 mW/cm^2) not only enhanced BMMSCs differentiation into neuron and osteoblast but also increased BMMSCs proliferation *in vitro* [20]. Wu et al. reported some genes that maybe playing an important role in mediating the effects of PBM (diode laser, 635 nm, 0.5 J/cm^2, 6.61 mW/cm^2) on the proliferation of MSCs [21]. Wu et al. in another study reported the anti-inflammatory effect of PBM (red, 660 nm, 4 and 8 J/cm^2) on human adipose-derived (hAD) SCs in an inflammatory situation [22]. Guo et al. reported that PBM (Light-emitting diode (LED) laser, 633 nm and 830 nm, 0.5–2.0 J/cm^2) on different cell types demonstrated differential gene expression [23]. They suggested these differences in gene expression have the potential to be exploited for therapeutic purposes [23].

As a matter of fact, before the present research, there were data about the positive effect of PBM with 890 nm wavelength, and 80 Hz frequency on both intact bone and fracture healing by biomechanical and histological parameters evaluating methods [12–14,24,25]. It seems in these studies [12–14,24,25] because of higher wavelength (890 nm) and frequency (80 Hz) used in the PBM protocol, it (PBM) is able to easily penetrate into the bony tissues [26,27], and reaching bone marrow zone, resulting in probable biostimulatory effects on MSC.

However based on our extensive literature review, there are no studies conducted looking for the biostimulatory effects of pulse wave (PW) PBM on healthy and OVX-MSCs viability *in vivo*.

The goal of this study was to assess the *in vivo* effects of PBM with 890 nm wavelength and 80 Hz frequency on viability, calcium ion release, and osteogenic differentiation of healthy BMMSCs and OVX-BMMSCs. It is important to note that, recently it was reported that transplanting *in vitro* cultured BMMSCs with suitable osteogenic phenotype into sites, particularly at danger of developing OP, improved bone organization and enhanced its biomechanical properties [28]. Furthermore, allogeneic MSCs are indicated as cells that are responsible for exerting wide-ranging and local signals, predominantly suppressive and affect innate and adaptive immunity. Nonetheless, these cells also keep a degree of immunogenicity in some conditions which may limit MSC longevity and reduce their beneficial effects [29]. Thus, a laser-treated -healthy and or osteoporotic autologous BMMSCs may represent a promising therapeutic agent for the treatment of OP and fractures in healthy and or osteoporotic patients.

2. Material and Methods

2.1. Animals and Study Design

14 week-old female *Wistar* rats weighing about 210 g were used. The rats were fed standard pellet and water *ad libitum*, and were housed in standard cage placed in a standard animal home for the length of the research, and were weighed on a weekly basis throughout the study. 18 rats randomly were divided into the following groups: 1) control healthy, 2) LASER healthy (890 nm, 80 Hz, 1.5 J/cm^2, three days per week, 60 days), 3) control-OVX, 4) LASER-OVX, 5) Alendronate (Alen.)-OVX [0.5 mg/kg, 5 days per week, 60 days], and 6) Alen. + LASER-OVX. Ovariectomy was done on rats of groups 3,4, 5 and 6. Ovariectomized animals were kept for 14 weeks after surgery to develop OP. Rats of groups 5, and 6 received Alen., and rat of groups 2, 4, and 6 received LASER. Treatments were continued for 60 days respectively. After that all rats were euthanized and their MSC harvested and cultured in complete osteogenic medium. In all groups, BMMSC viability, Alizarin red staining, and calcium colorimetric assay were evaluated. Each group of cell viability, and calcium colorimetric assays has three different samples (rats). And in case of cell viability assay, there were 5 repetitions for each sample. In Alizarin red, and calcium colorimetric assays each sample had one repetition. The Medical Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (protocol no. 7003) approved all the techniques.

2.2. Ovariectomy

The OVX-rats were subjected to total ovariectomy as reported previously [30]. At 14 weeks post-surgery, the healthy and OVX-rats were submitted for computerized tomography (CT) scanning examination in order to establish OP.

2.3. CT Scanning

OP progress of ovariectomized animals was assessed qualitatively by CT scanning using multislides (kV = 100, ma = 50, sections = 2 mm, FOV = 240 mm, Toshiba, Aquilion 16, Japan) as compared to the healthy group. Bone density of tibias was reported in Hounsfield units (HU).

2.4. PBM and Alendronate Administration

An IR laser (Mustang 2000; Technical Co., Moscow, Russia) was used. The specifications of the laser was shown in Table 1.

The pulse wave (PW) PBM parameters of the current study was applied successfully on the experimental rat model complete fracture healing in healthy rats [24]. PWPBM started 14 weeks after surgery and was done on the both tibia three times weekly for 60 days (25 sessions). And whole length of tibias were completely irradiated with three laser shoots while the laser probe held vertical to the tibia from a distance lesser than 0.5 cm. Rats of groups 5 and 6 were received 0.5 mg/kg alendronate (Alborzdarou Company, Tehran, Iran) subdermally five days per week for 60 days. After that, all the rats were euthanized and the both tibiae were removed.

2.5. BMMSCS Preparation and Cell Culture

The BMMSCs were isolated from tibiae of entire euthanized rats and were cultured as reported previously [31].