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Ebselen rescues oxidative-stress-suppressed osteogenic differentiation of bone-marrow-derived mesenchymal stem cells via an antioxidant effect and the PI3K/Akt pathway

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ABSTRACT

Background: Patients with metabolic bone diseases often have high risk of titanium implant failure due to compromised bone regeneration ability. Clinical evidence indicates that the poor osteogenic ability is partly because of excessive oxidative stress. To date, specific treatments for these patients are urgently needed. Ebselen, a non-toxic organoselenium compound, is reported to be a potent antioxidant agent. In this study, we hypothesized that ebselen exerted protective effects on osteogenic differentiation of bone-marrow-derived mesenchymal stem cells (BMSCs) under oxidative stress.

Methods: BMSCs were isolated from SD rats, and their morphology and multiple differentiation abilities were characterized. Proliferation rates of BMSCs treated with different concentrations of ebselen were analyzed. Then BMSCs were pretreated by hydrogen peroxide (H_2O_2), after which ebselen at different concentrations (0, 1, 5, 10 μM) was added, alkaline phosphatase (ALP) activity, mineralization and osteogenic-related protein levels were evaluated and an optimum concentration of ebselen was selected. Subsequently, intracellular reactive oxygen species (ROS) generation and the role of the PI3K/AKT pathway were also investigated.

Results: Ebselen within a proper range could promote the proliferation of BMSCs. H_2O_2-induced oxidative stress suppressed osteogenic differentiation of BMSCs, which was verified by the decrease in ALP activity, calcium deposition, Runx2 and β-catenin expression. However, ebselen could alleviate osteogenic dysfunction of BMSCs. We also observed that ebselen reduced ROS accumulation in H_2O_2-pretreated BMSCs. Moreover, the pro-osteogenic effects afforded by ebselen were almost abolished by the Akt inhibitor.

Conclusion: We concluded that ebselen could attenuate osteogenic dysfunction of BMSCs induced by H_2O_2 through an antioxidant effect and the activation of the PI3K/Akt pathway, suggesting that ebselen has a potential therapeutic effect for patients with metabolic bone diseases.

1. Introduction

Titanium and its alloys are widely applied as bone implants, such as dental implants, prostheses, joint replacement, maxillofacial and craniofacial treatments, due to their favorable mechanical properties, including high corrosion resistance, superior biocompatibility and capability of osteointegration [1]. The success rate of titanium implants in the general population has been reported at a minimum of 95% [2]. However, the success rate in special populations such as those suffering with metabolic bone diseases (osteoarthritis, osteoporosis, etc.) is often compromised due to their unfavorable microenvironment in bone tissue, such as reactive oxygen species (ROS) accumulation and stem cell aging [3–5]. In recent years, many researchers have confirmed that oxidative stress caused by the overproduction of ROS heavily influenced the activity of osteoblasts and the process of new bone formation, thus inducing poor osteointegration and even implant failure [6–9]. Therefore, it is urgent that antioxidant drugs be discovered for patients suffering with metabolic bone diseases.

Ebselen [2-Phenyl-1,2-benzisoselenazol-3(2 H)-1] is an organoselenium compound of low molecular weight (274.18 Da) and with glutathione peroxidase (GPx)-like, thiol-dependent, hydroperoxide-reducing activity [10]. It can catalyze several essential reactions for the

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protection of cellular components from oxidative and free radical damage [11–13]. As a pleiotropic molecule, it interacts with multiple molecular targets inside cells, directly or indirectly. Reports have indicated that it is involved in many biological pathways, such as apoptosis [14,15], genomic integrity [16], immune systems [17] and cellular transport [18]. In addition, ebselen has achieved a good therapeutic effect in many diseases, such as attenuating neuronal cell death induced by ischemia/reperfusion [19], treating diabetes-related disorders or heart disorders [20,21] and acting as an effective detoxifying [22], antimicrobial [23] and chemotherapeutic agent [24]. It has also been reported that ebselen has the potential to prevent bone loss by suppressing receptor activator of nuclear factor kappa-B ligand-induced osteoclast differentiation *in vitro* and lipopolysaccharide-induced inflammatory bone destruction *in vivo*, which provides a clue that ebselen might modulate the process of bone regeneration [25].

However, it has not been elucidated whether ebselen as an exogenous agent could protect pre-osteoblasts from oxidative stress stimuli. Consequently, this study aimed to determine if ebselen could promote the osteogenic differentiation of BMSCs by inhibiting H_2O_2-induced oxidative stress and exploring the underlying mechanisms, thus providing a theoretical foundation for clinical applications.

2. Materials and methods

2.1. BMSC isolation, culture and characterization

BMSCs were harvested from the femurs and tibiae of 2-week-old male Sprague-Dawley (SD) rats. All animal experiments were approved by the Animal Care and Research Committee of Sun Yat-sen University. Briefly, rats were sacrificed by cervical dissection, after which the bilateral femur and tibia were isolated and the distal and proximal ends of the bones were dissected. Then, the whole bone marrow was flushed out with DMEM/F12 medium (Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) with the aid of a syringe. The cells were then cultured with DMEM/F12 medium containing 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (P/S; Gibco) in a humidified 5% CO_2 incubator at 37 °C. The BMSCs were digested with 1× trypLE reagent (Gibco) and passaged when 80–90% confluence was reached. Passages 3 to 5 were used in this study.

For characterization, BMSCs were seeded at a density of 2×10^5 cells/well in six-well plates. For evaluation of osteogenic differentiation, osteogenic induction medium [DMEM/F12 complete medium supplemented with 10^{-7} M dexamethasone (Sigma), 50 μM ascorbic acid (Sigma) and 10 mM β-glycerol phosphate (Sigma)] was applied to BMSCs. After 21-day incubation, cells were washed with PBS and fixed with 4% paraformaldehyde at room temperature for 30 min. After being washed with distilled water, cells were stained with alizarin red (Cyagen Biosciences, Guangzhou, China) for 60 min, after which calcium nodules were captured by inverted microscopy (Axio observer Z1, Zeiss, Jena, Germany). For evaluation of adipogenic differentiation ability, adipogenic induction medium (Cyagen Biosciences) was applied to BMSCs. In brief, BMSCs were cultured with component A for 3 days, after which A was removed and component B was added. After 1 day, B was replaced by A again. After 3 cycles, BMSCs were cultured with B for 7 days. At the indicated times, cells were washed with PBS and fixed with 4% paraformaldehyde at room temperature for 30 min. After being washed with distilled water, cells were stained with oil red O (Cyagen Biosciences) for 30 min, and then lipid droplets were captured by inverted microscopy.

2.2. Preparation of ebselen

Ebselen was purchased from Aladdin (Shanghai, China) and dissolved in dimethyl sulfoxide. DMEM/F12 complete medium or osteogenic induction medium containing different concentrations of ebselen was prepared. The final culture concentration of ebselen was lower than 0.5%.

2.3. Cell proliferation assays

Proliferation rates of BMSCs were analyzed by CCK8 assay. Cells were seeded at a density of 3000 cells/well in 96-well plates (n = 5). After being cultured for 12 h, the medium was replaced with DMEM/F12 medium containing different concentrations of ebselen (0, 1, 5, 10 and 20 μM). The culture medium was removed after 1, 3 and 5 days, then serum-free medium containing 10% CCK8 (Dojindo Molecular Technology, Kumamoto, Japan) was added to the 96-well plates followed by incubation at 37 ℃ for 2 h. Absorbance at 450 nm was measured with an automatic microplate reader (Infinite200, Tecan, Beijing, China).

2.4. Alkaline phosphatase (ALP) assay

BMSCs were seeded in 24-well plates at a density of 5×10^4 cells/well. After culture for 12 h, cells were treated with 100 μM H_2O_2 for 1 h, after which the medium was replaced by DMEM/F12 osteogenic induction medium containing different concentrations of ebselen. The medium was replaced every 3 days. After 7 days of culture, cells were washed 3 times with cold PBS and subjected to lysis with RIPA (KeyGen BioTECH, Nanjing, China) supplemented by 1% protease inhibitor cocktail and phosphate protease inhibitor (CWBIO, Beijing, China) for the extraction of total protein. The concentrations of protein were measured by means of a BCA protein assay kit (CWBIO). The ALP assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) was used to test the content of ALP by measuring hydrolysis of p-nitrophenyl phosphate according to the manufacturer's instructions. The ALP content was then normalized to total protein content.

2.5. Calcium deposition assay

BMSCs were seeded in 6-well plates at a density of 2×10^5 cells/well. After being treated with 100 μM H_2O_2 for 1 h, cells were incubated with osteogenic induction medium with the indicated concentrations of ebselen for 21 days. The calcium nodules were then stained by alizarin red as described in paragraph 2.1 above. For quantification, the bound stain was treated with 10% cetylpyridinium chloride solution for 30 min. The absorbance at 562 nm was tested by an automatic microplate reader (Infinite200, Tecan).

2.6. Protein extraction and Western blot

To assess the effect of ebselen on the osteogenic differentiation of BMSCs under oxidative stress stimuli, BMSCs were seeded in 6-well plates at a density of 2×10^5 cells/well. After treatment with 100 μM H_2O_2 for 1 h, cells were incubated with osteogenic induction medium at the indicated concentrations of ebselen for 10 days. For exploration of the role of the PI3K/Akt signaling pathway, BMSCs pretreated with H_2O_2 as mentioned above were divided into 3 groups, then cultured with osteogenic medium, osteogenic medium containing 5 μM ebselen and osteogenic medium containing 5 μM ebselen and 10 μM Akt inhibitor (LY294002, MedChemExpress, Monmouth Junction, NJ, USA), respectively, for 10 days.

At indicated time points, culture medium was removed and cells were washed 3 times with cold PBS on ice. The cells then underwent lysis in RIPA buffer containing 1% protease inhibitor cocktail and phosphate protease inhibitor. Then the lysates were collected and centrifuged at 12,000 rpm for 20 min at 4 °C. Protein concentrations were measured by BCA protein assay. A 30-μg quantity of total protein from each group was loaded, separated by 10% SDS-polyacrylamide gel electrophoresis and then transferred to PVDF membrane (EMD Millipore, Billerica, MA, USA). After non-specific-binding sites were blocked with 5% bovine serum albumin (BSA, CWBIO) for 60 min at room temperature, the membranes were incubated overnight at 4 °C with primary monoclonal antibody against runt-related transcription factor-2 (Runx2), β-catenin, p-AKT, AKT and GAPDH at 1:1000 (Cell Signaling Technology, Inc., Danvers, MA, USA) at 4 °C overnight. Then the membranes were washed 3 times with TBST for 10 min each and then incubated with horseradish-peroxidase-conjugated secondary IgG secondary antibody (Cell Signaling Technology, Inc.) at a 1:5000 dilution for 60 min at room temperature. The membranes were then washed 3 times with TBST, and the immunoreactive bands were visualized by the enhanced chemiluminescent detection system (Millipore). The intensities of the protein bands were quantified by densitometric scanning with ImageJ software (NIH, Bethesda, MD, USA). Runx2 and β-catenin were normalized to GAPDH, and p-AKT was normalized to AKT.

2.7. Intracellular reactive oxygen species (ROS) generation

The generation of ROS was measured by means of a 2,7-dichlorofluorescein diacetate (DCFH-DA) fluorescent probe (Beyotime Institute of Biotechnology, Haimen, Jaingsu, China). For visual inspection, BMSCs were seeded in a confocal laser dish and cultured with DMEM/F12 complete medium for 24 h. Then, BMSCs were treated by 100 μM H_2O_2 for 1 h, washed 3 times in DMEM/F12 medium and cultured with osteogenic induction medium supplemented with or without 5 μM ebselen for 24 h. Then the medium was removed, cells were washed with PBS and incubated with 10 μmol/L DCFH-DA at 37 ℃ for 20 min in the dark. After being washed 3 times with serum-free medium, DCF fluorescence was observed by confocal laser scanning microscopy (LSM780, Zeiss). For quantification of ROS, BMSCs were cultured in 6-well plates and treated as described above. Then cells were collected and incubated with DCFH-DA at 37 ℃ for 20 min in the dark. Subsequently, cells were washed 3 times with serum-free medium, centrifuged and resuspended in 500 μL PBS. Finally, the mixture was analyzed by flow cytometry (FC500ML, Beckman, Indianapolis, IN, USA).

2.8. Statistical analysis

All experiments were run in triplicate. All statistical data were analyzed with SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA). The results with normal distribution were expressed as means ± standard deviation. Statistical analyses were carried out with Student’s t-test or one-way analysis of variance (ANOVA). *P* values < 0.05 were considered to be significant.

3. Results

3.1. Characterization of BMSCs

BMSCs derived from SD rats were visualized by inverted microscopy and were found to display a fibroblast-like morphology (Fig. 1a,b). A key property of any MSC is its multi-directional differentiation ability. After treatment by osteogenic- and adipogenic-induced media for the indicated times, calcium nodules and lipid droplets formed (Fig. 1c,d), which indicated that BMSCs have multiple differentiation abilities.

3.2. Ebselen promoted the proliferation of BMSCs

The effect of ebselen on BMSC viability was measured by the CCK8 assay. BMSCs were treated with different concentrations of ebselen (0, 1, 5, 10, 20 μM), and different proliferation rates were observed. Results showed that, within 10 μM, cell proliferation rates increased with the increase of ebselen concentration. Ebselen at 10 μM had the highest effect on cell viability and was significantly higher than that in other groups, but when the concentration reached 20 μM, the proliferation rate showed a declining trend (Fig. 2). Therefore, we applied 0, 1, 5 and 10 μM ebselen in the following studies.

3.3. Ebselen improved the ALP activity of oxidative-damaged BMSCs

ALP is an early marker of osteogenic differentiation. The ALP activity of BMSCs was obviously weakened by H_2O_2, as shown in Fig. 3 (H_2O_2 group *vs* control group, $P < 0.01$). With the addition and increasing concentrations of ebselen, ALP activity recovered progressively. Activity in the 10 μM group was significantly higher than that in the 1 μM and 5 μM groups ($P < 0.05$) but was still lower than that in the control group ($P < 0.05$), which indicated that ebselen rescued the osteogenic differentiation of oxidative-damaged BMSCs.

3.4. Ebselen increased calcium deposition of oxidative-damaged BMSCs

Pretreatment of BMSCs with H_2O_2 reduced calcium deposition but could be reversed by ebselen. As shown in Fig. 4, visual inspection of alizarin red staining and quantification of calcium deposition *via* cetylpyridinium chloride dissolution indicated that H_2O_2 obviously decreased the formation of calcium nodules (H_2O_2 group *vs* control group, $P < 0.05$). However, quantitative analysis showed that the 5 μM and 10 μM ebselen groups obviously promoted calcium deposition (5 μM and 10 μM *vs* H_2O_2 group, $P < 0.05$). Moreover, the levels of calcium deposition in the 5 and 10 μM groups were all slightly higher than those in the control group but were not significantly different ($P > 0.05$). These results suggested that ebselen could recover the osteogenic differentiation ability of oxidative-damaged BMSCs.

3.5. Ebselen enhanced osteogenic-related protein expression levels in oxidative-damaged BMSCs

To assess the influence of ebselen on the osteogenic differentiation of BMSCs after oxidative damage, we also analyzed Runx2 and β-catenin expression levels after 10-day incubation (Fig. 5). Results revealed that treatment with H_2O_2 resulted in decreased Runx2 expression in BMSCs (H_2O_2 group *vs* control group, $P < 0.05$). However, the expression levels of Runx2 were distinctly improved in the 5 μM and 10 μM groups and were not significantly different from those in the control group ($P > 0.05$). As for β-catenin, results showed that the expression level in oxidative-damaged BMSCs was obviously elevated in the 5 μM and 10 μM groups compared with that in the H_2O_2 group, which had the same trend with Runx2.

3.6. Ebselen alleviated ROS production in H2O2-mediated oxidative-damaged BMSCs

To determine whether ebselen could alleviate H_2O_2-induced oxidative damage in BMSCs, intracellular ROS production was evaluated. Confocal laser scanning microscopy inspection and flow cytometry fluorescence quantification both showed that BMSCs treated by H_2O_2 had the highest level of fluorescence (Fig. 6). With the addition of ebselen, fluorescence levels decreased and were significantly lower than those in the H_2O_2 group ($P < 0.05$). These results indicated that ebselen was involved in radical scavenging, which was beneficial to the osteogenic differentiation of BMSCs.

3.7. The effect of ebselen in promoting oxidative-damaged BMSC osteogenic differentiation was abolished by inhibition of the PI3K/Akt pathway

To elucidate the association between the PI3K/AKT pathway and ebselen-enhanced osteogenic differentiation, H_2O_2-pretreated BMSCs were incubated with or without 5 μM ebselen and the 10 μM Akt inhibitor. Results showed that ebselen significantly increased Runx2 and p-Akt expression levels in oxidative-damaged BMSCs (H_2O_2 + ebselen group *vs* H_2O_2 group, $P < 0.05$). However, BMSCs treated with both ebselen and the Akt inhibitor did not show enhanced Runx2 and p-Akt

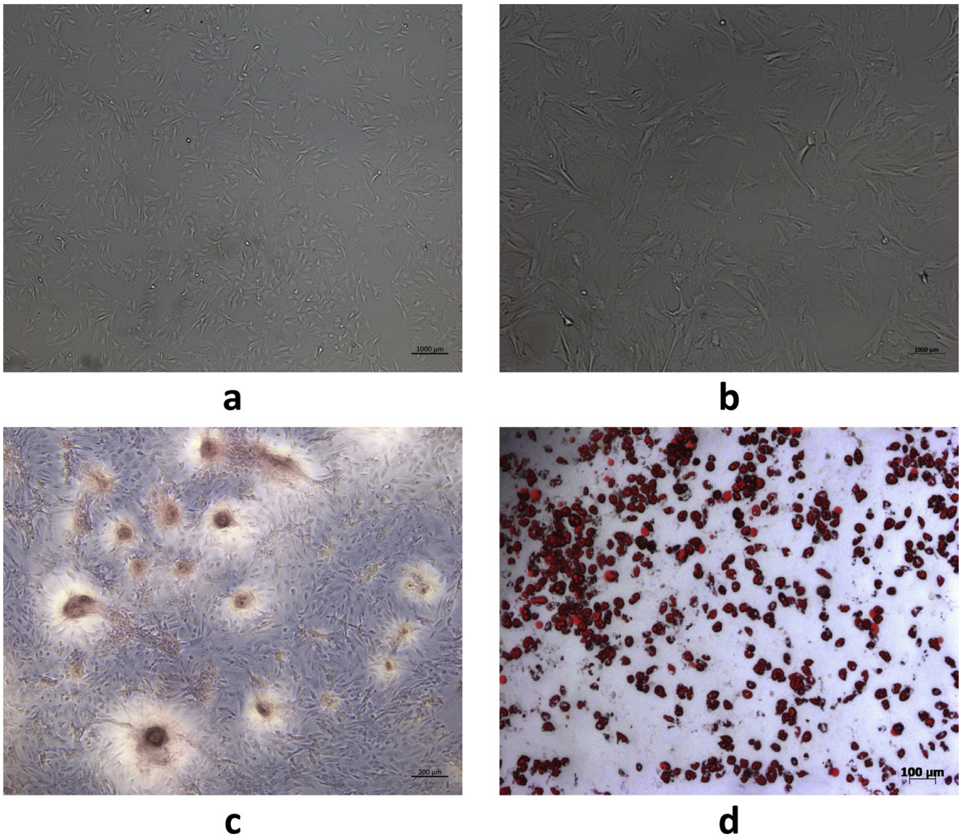

Fig. 1. Characterization of bone-marrow-derived mesenchymal stem cells (BMSCs). BMSCs (P3) were cultured in complete DMEM/F12 medium for 3 days and visualized by inverted microscopy at low (**a**) and high (**b**) magnifications. **c:** Calcium nodules formed after osteogenic induction (stained with Alizarin red). **d:** Lipid droplets formed after adipogenic induction (stained with oil red O).

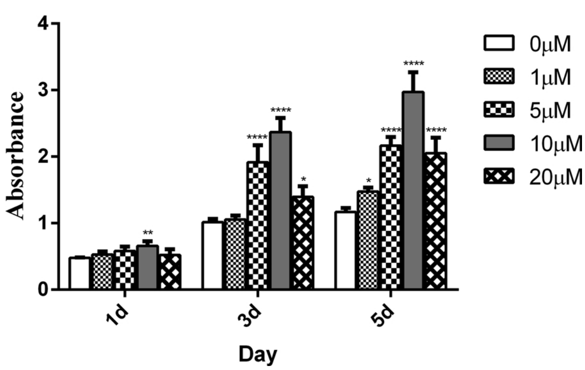

Fig. 2. BMSC proliferation rates after co-culture with different concentrations of ebselen for 1, 3, 5 days. *$P < 0.05$, **$P < 0.01$ and ****$P < 0.0001$ *vs* the 0 μM group (n = 5).

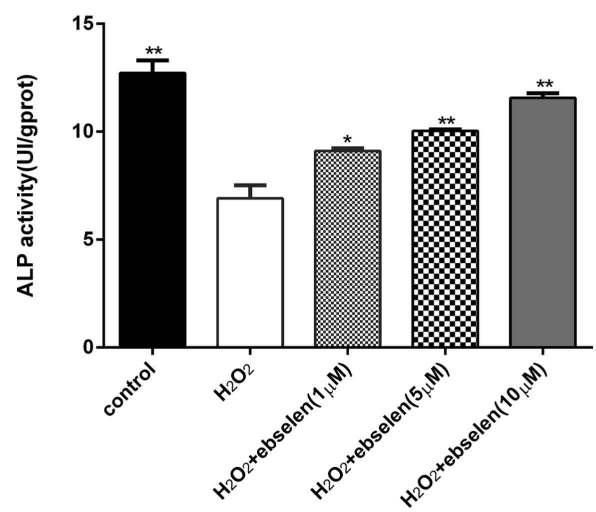

Fig. 3. Effects of ebselen on ALP activity after 7 days' osteogenic differentiation; *$P < 0.05$ and **$P < 0.01$ *vs* the H_2O_2 group.

expression levels (Fig. 7). These results suggested that the protective effects of ebselen might be partly due to the activation of the PI3K/AKT signaling pathway.

4. Discussion

Titanium implants are considered a successful treatment for replacing joints and missing teeth and for restoring esthetics due to their excellent biological and mechanical properties [2]. Recently, it has been reported that patients with metabolic bone diseases often have higher failure rates than individuals in the general population due to oxidative stress conditions [26]. With aging and social development, the number of patients with metabolic bone diseases is growing rapidly, so drugs that can specifically modulate the microenvironment for these patients are urgently needed. In the present study, we aimed to investigate whether ebselen had an antioxidant effect on pre-osteoblasts. The major findings of this study are as follows: first, H_2O_2 resulted in the inhibition of the osteogenic differentiation ability of BMSCs; second, the addition of ebselen rescued osteogenic differentiation of BMSCs under oxidative stress stimuli; finally, the protective effect of ebselen was partly due to the antioxidant effect and activation of the PI3K/Akt pathway. Thus, ebselen could be an effective strategy to improve bone regeneration ability in patients with metabolic bone diseases.

Metabolic bone diseases are a diverse group of conditions characterized by abnormalities in calcium metabolism and/or bone cells, which often lead to increased risk of bone fracture and low bone regeneration ability [27]. The incidence of metabolic bone diseases is closely related to aging, and one of the existing theories accounting for this is free radical (FR) or ROS accumulation [28]. It is known that ROS, as an important inflammatory mediator with local and distant

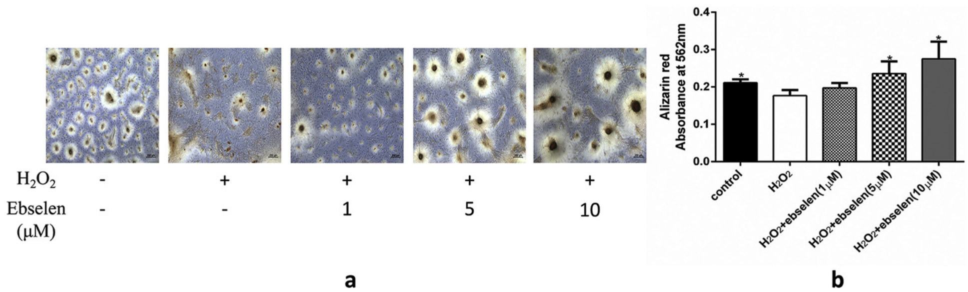

Fig. 4. Mineralization of BMSCs after osteogenic induction for 21 days. **a:** Alizarin red staining showed formation of calcium nodules. **b:** Quantification of calcium nodules after being dissolved in 10% cetylpyridinium chloride solution. *$P < 0.05$ *vs* the H_2O_2 group.

pathophysiological effects, has been demonstrated to link with osteogenic differentiation and bone regeneration. Low levels of ROS act as regulatory mediators in signaling processes [26,29,30], which are beneficial to bone regeneration. However, excessive generation of ROS often leads to suppression of Wnt/β-catenin and the Hedgehog signaling pathway and activation of the FOXO signaling pathway, thus inducing impaired bone regeneration [31–33]. Consistent with previous studies, we found that H_2O_2-induced oxidative stress resulted in reduction of ALP activity, calcium deposition and osteogenic-related protein expression levels in BMSCs [34]. Therefore, drugs that specifically target oxidative stress can improve the osteogenic differentiation of BMSCs.

Ebselen, as a synthetic organoselenium compound, has attracted much attention as therapy for diseases related to the neural and cardiovascular systems, due to its antioxidant effect [12,35]. Coincident with these results, we found that ebselen could rescue the osteogenic differentiation ability of oxidative-damaged BMSCs. Further, we also found that the effect of ebselen varied with its concentrations. Results showed that ALP, which is a cell-membrane-associated enzyme and acts as an early osteogenic differentiation marker, was elevated in 1, 5 and 10 μM groups at day 7 compared with the H_2O_2 group, and all were lower than in the control group (Fig. 3). However, on day 10, the expression levels of the osteogenic-related proteins Runx2 and β-catenin in the 5 μM and 10 μM groups were all obviously improved compared with those in the H_2O_2 group and were not significantly different from those in the control group (Fig. 5). Finally, on day 21, alizarin red staining was done to assess matrix mineralization formation, which indicated the final effect of ebselen on oxidant-damaged BMSCs. Calcium deposition in the 5 μM and 10 μM groups were obviously improved compared with the H_2O_2 group. Moreover, we found that 5 μM and 10 μM groups slightly exceeded the control group, while the differences between and among them were not significant (Fig. 4). Therefore, 5 μM ebselen was selected to be the optimum concentration in the present study. Intracellular ROS levels detected by a DCFH-DA fluorescence probe showed that ebselen down-regulated DCF fluorescence in oxidative-damaged BMSCs, indicating that ebselen could confront oxidative stress and be beneficial to bone regeneration.

It is known that the osteogenic differentiation of osteoblasts is closely connected with many signaling pathways. Among them, the PI3K/Akt signaling pathway has been reported to play a critical role in cell proliferation, differentiation and mineralization. The phosphorylation level of Akt is an indicator of the osteogenic differentiation of osteoblasts [36,37]. Moreover, the activation of the PI3K/Akt pathway has also been reported to serve as a negative regulator of oxidative stress [38]. Ying and colleagues [39] showed that high glucose-induced oxidative stress suppressed osteogenic differentiation and PI3K and Akt

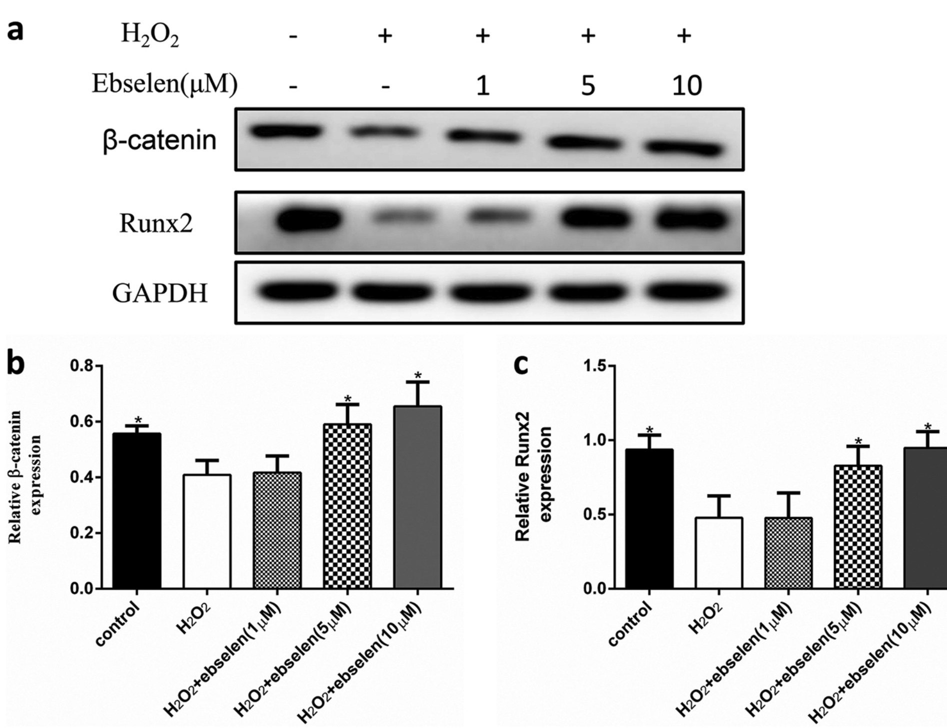

Fig. 5. The effects of ebselen on expression levels of osteogenic-related proteins. **a:** Representative Western blot scans of each protein. **b, c:** ImageJ software was used for semiquantitative analyses of β-catenin and Runx2 expression. *$P < 0.05$ *vs* the H_2O_2 group.

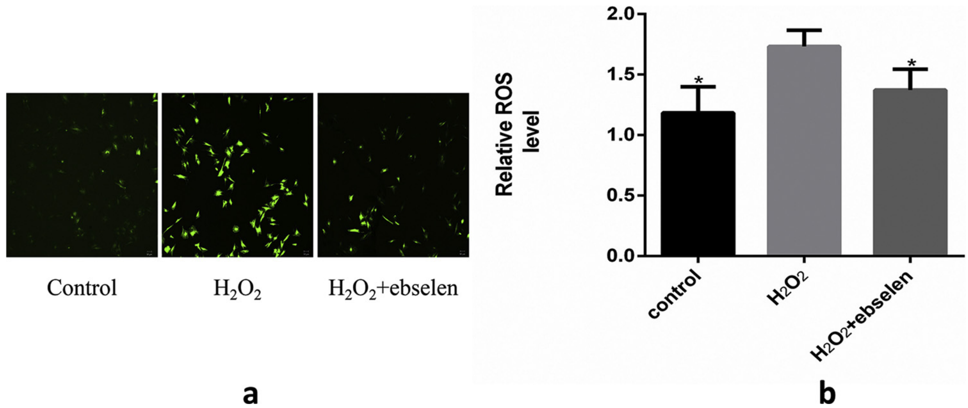

Fig. 6. Effects of ebselen on intracellular reactive oxygen species (ROS) production. **a:** ROS production in BMSCs was observed under confocal laser scanning microscopy by DCFH-DA. **b:** ROS production in BMSCs was assessed by flow cytometry. *$P < 0.05$ *vs* the H_2O_2 group.

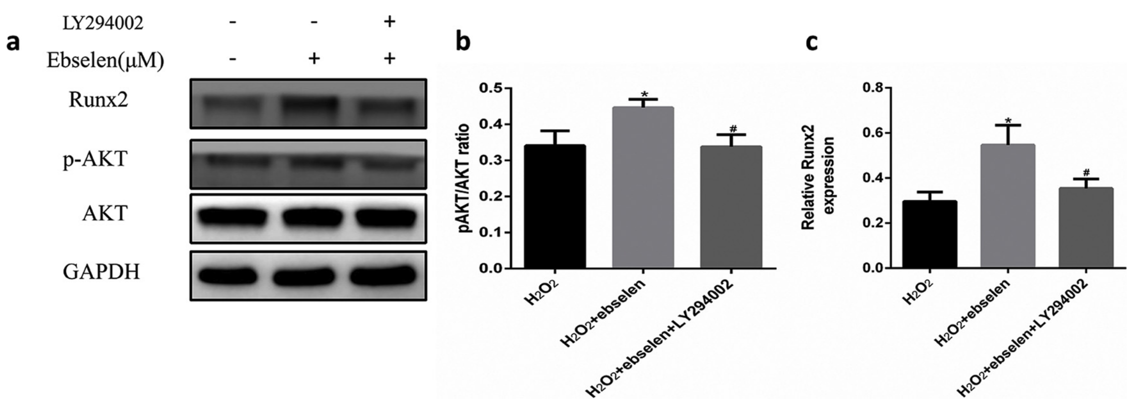

Fig. 7. Runx2 and p-Akt expression levels in H_2O_2-pretreated BMSCs were determined after incubation with or without ebselen and Akt inhibitor. **a:** Representative Western blot scans of each protein. **b, c:** ImageJ software was used for semiquantitative analyses of p-AKT and Runx2 expression. *$P < 0.05$ *vs* the H_2O_2 group; #$P < 0.05$ *vs* the H_2O_2 + ebselen group.

phosphorylation expression levels, but when treated together with silibinin, PI3K, Akt phosphorylation expression levels and osteogenic differentiation were enhanced simultaneously. Similarly, Ma et al. [40] also proved that glimepiride promoted osteogenic differentiation of rat osteoblasts *via* the PI3K/Akt/eNOS pathway in a high-glucose microenvironment. In accord with these studies, we found that when we treated BMSCs with ebselen after H_2O_2 stimulation, the Akt phosphorylation levels increased compared with those in the H_2O_2 group, which had the same trend with Runx2 expression levels. But when Akt was inhibited, increased Runx2 expression levels were eliminated, suggesting that ebselen promoted the osteogenic differentiation of BMSCs under oxidative stress, partly by the modulation of the PI3K/Akt signaling pathway.

In conclusion, our study demonstrated that oxidative stress stimuli by H_2O_2 suppressed osteogenic differentiation of BMSCs, which was verified by reductions in ALP activity, calcium deposition and Runx2 and β-catenin expression levels. Importantly, ebselen scavenged free radicals and promoted osteogenic differentiation of oxidative-stress-damaged BMSCs, and 5 μM ebselen was proved to be the optimum concentration. The recovery of osteogenic ability was partly through the modulation of the PI3K/Akt signaling pathway.

In addition to our findings in this study, future studies should aim to elucidate other molecular signaling pathways that may mediate the protective effect of ebselen against oxidative stress. Since *in vitro* studies cannot totally mimic the *in vivo* situation, studies are still needed to confirm the *in vivo* biological function of ebselen. Further, ebselen-modified titanium implants will be developed to explore its potential applications in patients with metabolic bone diseases.

Conflict of interest

The authors declare that they have no conflicts of interest.

Declaration of interests

Yiming Li, Guanhui Chen, Yi He, Xiliu Zhang, Binghui Zeng, Chao Wang, Chen Yi, Dongsheng Yu declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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