Pterostilbene promotes the osteogenic differentiation of MC3T3-E1 cells inhibited by H_2O_2 by activating the AMPK signaling pathways

Haiyan Li[1], Jiang Guo[2], Dong Zhang[3], Jihong Huang[3], Xuejia Li[3]*

[1]Department of Endocrinology, Shenzhen Second People's Hospital (the First Affiliated Hospital of Shenzhen University), Shenzhen, Guangdong Province, China; [2]Department of Ophthalmology, Shenzhen Second People's Hospital (the First Affiliated Hospital of Shenzhen University), Shenzhen, Guangdong Province, China; [3]Department of Orthopedics, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

***Corresponding Author:** Xuejia Li, Department of Orthopedics, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, No. 110 Ganhe Road, Hongkou District, Shanghai 200437, China. Email: lixuejia11115@163.com

Received: 16 November 2021; Accepted: 7 December 2021; Published: 20 January 2022

ORIGINAL ARTICLE

Abstract

Osteoporosis is one of the common degenerative skeletal diseases whose essential mechanism is the imbalance between bone resorption and formation. Currently, the close coordination of signaling pathways at genetic level during bone development is a hot topic of osteoporosis research. The present study is focused on whether pterostilbene protects against hydrogen peroxide (H_2O_2)-induced osteoblastic cell apoptosis, oxidative stress, and reveals the related underlying mechanisms. MC3T3-E1 osteoblastic cells were cultured and treated with different concentrations of H_2O_2 (0–1 mM) along with or without pterostilbene, and MTT assay or Annexin V-FITC/propidium iodide staining was applied for measuring cell viability and apoptosis. The *in vitro* cellular antioxidant analysis was performed using 2,7-dichlorodihydrofluorescein diacetate assay and enzyme-linked-immunosorbent serologic assay against glutathione peroxidase and malondialdehyde. In addition, cellular alkaline phosphatase activity was executed by Alizarin Red S staining. Western blot assay was conducted to determine the expression levels of osteogenic-related markers, including type I collagen, osteopontin, runt-related transcription factor 2, and the 5′ adenosine monophosphate-activated protein kinase (AMPK) signaling pathways related proteins. The key finding was that pterostilbene could attenuate the H_2O_2-induced cellular apoptosis and oxidative stress. Pterostilbene also promoted osteogenic differentiation in H_2O_2-treated MC3T3-E1 cells through activation of the AMPK pathway. In conclusion, pterostilbene blocked the H_2O_2-induced MC3T3-E1 cells dysfunction, indicating its potential to be a promising medication for treating osteoporosis.

Keywords: pterostilbene; osteogenic differentiation; osteoporosis; AMPK pathway; MC3T3-E1

Introduction

Osteoporosis is a systemic bone disease characterized by reduction in mineral density and bone mass and deterioration of skeletal structure. The pathological progress of osteoporosis may be caused by imbalance between bone formation and resorption. Recent studies have reported that production of intracellular reactive oxygen species (ROS) can induce osteoblast apoptosis, thereby promoting the progression of osteoporosis (Liu *et al.*, 2019b).

In addition, overexpression of ROS could significantly attenuate osteogenic differentiation of MC3T3-E1 cells (Zhang *et al.*, 2020). Accumulating evidence suggests that protective autophagy in cells could alleviate the development process of osteoporosis (Li *et al.*, 2017). Furthermore, appropriate activation of autophagy can moderate the apoptosis of MC3T3-E1 cells induced by oxidative stress, and autophagy can also promote osteogenic differentiation in MC3T3-E1 cells (Feng *et al.*, 2020; Li *et al.*, 2019).

Pterostilbene (trans-3,5-dimethoxy-4-hydroxystilbene), a natural component extracted from blueberry, is a dimethylated analog of resveratrol which has greater lipophilicity and a higher potential for cellular uptake with powerful physiological functions and bioavailability. Pterostilbene's characteristic feature is that as an antioxidant it has suppressive effects on inflammatory response, oxidative stress, and apoptosis in mammalian cells. Moreover, it is also evidenced as an effective anticancer agent in several common malignancies. A previous study has suggested that pterostilbene could inhibit the cell stemness of cervical cancer (Shin *et al.*, 2020). Pterostilbene prevents hepatocyte epithelial–mesenchymal transition in fructose-induced liver fibrosis by suppressing miR-34a/Sirt1/p53 and TGF-β1/Smads signaling pathways (Song *et al.*, 2019). Pterostilbene can also improve myocardial fibrosis by modulating the Pitx2c/miR-15b pathway induced by ROS (Ko *et al.*, 2015). Furthermore, pterostilbene demonstrated neuroprotective effect against astrocyte inflammation and neuronal oxidative stress, thereby alleviating ischemia/reperfusion-induced nerve injury through inhibition of NF-κB pathway (Liu *et al.*, 2019c). Pterostilbene also has potential in orthopedic treatment and might restrain the formation of osteoclasts and reduce bone loss via suppressing the phosphorylation of Mitogen-activated protein kinase (MAPK) pathway (Nikhil *et al.*, 2015). Additionally, numerous studies have demonstrated that pterostilbene could promote autophagy (He *et al.*, 2018; Wang *et al.*, 2020) and activate the 5′ adenosine monophosphate-activated protein kinase (AMPK) pathway by stimulating the osteogenic differentiation of MC3T3-E1 cells (Cheng *et al.*, 2019; Liu *et al.*, 2019a; Yang *et al.*, 2015). The present investigation scrutinized pterostilbene's therapeutic potency and explored the underlying signaling mechanism on hydrogen peroxide (H_2O_2)-induced cell apoptosis, oxidative stress, and inhibition of osteogenic differentiation.

Methods

Cell culture

MC3T3-E1 is an osteoblast precursor cell line derived from newborn mouse calvaria. The cells were purchased from the American Type Culture Collection and maintained in α-minimum essential medium containing 10% fetal bovine serum (FBS) at 37°C in 5% CO_2. Cells were harvested when reached approximately 80% confluence to execute the following experiments. MC3T3-E1 cells were treated with H_2O_2 (0–1 mM) and pterostilbene (0.125–2 mM) for 12 h. MC3T3-E1 cells were divided into the following five groups: control, H_2O_2, H_2O_2 + 0.25-mM pterostilbene, H_2O_2 + 0.5-mM pterostilbene, and H_2O_2 + 1-mM pterostilbene. All the experiments were repeated thrice.

MTT assay

MC3T3-E1 cells were seeded at a concentration of 1 × 10^5/well on 96-well plates for 24 h. When cells reached 80% confluence, different concentrations of pterostilbene (0.125–2 mM) were added 1 h prior to H_2O_2 treatment. After treatment, cells were incubated with 10 μL of 5 mg/mL 3-(4, 5-dimethylthiazol-2-Yl)-2, 5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA). After incubation for 4 h at 37°C, formazan crystals were solubilized in dimethyl sulfoxide (DMSO). The microplate reader was used to recorded the OD value of each well at 570 nm.

Flow cytometry

The flow cytometry Annexin V-FITC/PI apoptosis detection kit (Thermo Fisher Scientific, Inc., USA) was used to determine the apoptosis of MC3T3-E1 cells after exposure to H_2O_2 and pterostilbene. MC3T3-E1 cells were harvested and resuspended at a concentration of 5 ×10^5 cells/mL. FITC-Annexin V and p*ropidium Iodide* (PI) were added and kept on ice in the dark for 20 min. Finally, the apoptotic positive cell ratio was quantified by flow cytometry.

Enzyme-linked immunosorbent assay (ELISA)

The expression levels of glutathione peroxidase (GPx) and malondialdehyde (MDA) in the supernatants of different groups of MC3T3-E1 cells were determined at room temperature using commercial ELISA kits according to the manufacturer's protocol (R&D Systems, USA).

ROS measurement (DCFDA staining)

MC3T3-E1 cells were seeded at a concentration of 1 × 10^6 cells/well on 24-well plates, allowed to confluence to 80% overnight, and then treated with H_2O_2 (0–1 mM)

and pterostilbene (0.125–2 mM) for 12 h. After that, the cells were cultured with 2',7'-dichlorodihydrofluorescein-diacetate (H2DCFDA; Sigma-Aldrich), used as an ROS indicator, to detect intracellular ROS level. The mean fluorescence intensity was determined with an inverted fluorescent microscope using an excitation/emission wavelength of 535 nm.

Western blot assay

MC3T3-E1 cells were extracted using lysis buffer, including protease inhibitors, according to manufacturer's instructions. Protein concentration was quantified by bicinchoninic acid (BCA) protein assay kit (Sigma-Aldrich), and 30 μg of protein was separated with 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Millipore Sigma, Burlington, MA, USA). The membranes were incubated overnight at 4°C with primary antibodies against type I collagen (COL1A1, 1:3,000; Cell Signaling Technology, USA), osteopontin (OPN, 1:2,000; Invitrogen, Carlsbad, CA, USA), runt-related transcription factor 2 (Runx2, 1:3,000; Abcam, Cambridge, UK), p-AMPK (1:3,000; Abcam), AMPK (1:8,000; Abcam), β-actin (1:10,000; Invitrogen), and subsequently incubated with secondary antibodies for 1 h. Protein signals were measured using the enhanced chemiluminescence system (Bio-Rad Clarity Western ECL, Hercules, CA, USA) and analyzed using the ImageJ software (http://rsb.info.nih.gov/ij/; Bethesda, MD, USA) (Shu *et al*., 2020).

Cellular alkaline phosphatase (ALP) measurement (Alizarin Red S Staining)

MC3T3-E1 cells were seeded at a concentration of 1×10^5/well on 12-well plates to assess the situation of osteogenic differentiation. After exposure to H_2O_2 and pterostilbene, MC3T3-E1 cells were fixed with 4% paraformaldehyde and stained using Alizarin red S dye (Abcam) for 30 min at room temperature. The stained cells were washed again with Dulbecco's phosphate-buffered saline (DPBS), and images were captured using a light microscope with digital camera.

Statistics

All results were performed in triplicate to ensure reproducibility, and analyzed using SPSS-17.0. All data were presented as mean ± SD. One-way ANOVA followed by Bonferroni's post-hoc test was used to make comparison, and $P < 0.05$ indicated that the difference was statistically significant.

Results

Effect of pterostilbene on the viability and apoptosis of H_2O_2-treated MC3T3-E1 cells

In order to determine the effect of H_2O_2 on cell viability, MC3T3-E1 cells were treated with H_2O_2 at different concentrations (0–1 mM) for 12 h, and the viability was measured by MTT assay. The results revealed that H_2O_2 attenuated the cell viability of MC3T3-E1 cells in a dose-dependently manner (Figure 1A). On the other hand, compared to the control group, the cell viability demonstrated no significant difference in MC3T3-E1 cells treated with pterostilbene at different concentrations (0.125–2 mM; Figure 1B). Next, effect of pterostilbene on the viability and apoptosis of H_2O_2-treated MC3T3-E1 cells was evaluated. Results from MTT and Annexin V apoptosis assays indicated that pterostilbene promoted cell viability but suppressed the apoptosis of H_2O_2-treated MC3T3-E1 cells in a dose-dependent manner (Figures 1C and D). These data suggested that pterostilbene had potential for attenuating the apoptosis of H_2O_2-induced MC3T3-E1 cells.

Effect of pterostilbene on oxidative stress in H_2O_2-treated MC3T3-E1 cells

In order to determine whether pterostilbene had protective effect on H_2O_2-induced oxidative stress, the levels of GPx and MDA were evaluated in MC3T3-E1 cells. After treatment with pterostilbene (0.25, 0.5, and 1.0 mM) and H_2O_2 (1 mM), the MDA level was remarkably enhanced in the H_2O_2 treatment group but pterostilbene suppressed the simulative effects of H_2O_2 on MDA level in a dose-dependent manner (Figure 2A). The level of GPx was significantly reduced after H_2O_2 treatment. Conversely, co-treatment of H_2O_2 with pterostilbene could significantly reverse the suppressive effects of H_2O_2 on GPx level in a dose-dependent manner (Figure 2B). Moreover, the 2'-7'dichlorofluorescin diacetate (DCFH-DA) staining was conducted to evaluate ROS production in MC3T3-E1 cells. The level of ROS in H_2O_2-treated group had increased significantly whereas ROS production was suppressed after co-treatment with different concentrations of pterostilbene (Figure 2C). These results indicated that pterostilbene produced significant reduction in the H_2O_2-induced oxidative stress in MC3T3-E1 cells.

Effect of pterostilbene on osteogenic differentiation in H_2O_2-treated MC3T3-E1 cells

In order to understand the effects of pterostilbene on the osteogenic differentiation of H_2O_2-treated MC3T3-E1 cells, MC3T3-E1 cells were exposed to

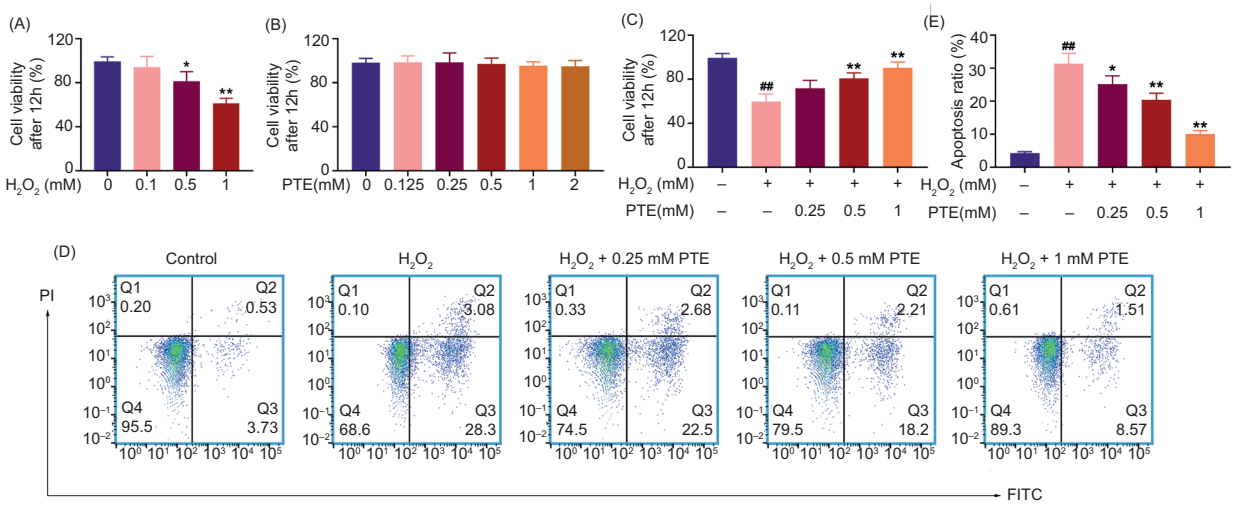

Figure 1. Effect of pterostilbene on the viability and apoptosis of H_2O_2-treated MC3T3-E1cells. (A) The relative viability of MC3T3-E1 cells treated with H_2O_2 at concentrations of 0.1, 0.5, and 1.0 mM. (B) The relative viability of MC3T3-E1 cells treated with pterostilbene at concentrations of 0.125, 0.25, 0.5, 1.0 and 2.0 mM. (C) The relative viability of MC3T3-E1 cells treated with pterostilbene at concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 mM in H_2O_2 exposure. (D) and (E) The cell apoptotic rates in different groups were quantified by Annexin-V and PI double-stained flow cytometry. Data were presented as mean ± SD. $^{*}P < 0.05$ compared with the control group or H_2O_2 group, $^{**}P < 0.01$ compared with the control group or H_2O_2 group; $^{\#\#}P < 0.01$ compared with the control group.

different concentrations of pterostilbene after treating with 1-mM H_2O_2. The osteogenic-related markers, including COL1A1, OPN, and Runx2, were determined by Western blot assay. In this study, H_2O_2 treatment suppressed the levels of osteogenic-related proteins. As shown in Figure 3A, the expression levels of COL1A1, OPN, and Runx2 in the co-treated H_2O_2 + pterostilbene groups were significantly higher than in the H_2O_2 group. To further determine whether pterostilbene had protective effect on osteogenic differentiation, we examined the activity of ALP, which is a biochemical marker of osteoblasts, after exposure to pterostilbene and H_2O_2. The ALP activity was visualized by ALP enzyme histochemistry, where 1-mM pterostilbene generated the highest improvement in ALP activity compared to the H_2O_2 group (Figure 3B). These findings strongly suggested that pterostilbene could protect against the H_2O_2-induced inhibition of osteogenic differentiation.

Effect of pterostilbene on the activation of AMPK signaling pathway

The AMPK signaling pathway is reported to be involved in osteogenic differentiation. Therefore, the molecular mechanisms underlying the relationship between AMPK signaling pathway and pterostilbene-induced osteogenic differentiation in MC3T3-E1 cells was explored. Compared to the control group, the phosphorylation level of AMPK was evidently reduced in the cells exposed to 1-mM H_2O_2. Conversely, Western blot analysis revealed that pre-incubation with pterostilbene improved the phosphorylation level of AMPK and reversed the H_2O_2-induced effect in a dose-dependent manner (Figure 4). Taken together, these results indicated that pterostilbene could reverse the inhibited osteogenic differentiation of MC3T3-E1 cells caused by H_2O_2 through the activation of AMPK pathway.

Discussion

The key finding of this study was that pterostilbene has potential in attenuating the H_2O_2-induced cell apoptosis and oxidative stress. Pterostilbene could promote the osteogenic differentiation of inhibited H_2O_2-treated MC3T3-E1 cells through the activation of AMPK pathway. Osteoporosis is one of the common degenerative skeletal diseases that causes millions of bone fractures yearly (Pouresmaeili *et al.*, 2018). It is commonly encountered in elderly population and postmenopausal women. The treatment of osteoporosis makes the world to spend billions of dollars every year, including drug intervention and hospitalization costs. Osteoporosis is characterized by destruction of bone microstructure and bone density reduction, and is caused by imbalance between bone resorption and formation (Bouxsein, 2003; Choksi *et al.*, 2018). The current limited understanding of the potential mechanisms of osteoporosis obstructs the development of therapeutic approach to osteoporosis. Although there are multiple treatment methodologies, people are more concerned about the adverse and long-term effects

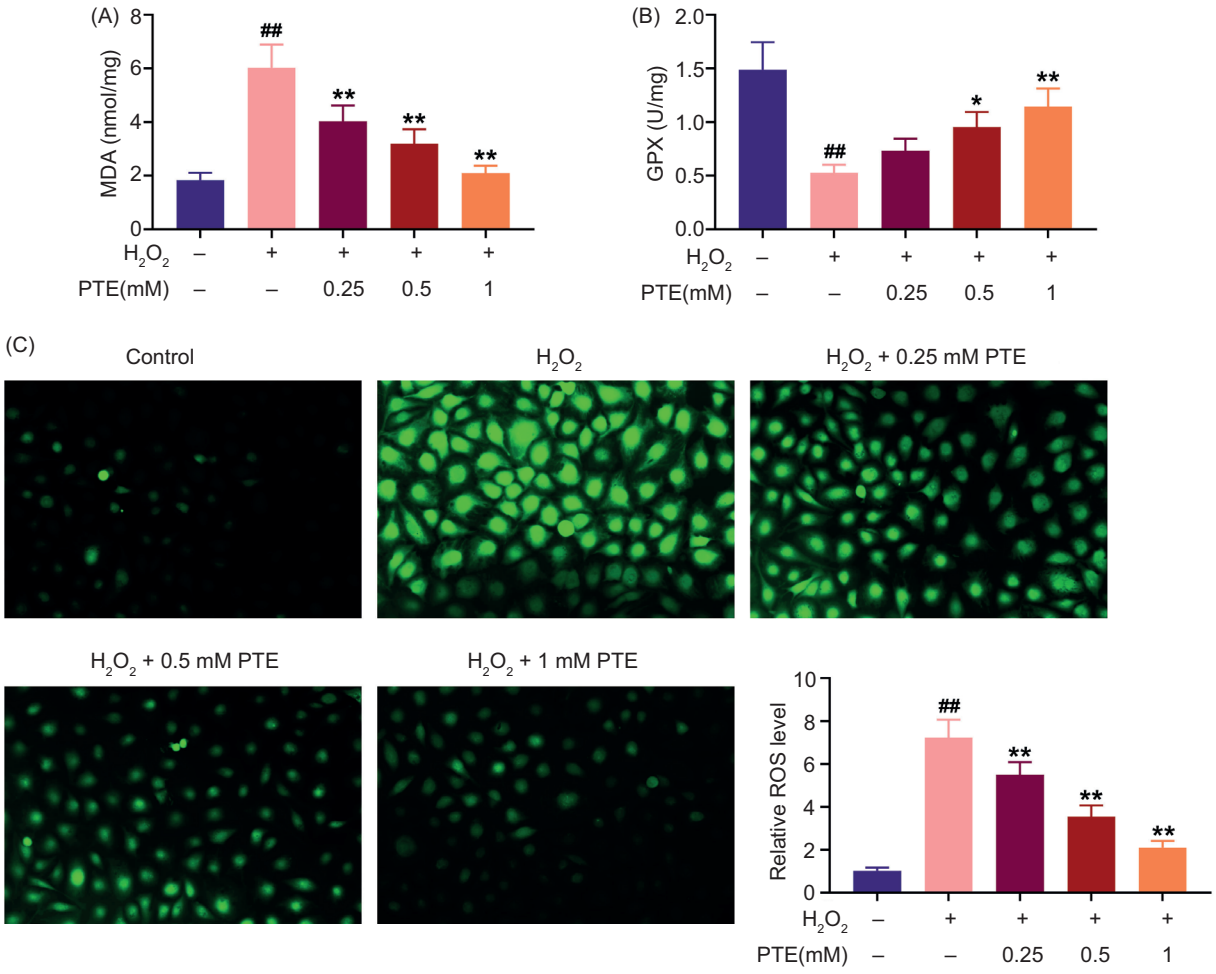

Figure 2. Effect of pterostilbene on H_2O_2-induced oxidative stress in MC3T3-E1 cells. (A) The level of MDA was measured by MDA ELISA kit. (B) The level of GPx was measured by GPx ELISA kit. (C) The ROS production was detected by DCFH-DA staining and observed under fluorescence microscopy. Data were presented as mean ± SD. $^{*}P < 0.05$ compared with the H_2O_2 group, $^{**}P < 0.01$ compared with the H_2O_2 group; $^{\#\#}P < 0.01$ compared with the control group.

of currently available palliative drugs (Chan *et al.*, 2016). Currently, many factors are known to induce osteoporosis, and the molecular basis of pathological progress may be having diversity under different conditions of osteoporosis. Especially, close coordination between transcription network and signaling pathways in the process of bone development at the genetic level is the hot topic of recent research in osteoporosis. Pterostilbene, as an antioxidant, has gained increasing attention because of its functions in the potential prevention and treatment of chronic diseases, including cancer, atherosclerosis, coronary heart disease, diabetes, and neurological degeneration (McCormack and McFadden, 2012, 2013). Previous studies have suggested that ROS concentration beyond a certain level could disturb the oxidant–antioxidant balance and lead to many inflammatory diseases, such as osteoporosis (Abdollahi *et al.*, 2005; Schröder, 2015). ROS could induce cell damage through DNA destruction, and stimulation of some signaling pathways trigger the osteoclastic bone resorption. Recently, pterostilbene has been established as a promising medication to alleviate the inflammation-induced degeneration of cartilage, since it could inhibit the expression of inflammatory mediators, including inducible nitric oxide synthase (iNOS), nitric oxide (NO), and intracellular ROS production, in chondrocytes (Pan *et al.*, 2008; Xue *et al.*, 2017). The present study rigorously investigated the cellular mechanisms responsible for the protective effect of pterostilbene on osteogenic differentiation. Therefore, it explored the role of pterostilbene in the inhibition of ROS production and apoptosis in osteoblast induced by H_2O_2. The results revealed that cell viabilities increased after treatment with H_2O_2 and caused the inactivation of AMPK signaling pathway. However, pterostilbene suppressed the H_2O_2-induced cell apoptosis and oxidative stress, and improved the survival rate of MC3T3-E1

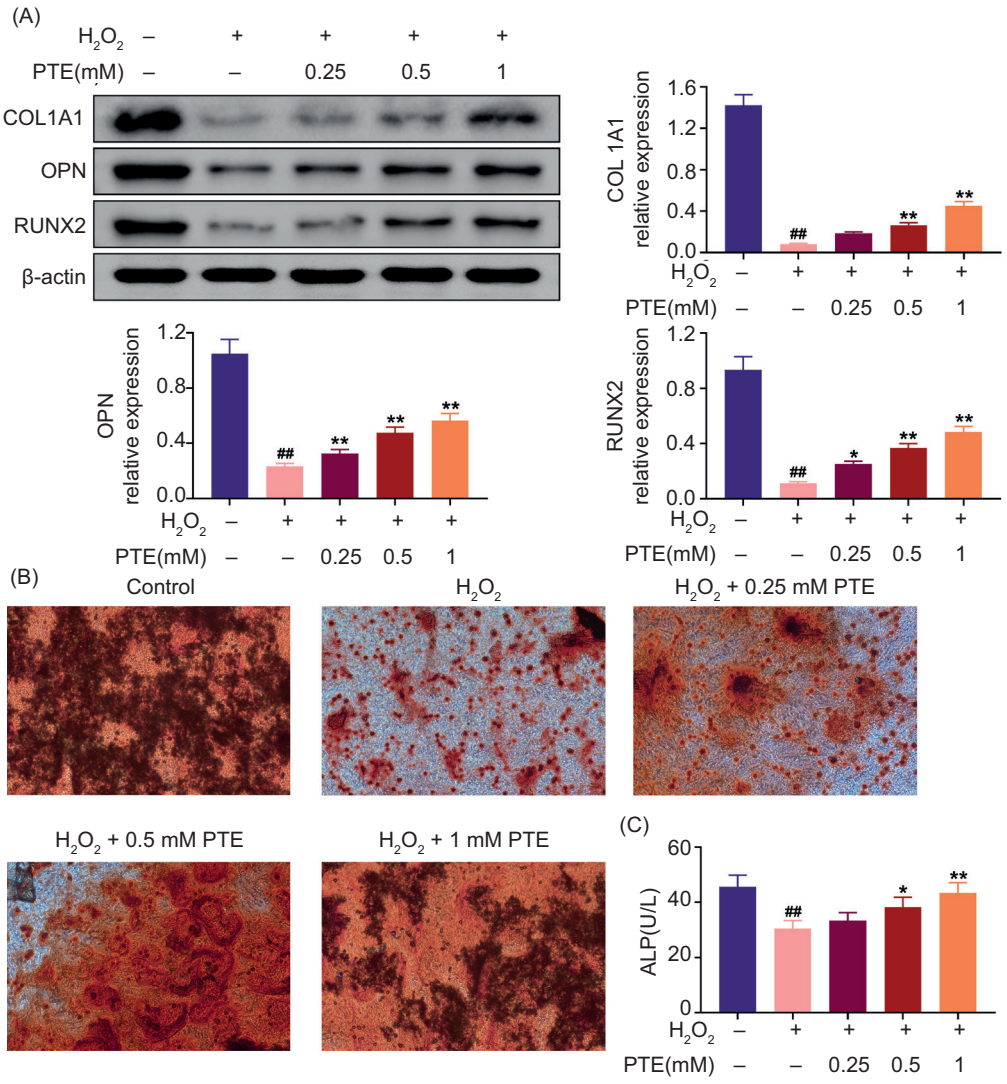

Figure 3. Effect of pterostilbene on osteogenic differentiation in MC3T3-E1 cells. (A) The protein expression levels of COL1A1, OPN, and Runx2 were analyzed with Western blot assay. (B) MC3T3-E1 cells were incubated at different concentrations of pterostilbene and fixed for ALP staining. Data were presented as mean ± SD. $^{*}P < 0.05$ compared with the H_2O_2 group, $^{**}P < 0.01$ compared with the H_2O_2 group; $^{\#\#}P < 0.01$ compared with the control group.

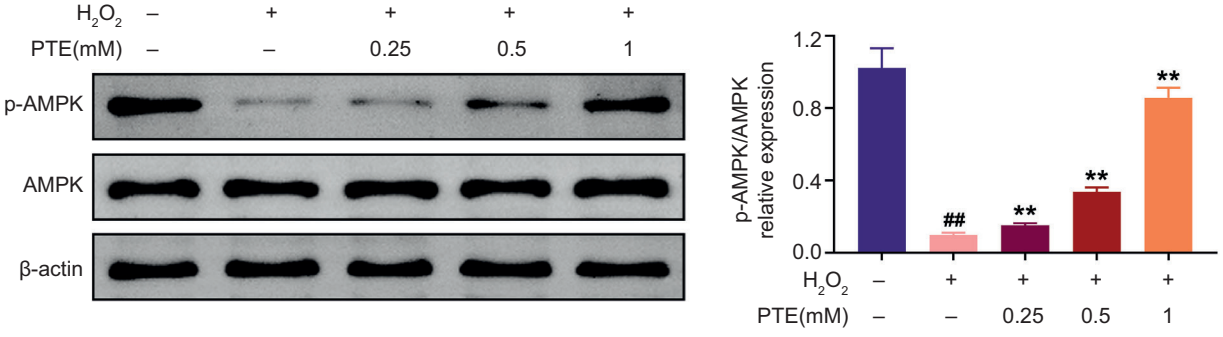

Figure 4. Pterostilbene attenuated the inhibitory effects of H_2O_2 on the AMPK signaling pathway. MC3T3-E1 cells were treated with or without different concentrations of pterostilbene (0.25, 0.5, and 1.0 mM) before exposure to H_2O_2. The expression levels of p-AMPK and AMPK were measured by Western blot analysis. Quantitative analysis of p-AMPK and AMPK was manifested. Data were presented as mean ± SD. $^{**}P < 0.01$ compared with the H_2O_2 group; $^{\#\#}P < 0.01$ compared with the control group.

cells. These results were consistent with other investigations, demonstrating that pterostilbene could attenuate herbicide-induced oxidative damage to the growth and metastatic activity of B16 melanoma (Ko *et al.*, 2015). Together with these foundations, it was concluded that pterostilbene could promote cell viability by reducing oxidative stress.

In order to clarify the protective effect of pterostilbene and its downstream signaling pathway on osteogenic differentiation after H_2O_2 exposure, this study investigated the expression of osteogenic-related biomarkers and molecular mechanisms of AMPK signaling pathway. The mechanisms mediating the protective effects of pterostilbene included multiple signaling pathways such as extracellular regulated kinase, AMPK, and nuclear factor *kappa B* (NF-κB) pathways, among which the activation of AMPK pathway was established as a promising approach (Lin *et al.*, 2012; Xue *et al.*, 2017). Previous studies have established that AMPK signaling pathway is related to the modulation of cellular oxidative stress (Auciello *et al.*, 2014; Wu snd Wei, 2012). Oxidative stress could trigger the activation of AMPK, which stimulates nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream antioxidant enzymes (Zimmermann *et al.*, 2015). It has been reported that AMPK activation regulates cellular energy content by promoting generation of adenosine triphosphate (ATP) through upregulation of mitochondrial biogenesis, which is a requisite of oxidative stress inhibition. These results indicated that the expression levels of COL1A1, OPN, and Runx2 in the co-treatment H_2O_2 + pterostilbene groups were obviously higher than in the H_2O_2 group. In addition, the co-treatment of H_2O_2 + pterostilbene generated the highest ALP activity compared to the H_2O_2 group. It was also determined that pre-incubation with pterostilbene improved the phosphorylation level of AMPK and reversed the H_2O_2-induced injury.

Conclusion

In conclusion, results of the present study indicated that pterostilbene led to a significant reduction in the H_2O_2-induced apoptosis and oxidative stress of MC3T3-E1 cells. It was also revealed that pterostilbene induced AMPK signaling pathway activation to exert antioxidant effect and inhibit ROS production after H_2O_2 exposure. Importantly, pterostilbene blocked the H_2O_2-induced MC3T3-E1 cells dysfunction, indicating that pterostilbene could be a potential medication for treating osteoporosis. Although additional studies are required to confirm the efficacy of pterostilbene in the treatment of osteoporosis *in vivo*, these results indicated that pterostilbene could be a potential therapeutic target for disorders associated with bone loss.

Competing Interests

The authors stated that there were no conflicts of interest to disclose.

Author Contributions

Haiyan Li and Jiang Guo designed and conducted the study. Dong Zhang supervised the data collection, and analyzed and interpreted the data. Jihong Huang and Xuejia Li prepared the manuscript for publication and reviewed the draft manuscript. All authors read and approved the final manuscript.

References

Abdollahi, M., Larijani, B., Rahimi, R. and Salari, P., 2005. Role of oxidative stress in osteoporosis. Clinical Practice 2: 787. https://doi.org/10.2217/14750708.2.5.787

Auciello, F.R., Ross, F.A., Ikematsu, N. and Hardie, D.G., 2014. Oxidative stress activates AMPK in cultured cells primarily by increasing cellular AMP and/or ADP. FEBS letters 588: 3361–3366. https://doi.org/10.1016/j.febslet.2014.07.025

Bouxsein, M.L., 2003. Mechanisms of osteoporosis therapy: a bone strength perspective. Clinical cornerstone. 5: S13–S21. https://doi.org/10.1016/S1098-3597(03)90043-3

Chan, C.K., Mason, A., Cooper, C. and Dennison, E., 2016. Novel advances in the treatment of osteoporosis. British Medical Bulletin 119: 129–142. https://doi.org/10.1093/bmb/ldw033

Cheng, Y., Huang, L., Wang, Y., Huo, Q., Shao, Y., Bao, H., et al. 2019. Strontium promotes osteogenic differentiation by activating autophagy via the the AMPK/mTOR signaling pathway in MC3T3-E1 cells. International Journal of Molecular Medicine 44: 652–660. https://doi.org/10.3892/ijmm.2019.4216

Choksi, P., Jepsen, K.J. and Clines, G.A., 2018. The challenges of diagnosing osteoporosis and the limitations of currently available tools. Clinical Diabetes and Endocrinology 4: 1–13. https://doi.org/10.1186/s40842-018-0062-7

Feng, Q., Cheng, S.Y., Yang, R., Zeng, X.W., Zhao, F.M. and Zhan, X.Q., 2020. Puerarin promotes the viability and differentiation of MC3T3-E1 cells by enhancing LC3B-mediated autophagy through downregulation of miR-204. Experimental and Therapeutic *Medicine* 19: 883–890. https://doi.org/10.3892/etm.2019.8291

He, J.L., Dong, X.H., Li, Z.H., Wang, X.Y., Fu, Z.A. and Shen, N., 2018. Pterostilbene inhibits reactive oxygen species production and apoptosis in primary spinal cord neurons by activating autophagy via the mechanistic target of rapamycin signaling pathway. Molecular Medicine Reports 17: 4406–4414. https://doi.org/10.3892/mmr.2018.8412

Ko, C.-P., Lin, C.-W., Chen, M.-K., Yang, S.-F., Chiou, H.-L. and Hsieh, M.-J., 2015. Pterostilbene induces autophagy on human oral cancer cells through modulation of Akt and mitogen-activated protein kinase pathway. Oral Oncology 51: 593–601. https://doi.org/10.1016/j.oraloncology.2015.03.007

Li, D., Yu, J., Xiao, L., Miao, W., Ji, K., Wang, S. and Geng, Y., 2017. Autophagy attenuates the oxidative stress-induced apoptosis of Mc3T3-E1 osteoblasts. European Review for Medical and Pharmacological Sciences 21: 5548–5556.

Li, W., Zhang, S., Liu, J., Liu, Y. and Liang, Q., 2019. Vitamin K2 stimulates MC3T3-E1 osteoblast differentiation and mineralization through autophagy induction. Molecular Medicine Reports 19: 3676–3684. https://doi.org/10.3892/mmr.2019.10040

Lin, V. C.-H., Tsai, Y.-C., Lin, J.-N., Fan, L.-L., Pan, M.-H., Ho, C.-T., et al. 2012. Activation of AMPK by pterostilbene suppresses lipogenesis and cell-cycle progression in p53 positive and negative human prostate cancer cells. Journal of Agricultural and Food Chemistry 60: 6399–6407. https://doi.org/10.1021/jf301499e

Liu, D., Ma, Z., Xu, L., Zhang, X., Qiao, S. and Yuan, J., 2019a. PGC1α activation by pterostilbene ameliorates acute doxorubicin cardiotoxicity by reducing oxidative stress via enhancing AMPK and SIRT1 cascades. Aging (Albany, NY) 11: 10061. https://doi.org/10.18632/aging.102418

Liu, Y., Wang, C., Wang, G., Sun, Y., Deng, Z., Chen, L., et al. 2019b. Loureirin B suppresses RANKL-induced osteoclastogenesis and ovariectomized osteoporosis via attenuating NFATc1 and ROS activities. Theranostics 9: 4648. https://doi.org/10.7150/thno.35414

Liu, H., Wu, X., Luo, J., Wang, X., Guo, H., Feng, D., et al. 2019c. Pterostilbene attenuates astrocytic inflammation and neuronal oxidative injury after ischemia-reperfusion by inhibiting NF-κB phosphorylation. Frontiers in Immunology 10: 2408. https://doi.org/10.3389/fimmu.2019.02408

McCormack, D. and McFadden, D., 2012. Pterostilbene and cancer: current review. Journal of Surgical Research 173: e53–e61. https://doi.org/10.1016/j.jss.2011.09.054

McCormack, D. and McFadden, D., 2013. A review of pterostilbene antioxidant activity and disease modification. Oxidative Medicine and Cellular Longevity 2013: 575482. https://doi.org/10.1155/2013/575482

Nikhil, K., Sharan, S. and Roy, P., 2015. A pterostilbene derivative suppresses osteoclastogenesis by regulating RANKL-mediated NFκB and MAPK signaling in RAW264. 7 cells. Pharmacological Reports 67: 1264–1272. https://doi.org/10.1016/j.pharep.2015.05.009

Pan, M.-H., Chang, Y.-H., Tsai, M.-L., Lai, C.-S., Ho, S.-Y., Badmaev, V. and Ho, C.-T., 2008. Pterostilbene suppressed lipopolysaccharide-induced up-expression of iNOS and COX-2 in murine macrophages. Journal of Agricultural and Food Chemistry 56: 7502–7509. https://doi.org/10.1021/jf800820y

Pouresmaeili, F., Kamalidehghan, B., Kamarehei, M. and Goh, Y.M., 2018. A comprehensive overview on osteoporosis and its risk factors. Therapeutics and Clinical Risk Management 14: 2029. https://doi.org/10.2147/TCRM.S138000

Schröder, K., 2015. NADPH oxidases in bone homeostasis and osteoporosis. Cellular and Molecular Life Sciences 72: 25–38. https://doi.org/10.1007/s00018-014-1712-2

Shin, H.J., Han, J.M., Choi, Y.S. and Jung, H.J., 2020. Pterostilbene suppresses both cancer cells and cancer stem-like cells in cervical cancer with superior bioavailability to resveratrol. Molecules 25: 228. https://doi.org/10.3390/molecules25010228

Shu, Q., Zhao, X., Geng, X. and Wang, X., 2020. CD82 aggravates sevoflurane-induced neurotoxicity by regulating TRPM7 in developing neurons. Signa Vitae 16: 142–147.

Song, L., Chen, T.Y., Zhao, X.J., Xu, Q., Jiao, R.Q., Li, J.M. and Kong, L.D., 2019. Pterostilbene prevents hepatocyte epithelial–mesenchymal transition in fructose-induced liver fibrosis through suppressing miR-34a/Sirt1/p53 and TGF-β1/Smads signalling. British Journal of Pharmacology 176: 1619–1634. https://doi.org/10.1111/bph.14573

Wang, Y.-J., Chen, Y.-Y., Hsiao, C.-M., Pan, M.-H., Wang Jr, B., Chen, Y.-C., et al. 2020. Induction of autophagy by pterostilbene contributes to the prevention of renal fibrosis via attenuating NLRP3 inflammasome activation and epithelial-mesenchymal transition. Frontiers in Cell and Developmental Biology 8: 436. https://doi.org/10.3389/fcell.2020.00436

Wu, S.-B. and Wei, Y.-H., 2012. AMPK-mediated increase of glycolysis as an adaptive response to oxidative stress in human cells: implication of the cell survival in mitochondrial diseases. Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease 1822: 233–247. https://doi.org/10.1016/j.bbadis.2011.09.014

Xue, E.-X., Lin, J.-P., Zhang, Y., Sheng, S.-R., Liu, H.-X., Zhou, Y.-L. and Xu, H., 2017. Pterostilbene inhibits inflammation and ROS production in chondrocytes by activating Nrf2 pathway. Oncotarget 8: 41988. https://doi.org/10.18632/oncotarget.16716

Yang, J.Y., Park, M.Y., Park, S.Y., Yoo, H.I., Kim, M.S., Kim, J.H., et al. 2015. Nitric oxide-induced autophagy in MC3T3-E1 cells is associated with cytoprotection via AMPK activation. Korean Journal of Physiology & Pharmacology 19: 507–514. https://doi.org/10.4196/kjpp.2015.19.6.507

Zhang, W., Cui, S.-Y., Yi, H., Zhu, X.-H., Liu, W. and Xu, Y.-J., 2020. MiR-708 inhibits MC3T3-E1 cells against H_2O_2-induced apoptosis through targeting PTEN. Journal of Orthopaedic Surgery and Research 15: 1–8. https://doi.org/10.1186/s13018-020-01780-w

Zimmermann, K., Baldinger, J., Mayerhofer, B., Atanasov, A.G., Dirsch, V.M. and Heiss, E.H., 2015. Activated AMPK boosts the Nrf2/HO-1 signaling axis—a role for the unfolded protein response. Free Radical Biology and Medicine 88: 417–426. https://doi.org/10.1016/j.freeradbiomed.2015.03.030