Bergenin increases osteogenic differentiation and prevents methylglyoxal-induced cytotoxicity in MC3T3-E1 osteoblasts

Kwang Sik Suh · Suk Chon · Eun Mi Choi

Received: 16 January 2017 / Accepted: 30 August 2017 / Published online: 11 September 2017
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Abstract Bergenin, an active component of plants in the genus *Bergenia*, has multiple biological activities, including anti-inflammatory and immunomodulatory properties. We investigated the effects of bergenin on MC3T3-E1 osteoblasts. Bergenin treatment significantly elevated collagen synthesis, alkaline phosphatase activity, osteocalcin synthesis, and mineralization in the cells ($p < 0.05$). Additionally, bergenin increased the ratio of osteoprotegerin to receptor activator of nuclear factor kappa-B ligand, and cyclophilin B release. Methylglyoxal (MG), a highly reactive dicarbonyl compound, is the major precursor in the formation of advanced glycation end products. Pretreatment of MC3T3-E1 cells with bergenin prevented MG-induced cell death. Furthermore, bergenin treatment significantly reduced the induction of activating transcription factor 6 and autophagy by MG. These results indicate that bergenin may have positive effects on critical osteoblastic cell functions.

Keywords Bergenin · Cytotoxicity · Differentiation · Methylglyoxal · Osteoblasts

Introduction

Bone is composed of two main cell types: bone-forming osteoblasts and bone-resorbing osteoclasts. The net balance between bone resorption and formation defines the rate of bone turnover and bone mass. Normal bone remodeling is necessary for fracture healing and skeleton adaptation to mechanical use (Dallas et al. 2013). On the other hand, an imbalance of bone resorption and formation results in several bone diseases. Thus, the equilibrium between bone formation and resorption is necessary and depends on the action of several local and systemic factors including hormones, cytokines, chemokines, and biomechanical stimulation (Phan et al. 2004; Crockett et al. 2011). Both animals with experimentally induced insulin deficiency syndromes and patients with type 1 diabetes mellitus have impaired osteoblastic bone formation, with or without increased bone resorption (Hough et al. 2016). Insulin deficiency appears to be a major pathogenetic mechanism involved, along with glucose toxicity, marrow adiposity, inflammation, adipokine and other metabolic alterations that may all play a role on altering bone turnover (Zofková 2003).

Chronic hyperglycemia and impaired glucose metabolism associated with diabetes mellitus produce a large number of reactive carbonyl compounds, such as methylglyoxal (MG) (Thornalley et al. 1999). Elevated MG levels trigger carbonyl stress and activate an inflammatory response, leading to

Kwang Sik Suh and Suk Chon have contributed equally to this work.

K. S. Suh · S. Chon · E. M. Choi (✉)
Department of Endocrinology and Metabolism, School of
Medicine, Kyung Hee University, 1, Hoegi-dong,
Dongdaemun-gu, Seoul 130-701, Republic of Korea
e-mail: cheunmi@hanmail.net

accelerated diabetic complications. Diabetic patients are known to be at increased risk for osteopenia and osteoporotic fracture. Oxidative stress caused by diabetic conditions plays a critical role in the development of diabetic osteopenia (Hamada et al. 2009), which can be regarded as a condition induced by the impairment of the anabolic functions of osteoblasts through oxidative stress (Hamada et al. 2007, 2009). Type I collagen is the major protein component of the extracellular matrix of bone. Several reports have described the effects of cyclophilin B (CypB) mutants on type I collagen modification and components of the prolyl 3-hydroxylation complex; CypB-deficient mice present with severe osteogenesis imperfecta-like phenotypes clinically characterized by bone disorders (van Dijk et al. 2009). Thus, CypB is believed to catalyze the rate-limiting step in collagen folding (Andreeva et al. 1999).

The endoplasmic reticulum (ER) is a critical cellular compartment responsible for proper protein-folding homeostasis. Unfolded proteins accumulate within the ER lumen under ER stress. If the stress is excessive or prolonged, apoptotic cell death occurs (Nishitoh et al. 2002). However, cells first attempt to control cell survival and adaptation by transmitting signals from the ER to the nucleus and cytoplasm via a regulatory system termed the unfolded protein response (UPR) (Schroder and Kaufman 2005). ER stress transducers play important roles in UPR signal transduction. In mammalian cells, activating transcription factor 6 (ATF6), one of the UPR transducers, senses the presence of unfolded proteins in the ER lumen. Autophagy is one of the ways unfolded proteins are degraded after ER stress, in addition to the protective pathway associated with the maintenance of cellular homeostasis activated in response to nutrient depletion and various metabolic stimuli. Autophagy can also induce apoptotic cell death (Levine et al. 2008). The induction of ER stress followed by autophagy can degrade unfolded aggregated proteins to reduce ER stress, thus enabling the cell to prevent ER stress-mediated cell death.

Therapeutic strategies for the prevention of diabetic complications include antihyperglycemic agents or advanced glycation end product (AGE) inhibitors, such as aminoguanidine (Thomas et al. 2005), carnosine (Blatnik et al. 2008), and tenilsetam (Price et al. 2001). The trapping of dicarbonyl species has also been investigated as a mode of inhibiting the

formation of AGEs and the development of diabetic complications. However, each drug class or inhibitor has adverse side effects (Cornish 2014), so there is great interest in developing natural interventions that combine higher efficacy and improved safety for managing diabetes and its complications. Bergenin, a bioactive plant component, is a C-glucoside of 4-O-methylgallic acid (Fig. 1). It occurs in several plants, including *Bergenia purpurascens*, *Mallotus japonicus*, *Ardisia crenata*, *Rodgersia sambucifolia*, *Peltophorum africanum*, *Flueggea virosa*, and *Sacoglottis gabonensis* (Yan et al. 2014). Bergenin reportedly has many other biological and medical functions, such as anti-arthritic, antitussive (Nazir et al. 2007; Xie et al. 1981), hepatoprotective (Lim et al. 2000), anti-HIV (Piacente et al. 1996), antidiabetic (Li et al. 2005), antiarrhythmic (Pu et al. 2002), and anti-inflammatory activities (Li et al. 2004). The natural antioxidant properties of bergenin are well established (Khan et al. 2016). In this study, we investigated the effects of bergenin on MG-induced cytotoxicity in MC3T3-E1 osteoblasts in vitro to determine the possible bioactivities of this compound on bone metabolism.

Materials and methods

Materials

Bergenin was purchased from ChromaDex Inc. (Irvine, CA, USA). α -Modified minimal essential medium (α -MEM) and fetal bovine serum (FBS) were

Fig. 1 Structure of bergenin

purchased from Gibco BRL (Grand Island, NY, USA). Other reagents were of the highest commercial grade available and purchased from Sigma Chemical (St. Louis, MO, USA).

Cell culture

Osteoblastic MC3T3-E1 Subclone 4 cells were obtained from ATCC (Manassas, VA, USA). MC3T3-E1 cells were cultured at 37 °C in 5% CO₂ atmosphere in α -MEM. Unless otherwise specified, the media contained 10% heat-inactivated FBS, 100 U/ml penicillin, and 100 μ g/ml streptomycin. The cells were treated at confluence with culture media containing 5 mM β -glycerophosphate and 50 μ g/ml ascorbic acid to initiate differentiation. After 6 days or 14 days (for osteocalcin and calcium deposition assay, respectively), the culture medium was removed and the cells were incubated with bergenin or 300 μ M aminoguanidine (AG) in medium containing 0.5% FBS prior to treatment with 400 mM MG for 48 h.

Collagen content

Collagen content was quantified by Sirius Red-based colorimetric assay (Tullberg-Reinert and Jundt 1999). Cultured osteoblasts were washed with phosphate buffered saline (PBS), followed by fixation in Bouin's fixative for 1 h. After fixation, the fixative was removed and the culture dishes were washed in running tap water for 15 min. The culture dishes were air-dried and stained with Sirius Red dye reagent for 1 h under mild shaking on a shaker. Thereafter, the solution was removed and the cultures were washed with 0.01 N HCl to remove unbound dye. The stained material was dissolved in 0.1 N NaOH and absorbance was measured at 550 nm. A standard curve was constructed using known concentrations of commercial collagen (Sigma).

Alkaline phosphatase activity

The cells were lysed with 0.2% Triton X-100, and the lysate was centrifuged at 14,000 $\times g$ for 5 min. The clear supernatant was used to measure alkaline phosphatase (ALP) activity, which was determined using an ALP activity assay kit (Asan Co., Seoul, Korea). Protein concentrations were determined using a Bio-Rad protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of osteocalcin

Osteocalcin content in cytosol was measured using a sandwich ELISA assay kit (Biomedical Technologies Inc., Stoughton, MA, USA) following the manufacturer's protocol. Two mouse osteocalcin antibodies were employed, each directed toward an end (C or N terminus) of the osteocalcin molecule. The N-terminal antibody was bound to the well, which binds the mouse osteocalcin standard or sample. The biotin labeled C-terminal mouse osteocalcin antibody completed the sandwich. Both carboxylated and decarboxylated mouse osteocalcin were recognized.

Calcium deposition assay

On harvesting, the cells were fixed in 70% ethanol for 1 h, and then stained with 40 mM Alizarin Red S for 10 min with gentle shaking. To quantify the bound dye, the stain was washed with DPBS and solubilized with 10% cetylpyridinium chloride by shaking for 15 min. The absorbance of the solubilized stain was measured at 561 nm.

Measurement of OPG, RANKL, IL-6, and CypB

Osteoprotegerin (OPG), receptor activator of nuclear factor kappa-B ligand (RANKL), and interleukin-6 (IL-6) content in the media were measured with an enzyme immunoassay system (R&D system Inc., Minneapolis, MN, USA). CypB release into culture media was determined by an ELISA kit (Elabscience Biotechnology, Wuhan, Hubei, China).

Cell viability

Surviving cells were counted using the MTT method, whereby 20 μ l of 5 mg/ml MTT in PBS solution, pH 7.4, was added to each well, and the plates were incubated for 2 h. After the removal of this solution, dimethyl sulfoxide was added to dissolve formazan products, and the plates were shaken for 5 min. The absorbance of each well was recorded on a microplate spectrophotometer at 570 nm.

Measurement of apoptosis

Apoptosis was assessed with the Cell Death Detection ELISA^{PLUS} kit (Roche Diagnostics GmbH,

Mannheim, Germany) according to the manufacturer's instructions. The assay is based on a quantitative sandwich enzyme immunoassay principle, using mouse monoclonal antibodies directed against DNA and histones. This allows for the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates.

LDH cytotoxicity assay

Cytotoxicity was evaluated by quantifying plasma membrane damage. Lactate dehydrogenase (LDH) is a stable enzyme that is present in all cell types, and it is rapidly released into the culture media when the plasma membrane is damaged. Cell membrane integrity was evaluated by measuring the levels of LDH leaking from the cells with the LDH Cytotoxicity Assay Kit (BioVision, Inc., Milpitas, CA, USA) according to the manufacturer's instructions.

Measurement of ATF6

The levels of ATF6 in the cytosol were determined using an ELISA kit (MyBioSource, Inc., San Diego, CA, USA). The assay was performed according to the instructions provided by the manufacturer.

Autophagy detection assay

An Autophagy Detection Kit (Abcam, Cambridge, MA, USA) was used according to the manufacturer's protocol with a fluorescence microplate reader. The 488 nm-excitable green fluorescent detection reagent supplied in the kit becomes brightly fluorescent in vesicles produced during autophagy, and has been validated under a wide range of conditions known to modulate autophagy pathways.

Statistical analysis

The results are expressed as means $\pm$ SEM. Statistical significance was determined by analysis of variance (ANOVA) and subsequently applying the Dunnett's *t* test, with significance set at $p < 0.05$.

Results

Effect of bergenin on the early differentiation markers of MC3T3-E1 cells

To investigate the effects of bergenin on the differentiation of MC3T3-E1 cells, we first assessed collagen content and ALP activity. Collagen is an early osteoblastic marker, and as shown in Fig. 2a, incubating cells with 0.01–1 μ M bergenin significantly increased collagen synthesis. We then examined ALP activity, another early-stage osteogenic

Fig. 2 Bergenin increases the early differentiation of MC3T3-E1 cells. MC3T3-E1 cells were treated, at confluence, with culture medium containing 10 mM β -glycerophosphate and 50 μ g/mL ascorbic acid to initiate differentiation for 6 days. After incubation with bergenin in differentiation medium for 2 day, collagen content (a) and alkaline phosphatase (ALP) activity (b) were measured. Data are expressed as mean $\pm$ SEM ($n = 6$). The control values for collagen content and ALP activity are $6.72 \pm 0.107 \mu\text{g}/10^6$ cells and 0.708 ± 0.006 U/mg protein, respectively. * $p < 0.05$ compared with the control, by Dunnett's *t* test

differentiation marker, in MC3T3-E1 cells. Culturing cells in the presence of 1 μM bergenin resulted in a significant increase in ALP activity (Fig. 2b).

Effect of bergenin on cyclophilin B (CypB) release in MC3T3-E1 cells

It was reported that CypB facilitates collagen folding directly, but also indirectly regulates collagen hydroxylation, glycosylation, cross-linking, and fibrillogenesis through its interactions with other collagen modifying enzymes in the ER (Cabral et al. 2014). We therefore investigated whether bergenin modulates the production of CypB in MC3T3-E1 cells (Fig. 3). When 0.01–1 μM bergenin was added to cells, the production of CypB increased significantly.

Effect of bergenin on the OPG/RANKL ratio in MC3T3-E1 osteoblasts

To further examine the regulation of osteoclastic differentiation in osteoblasts, we assessed the ratio of OPG/RANKL in MC3T3-E1 cells. Osteoblasts express RANKL, which binds to its receptor, RANK, on the surface of osteoclasts. This stimulates the differentiation of precursors into multinucleated osteoclasts. OPG secreted by osteoblasts protects the skeleton from excessive bone resorption by binding to RANKL and preventing it from interacting with RANK. The OPG/RANKL ratio in bone marrow is thus an important determinant of bone mass in normal

Fig. 3 Bergenin increases cyclophilin B level in MC3T3-E1 cells. Osteoblasts were incubated with bergenin for 48 h. Data are expressed as mean $\pm$ SEM ($n = 6$). The control value for cyclophilin B was 16.43 ± 0.195 pg/mg. * $p < 0.05$ compared with the control, by Dunnett's t test

and disease states (Boyce and Xing 2007). When 1 μM bergenin was added to MC3T3-E1 cells, the OPG/RANKL ratio increased significantly (Fig. 4).

Effect of bergenin on the late differentiation markers of MC3T3-E1 cells

When osteocalcin, a late-stage osteogenic differentiation marker, was measured in the cytosol, 0.01 and 0.1 μM bergenin supplementation significantly increased osteocalcin secretion compared with controls (Fig. 5a). Matrix mineralization, the final step in osteoblastic differentiation, plays a critical role in maintaining the mechanical integrity of bone tissues. To detect the effect of bergenin on mineralization, MC3T3-E1 cells were stained with Alizarin Red S. As shown in Fig. 5b, mineralization was significantly increased at bergenin concentrations of 0.01–1 μM . These results suggest that bergenin may induce osteogenic differentiation processes throughout early and late phases.

Cytoprotective effect of bergenin against MG in MC3T3-E1 osteoblasts

To examine the effect of bergenin itself on osteoblastic MC3T3-E1 cells, we treated the cells with various concentrations of bergenin for 48 h and measured cell

Fig. 4 Bergenin increases the OPG/RANKL ratio of MC3T3-E1 cells. MC3T3-E1 cells were treated, at confluence, with culture medium containing 10 mM β -glycerophosphate and 50 $\mu\text{g/mL}$ ascorbic acid to initiate differentiation for 6 days. After incubation with bergenin in differentiation medium for 2 day, cells were assayed for OPG and RANKL ELISA. Data are expressed as mean $\pm$ SEM ($n = 6$). * $p < 0.05$ compared with the control, by Dunnett's t test

Fig. 5 Bergenin increases the late differentiation of MC3T3-E1 cells. MC3T3-E1 cells were treated, at confluence, with culture medium containing 10 mM β -glycerophosphate and 50 μ g/mL ascorbic acid to initiate differentiation for 14 days. After incubation with bergenin in differentiation medium for 2 day, **a** osteocalcin and **b** mineralization were measured. Data are expressed as mean $\pm$ SEM ($n = 6$). The control values for osteocalcin and mineralization are 4.308 ± 0.2 ng/mg protein and 0.746 ± 0.042 OD/ 10^6 cells, respectively. * $p < 0.05$ compared with the control, by Dunnett's t test

viability. Bergenin at concentrations of ≤ 1 μ M had no effect on the viability of MC3T3-E1 cells (Fig. 6a). To determine whether bergenin has a protective effect against MG-induced cytotoxicity, cells were pre-incubated with bergenin for 1 h and then cultured with 400 μ M MG for 48 h. As shown in Fig. 6b, 400 μ M MG treatment induced death in nearly 50% of cells, compared with non-treated control cells, but 0.1 and 1 μ M bergenin inhibited MG-induced cytotoxicity. Apoptosis was evaluated with a Cell Death Detection ELISA kit. As shown in Fig. 6c, MG (400 μ M) induced apoptosis in MC3T3-E1 cells, but 0.01–1 μ M bergenin reduced this effect. Cytotoxicity was evaluated by quantifying plasma membrane

damage. LDH activity increases upon loss of cell membrane integrity. As shown in Fig. 6d, 400 μ M MG was cytotoxic to MC3T3-E1 cells, but 0.1 and 1 μ M bergenin reduced this effect. Aminoguanidine (AG, 300 μ M), a carbonyl scavenger, also inhibited MG-induced cytotoxicity. Thus, bergenin may prevent MG-induced cell death.

Bergenin inhibits MG-induced ER stress and autophagy in MC3T3-E1 cells

ER stress can be generally assessed by evaluating ATF6 levels. The level of ATF6 in MC3T3-E1 cells stimulated with MG was significantly inhibited by treatment with 0.01–1 μ M bergenin or AG (Fig. 7a), indicating reduced ER stress. We also evaluated autophagy in MC3T3-E1 cells pretreated with bergenin or AG in the presence of MG. More autophagy was observed in MG-treated cells than in the controls (Fig. 7b). However, when incubated with 0.01–1 μ M bergenin or AG, the autophagy of MC3T3-E1 cells was significantly decreased. Our experiments suggest that ER stress and autophagy are activated in MC3T3-E1 cells by MG treatment, and bergenin may inhibit these processes.

Discussion

Bergenin has a wide range of beneficial effects on human health (Nazir et al. 2007; Xie et al. 1981; Lim et al. 2000; Piacente et al. 1996; Li et al. 2005; Pu et al. 2002; Li et al. 2004). However, the stimulatory effect of bergenin on osteoblastic differentiation has not yet been determined. This study was therefore designed to elucidate the osteogenic effect of bergenin in an in vitro MC3T3-E1 osteoblastic cell model. In general, collagen synthesis increases as osteoblasts differentiate, especially from the early stage of osteogenesis. ALP activity is one of the most well-known early differentiation markers for osteoblastic mineralization and maturation. In our study, bergenin increased collagen synthesis and ALP activity in cultured cells, which could be interpreted as indicative of osteoblastic differentiation and bone matrix maturation. Enhancing the mineralization of late-stage osteoblastic cell differentiation is the ultimate objective of any bone regeneration therapy. Therefore, we investigated the in vitro effects of bergenin on mineralization with

Fig. 6 Bergenin reduces the MG-induced cytotoxicity of MC3T3-E1 cells. Osteoblasts were treated with bergenin (Berg) in the absence (a) or presence (b) of 400 μM methylglyoxal (MG) for 48 h, and the viability and extent of MG-mediated cytotoxicity were assessed using the MTT assay. Apoptosis

(c) and lactate dehydrogenase (LDH) levels (d) were also measured. Data are expressed as mean $\pm$ SEM ($n = 6$). # $p < 0.05$ compared with the untreated cells; * $p < 0.05$ compared with the cells treated with 400 μM MG alone, by Dunnett's t test

Alizarin Red S staining of mineralization nodules to assess bone formation in MC3T3-E1 osteoblasts. We observed that bergenin stimulates mineralization nodules at a minimum concentration of 0.01 μM. Osteocalcin synthesis is a well-known sign of later stages of osteogenesis (Ducy et al. 1996). In the present study, bergenin treatment increased osteocalcin synthesis in MC3T3-E1 osteoblasts, indicating the stimulation of osteoblastic maturation and mineralization.

The effect of bergenin on osteoclastic differentiation was further investigated by analyzing the amounts of the cytokines OPG and RANKL secreted into culture media. Osteoblast-released OPG and RANKL

are key components in bone remodeling (Khosla 2001). OPG has been identified as a natural decoy receptor for RANKL that prevents its interaction with RANK, thus inhibiting osteoclastic differentiation (Simonet et al. 1997). Taken together, the ratio of OPG/RANKL may be the ultimate determinant of bone resorption and bone remodeling (Boyle et al. 2003). In our study, bergenin significantly increased the OPG/RANKL ratio in MC3T3-E1 cells. Our data suggest that bergenin not only stimulates osteoblastic differentiation, but also inhibits osteoclastic differentiation, perhaps by up-regulating the OPG/RANKL ratio in the cellular microenvironment. Biosynthesis of procollagen is a complex process that requires several

Fig. 7 Bergenerin reduces the MG-induced ATF6 enhancement and autophagy of MC3T3-E1 cells. Osteoblasts were pre-incubated with bergenerin (Berg) before treatment with 400 μM MG for 24 h. Data are expressed as mean ± SEM ($n = 6$). The control value for ATF6 was 54.72 ± 0.498 ng/mg. Data for autophagy activation are expressed as mean relative percentage of fluorescence. [#] $p < 0.05$ compared with untreated cells; ^{*} $p < 0.05$ compared with cells treated with 400 μM MG alone, by Dunnett's t test

co- and post-translational modifications within the ER (Myllyharju and Kivirikko 2004). Cyclophilins (CyPs) are a family of ubiquitous, evolutionarily well-conserved proteins present in all prokaryotes and eukaryotes (Wang and Heitman 2005). CypB is an ER-localized member of the immunophilin family of proteins with peptidyl-prolyl cis-trans isomerase (PPIase) activity that catalyzes the rate-limiting step in collagen folding (Galat 2003). Cabral et al. (2014) demonstrated that CypB is the major peptidyl prolyl cis-trans isomerase that catalyzes the rate-limiting step in collagen folding. In the present study, CypB

release in MC3T3-E1 osteoblasts was increased with bergenerin treatment. CypB has been associated with collagen functions (Smith et al. 1995). Therefore, the enhancement of CypB by bergenerin may increase collagen folding.

In a previous study, we demonstrated that MG has detrimental effects on MC3T3-E1 osteoblasts through a mechanism involving oxidative stress and mitochondrial dysfunction (Suh et al. 2014). The findings of the present study suggest that pretreatment of MC3T3-E1 cells with bergenerin protects against MG-induced cytotoxicity. Additionally, we found that bergenerin inhibits MG-induced activation of ATF6, indicating that MG-induced ER stress is inhibited by bergenerin in MC3T3-E1 osteoblasts. Activation of the ATF6 pathway generates an active ATF6 fragment that translocates to the nucleus and upregulates the transcription of UPR genes (Ye et al. 2000). ATF6 knockout mice are viable and fertile and have no overt phenotype unless challenged by ER stress-inducing agents (Rutkowski et al. 2008). ER stress is reportedly involved in the induction of apoptosis during various pathophysiological processes, including osteoporosis (Park et al. 2012; He et al. 2013). Taken together, our results suggest that ER stress is involved in MG-induced cytotoxicity, and can be blocked by bergenerin. Several ER stress-activated kinases have been associated with ER stress-induced autophagy (Hoyer-Hansen and Jaattela 2007). Once activated, these kinases can ultimately upregulate autophagy-related genes and inhibit autophagic suppressors. Autophagy is a process of self-degradation that maintains cellular viability during periods of metabolic stress. Although autophagy is considered an important survival mechanism under cellular stress, extensive autophagy can also lead to cell death. Under normal conditions, basal autophagy removes aged and damaged organelles and proteins (Rubinsztein 2006). Excessive autophagy may, however, lead to cell death, referred to as type II programmed cell death (Galluzzi et al. 2009). In this study, autophagy increased in cells incubated with MG. A remarkable decrease in autophagy was observed in bergenerin-treated cells during incubation with MG. The autophagic pathway is involved in mitochondrial dysfunction, which serves as a major pathogenic mechanism (Banerjee et al. 2009). Reactive oxygen species (ROS) may increase mitochondrial membrane lipid peroxidation and mitochondrial dysfunction, causing autophagic cell death (Kirkland

et al. 2002; Xue et al. 1999). Furthermore, excessive intracellular accumulation of ROS may mediate autophagy (Chen and Gibson 2007). We found that ROS levels significantly increased when MC3T3-E1 cells were incubated with MG, and bergenin treatment decreased intracellular ROS levels. We speculate that MG induced autophagy by elevating ROS levels in MC3T3-E1 osteoblasts, and bergenin protected the cells by reducing ROS generation, thus weakening MG-induced autophagy.

In conclusion, bergenin may stimulate osteoblastic bone regeneration, in addition to preventing MG-induced cytotoxicity, ER stress, and autophagy in osteoblasts. Bergenin could contribute to the development of compounds useful for the prevention and treatment of diabetes-related bone disease.

Acknowledgements This research was supported by the Basic Science Research Program of the National Research Foundation of Korea (NRF), funded by the Ministry of Education (NRF-2016R1D1A1B03930082).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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