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Gu Ling Pian, a traditional Chinese medicine, regulates function and OPG/RANKL synthesis of osteoblasts via the p38 MAPK pathway

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Abstract

Osteoporosis is a common disease that makes bones prone to fracture and can affect both men and women. Many traditional Chinese medicine formulations have the potential effect of preventing osteoporosis. Gu Ling Pian (GLP), a traditional Chinese medicine formulation, comprised of tonifying kidney herbal medicines, has been demonstrated to prevent osteoporosis by increasing bone mineral density, however the exact mechanism has not yet been elucidated. Osteoprotegerin (OPG), a receptor activator of NF-κB (RANK), and RANK ligand (RANKL) play critical roles in bone remodelling by regulating the function of osteoclasts. In this study, we investigated the effect of GLP on osteoblasts, namely MG-63 cells. The cell proliferation and differentiation, synthesis of OPG/RANKL and p38 expression were tested on MG-63 cells exposed to serum from rats fed with GLP or not. The results showed that GLP significantly promoted MG-63 cell proliferation and differentiation. Up-regulation of OPG and down-regulation of RANKL at the protein and mRNA level were observed in GLP serum treated MG-63 cells using an enzyme-linked immunosorbent assay and real-time polymerase chain reaction. Further, treatment with GLP serum increased the level of p38 phosphorylation but did not affect the total p38 expression. These effects can be blocked by the p38 specific inhibitor SB203580. The results indicate that GLP can effectively promote the proliferation and differentiation of osteoblasts and regulate their OPG/RANKL expression, while the effects may be mediated via the p38 MAPK pathway. The findings suggest that GLP induces bone formation and may be beneficial for patients with osteoporosis.

Introduction

Osteoporosis is a skeletal disease characterized by low bone mass and microarchitectural deterioration, with a resulting increase in bone fragility and hence susceptibility to fracture (Sambrook & Cooper 2006). The underlying mechanism in all cases of osteoporosis is an imbalance between bone resorption and bone formation. Either bone resorption is excessive or bone formation is diminished. As the most important cells responsible for bone formation, osteoblasts are in charge of synthesis, secretion and mineralization of bony matrix. In-vivo, the functions of osteoblasts and osteoclasts are intimately linked. During skeletal development, cells from the osteoblast lineage synthesize and secrete molecules that initiate and control osteoclast differentiation and maturation (Teitelbaum 2000).

Many studies have investigated the interaction between osteoblasts and osteoclasts. Recently, a family of biologically related tumour necrosis factor (TNF) receptor (TNFR)/TNF-like proteins, osteoprotegerin (OPG), a receptor activator of nuclear factor-κB (RANK), and RANK ligand (RANKL), which together regulate osteoclast function, have been found to play critical roles in bone remodelling (Takahashi et al 1999). OPG is a member of the TNFR family and a soluble decoy receptor competitive against RANKL and soluble RANKL (Khosla 2001). OPG, produced by osteoblasts and other cells, has been found to be a key factor in the inhibition of differentiation and activation of osteoclasts (Hofbauer & Heufelder 2001). On the other hand, RANKL, a member of the TNF family, mainly expressed by osteoblasts, induces osteoclast differentiation and maturation, and activates mature osteoclasts by binding with their receptor RANK expressed on the surface, while soluble RANKL cleaved from RANKL behaves

similarly to RANKL as a soluble factor (Tran et al 2000). The increase of RANKL expression and soluble RANKL release leads to bone resorption and loss (Hofbauer & Heufelder 2001). The expression of OPG and RANKL regulates activation of osteoclasts and therefore profoundly affects bone remodelling (Khosla 2001). Many drugs that decrease bone resorption and increase density, such as oestrogen (Bord et al 2003) and bisphosphonate (Martini et al 2007), have the converse effect on the coupling between the osteoblasts and osteoclasts, up-regulating OPG and/or down-regulating RANKL, leading to decreased activation of RANK and subsequently the numbers of activated osteoclasts in the bone (Boyle et al 2003). The importance of OPG/RANKL in osteoclast differentiation indicates the central role played by osteoblasts in bone formation and resorption.

Osteoblast growth and differentiation, mediated by receptor tyrosine kinases, is regulated by many extracellular signals, most of which activate the Ras mitogen-activated protein kinase (MAPK) cascade (Takeuchi et al 1997). Three distinct subgroups within the MAPK family have been extensively investigated and are reported to have profound effects on osteoblast function. These include: (i) the extracellular signal-regulated kinase (ERK1/2) pathway; (ii) the *c*-Jun *N*-terminal kinase/stress activated protein kinase pathway; and (iii) the p38 MAPK pathway (Hipskind & Bilbe 1998). Recent studies have illustrated that the ERK1/2 pathway is responsible for the signal transduction of growth factors and mitogens, which is predominantly involved in osteoblast differentiation (Lovicu & McAvoy 2001). *c*-Jun *N*-terminal kinase, which is weakly activated by growth factors, plays a part in prostaglandin E_1-induced vascular endothelial growth factor synthesis in osteoblast-like cells (Kanno et al 2004). On the other hand, p38 MAPK, activated in cellular responses to various environmental stresses and factors, regulates expression of OPG and/or RANKL in osteoblasts (Kusumi et al 2005; Pantouli et al 2005; Luo et al 2006).

Gu Ling Pian (GLP), a traditional Chinese medicine, is comprised of Rhizoma Drynariae, Semen Cuscutae, Cornu Cervi Pantotrichum and Deerhorn GlueColla, which, according to a 1000 years of Chinese medicine, tonify the kidney. Modern pharmacological studies have shown that Rhizoma Drynariae, Semen Cuscutae, Cornu Cervi Pantotrichum and Deerhorn GlueColla are able to promote osteoblast differentiation (Jeong et al 2004; Lin et al 2005), increasing the secretion of sex hormone (Qin et al 2000), and preventing osteoporosis (Shen et al 2006). Our previous studies have demonstrated that Gu Ling Wan, a pill dosage form with the same composition of GLP, is able to prevent primary osteoporosis in a vitamin A acid induced animal model (Li et al 1999), and to regulate the factors related to bone formation and resorption, and increase bone mineral density in patients with osteoporosis (Li et al 2005). To our knowledge, the pharmacological mechanism by which GLP prevents osteoporosis has not yet been elucidated, In the present study, we used serum pharmacology to test in-vitro the effects of GLP on human osteoblastic MG-63 cell differentiation, mineralization and regulation of OPG/RANKL as well as p38.

Materials and Methods

GLP and other reagents

GLP was obtained from the School of Traditional Chinese Medicine, Southern Medical University, Guangdong, China. Rhizoma Drynariae (300 g) and Semen Cuscutae (300 g) were extracted twice with 5-fold volumes of 80% ethanol. The supernatant was then filtered and concentrated to 1.3 relative density at 60°C. After drying in vacuum (60°C, −0.08 Mpa), the dry mastic was obtained and mixed with the powder of Cornu Cervi Pantotrichum (37.5 g) and Deerhorn GlueColla (300 g). The mixed powder was dried, then tabletted and GLP was ultimately produced. Other drugs and reagents used in this study were as follows: alkaline phosphatase (ALP) assay kit, dimethylsulfoxide (DMSO), penicillin G, methyl thiazolyl tetrazolium (MTT), streptomycin, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1*H*-imidazole (SB203580) (Sigma, St Louis, USA), minimal essential medium (MEM), phosphate-buffered saline (PBS), fetal bovine serum (FBS) (Gibco RBL, NY, USA), mouse polyclonal anti-human p38, phospho-p38, GAPDH, HRP-conjugated rabbit anti-mouse antibody immunoglobulin IgG (Santa Cruz, CA, USA), OPG (R&D Systems, Minneapolis, MN, USA) and RANKL enzyme-linked immunosorbent assay (ELISA) kit (Biomedica, Vienna, Austria).

Component analysis

Component separation and analysis were performed according to the standard of the Chinese Pharmacopeia 2005. High-performance liquid chromatography (HPLC) was used to separate components of GLP and detect the content of naringin in it. Methanol/acetic acid/H_2O (35:4:65) was used as the mobile phase. GLP sample (0.1 g mL^{-1}) and standard solution were prepared by dissolving in methanol (60 μg/1 mL). The injection volume was 10 μL and the flow rate was 1 mL min^{-1}. The chromatographic system (HP1100; HP, USA) was used to detect the peaks at 283 nm (Chinese Pharmacopeia 2005, Section 1, Appendix VID). Thin-layer chromatography (TLC) was used to separate components of GLP. A 10-mL GLP sample (1 mg) and 10-μL positive sample (meletin, 1 μg) spotted on silica gel G (Merck, Germany) was developed with a solvent system of benzene/ethyl acetate/methanoic acid (5:4:2) solution. After development, it was observed under 365 nm UV irradiation (Chinese Pharmacopeia 2005, Section 1, Appendix VIB).

Preparation of rat serum containing GLP

Male SD rats (SPF grade, certificate no. A2004038), aged 12 weeks, 220–240 g, were obtained from the Laboratory Animal Center of Southern Medical University, Guangdong, China, and divided into four groups that were fed with media, low dose GLP (1.77 g kg^{-1} per day), high dose GLP (3.54 g kg^{-1} per day) and simvastatin (SVT; 120 mg kg^{-1} per day, batch no. J20040032; Merck Sharp & Dohme Ltd, UK) for 3 days, respectively. The serum with or without GLP or SVT

ingredients was prepared 2 h after the last feeding (Bochu & Liancai 2005). Blood samples collected from rats were centrifuged and serum was diluted in MEM to give a final concentration of 10% rat serum.

Cell culture

The human osteoblast-like cell line MG-63 (CRL-1427; American Type Culture Collection Manassas, VA, USA) was cultured in MEM with 10% FBS and antibiotics (100 IU mL^{-1} penicillin G and 100 mg mL^{-1} streptomycin) in a 25-cm^2 flask. To investigate the effect of GLP on MG-63 cells, cells were cultured with serum-free medium for 12 h and co-cultured with serum from rats fed with media, low dose GLP, high dose GLP and SVT for 48 h. To study the effect of p38 inhibitor SB203580, MG-63 cells were treated with 10 μM SB203580 for 2 h before rat serum treatment.

Cell proliferation assay

The MG-63 cells were adjusted to 1.25×10^5 mL^{-1} and plated in a 96-well plate, 0.2 mL/well. After treatment, the medium was removed and the cells were washed twice with RPMI-1640. Then, 200 μL RPMI-1640 supplemented with 10% FBS and 10% MTT. The MG-63 cells were adjusted to 1.25×10^5 mL^{-1} and plated in a 96-well plate, 0.2 mL/well. After treatment, the medium was removed and the cells were washed twice with RPMI-1640. Then, 200 μL RPMI-1640 supplemented with 10% FBS and 10% MTT (5 mg mL^{-1}) was added. After incubation for another 4 h, the reduced intracellular formazan product was dissolved by replacing 150 μL of RPMI-1640 with the same volume of DMSO. Optical density at 490 nm and 570 nm was determined using a Bio-Rad 680 plate reader (Bio-Rad, CA, USA).

ALP activity assay

MG-63 cells were seeded into 96-well plates at a density of 5×10^3 cells/well and cultured for 24 h. After exposure to rat serum for 48 h, cells were collected and washed with phosphate-buffered saline three times. Cell lysates were extracted with 0.1% Triton X-100. ALP activity in cell lysates was determined by ALP assay kits according to the manufacturer's recommendations and normalized by the protein content.

Mineralized bone nodule formation assay

MG-63 cells were plated in 24-well culture plates at a density of 2×10^4 cells/well and cultured in MEM with 10% FBS. When the cells reached confluence (Day 0), the medium was changed to MEM containing 10% rat serum to be tested and 10 mM β-glycerophosphate. The medium was changed every other day and fresh reagents were added. After 10-day incubation, the cells were fixed with 100% methanol and stained with the Alizarin Red method (Tanabe et al 2004). Mineralization was quantified by visual counting with an optical microscope (magnification ×40).

RNA isolation and real-time polymerase chain reaction

After treatment with rat serum for 48 h, MG-63 cells were collected. Total RNA was isolated using Trizol reagent (Invitrogen, Cergy Pontoise, France). The 260/280 absorbance ratio was measured for verification of the purity of RNA. Total RNA was reverse transcribed and quantitative real-time polymerase chain reaction was conducted using the Taqman system in a Perkin Elmer Prism 7000 (Applied Biosystems, Foster City, CA, USA). The primers used to amplify cDNA sequences and fluorescence labelled probes, all obtained from Daangene Co, Ltd (Guangzhou, China), were as follows. For OPG: forward: 5′-CGGCACATTGGACATGCTAA-3′; reverse: 5′-TCCCGGTAAGCTTTCCATCA-3′ (66 bp); probe: 5′-FAM-TCACCTTCGAGCAGCTTCGTAGCTAMRA-3′. For RANKL: forward: 5′-CGATGGTGGATGGCTCATG-3′; reverse: 5′-TGAGCAAAAGGCTGAGCTTCA-3′ (67 bp); probe: 5′-FAM-TTAGATCTGGCCAAGAGGAGCAAGCTAMRA-3′. For β-actin: forward: 5′-GCGCGGCTACAGCTTCA-3′; reverse: 5′-TCTCCTTAATGTCACGCACGAT-3′ (59 bp); probe: 5′-FAM-CACCACGGCCGAGCGGGATAMRA-3′. The amplification efficiency of the target gene and housekeeping gene was measured and the result of approximately equal efficiency between target gene and β-actin was obtained. Agarose gel electrophoresis of polymerase chain reaction products was performed to ensure correct amplicon size and reaction specificity. The mRNA level of each sample for each gene was normalized to that of the β-actin mRNA. The relative mRNA level was represented as $2^{-\Delta\Delta t}$ and expressed as the fold increase compared with the untreated cells where $\Delta\Delta t = (Ct_{target} - Ct_{actin})_{experiment} - (Ct_{target} - Ct_{actin})_{control}$ (Livak & Schmittgen 2001).

ELISA for OPG and soluble RANKL

Supernatants of MG-63 cells exposed to rat serum for 48 h were collected. OPG and RANKL in cell-free supernatants were detected by an ELISA kit according to the manufacturer's instructions. The lower detection limit of the OPG assay was 62.5 pg mL^{-1} and that of soluble RANKL was 1.6 pg mL^{-1}. Absorption was determined using an ELISA reader at 492 nm.

Western blot analysis

After treatment with rat serum for 48 h, 1×10^6 stimulated cells were lysed with lysis buffer (50 mM Tris-HCl, (pH 7.4), 150 mM NaCl, 1% Triton X-100 and protease inhibitors). Protein (20 μg) was denatured in the SDS sample buffer and separated on 10% polyacrylamide-SDS gel. The protein was transferred electrophoretically to polyvinylidene difluoride membranes. Membranes were immunoblotted with total p38, phospho-p38 and GAPDH antibody. Proteins were detected by an ECL kit according to the manufacturer's instruction.

Statistical analysis

All data are expressed as means ± s.d. and differences among groups were analysed using the Kruskal–Wallis test. Individual

differences were then detected using Dunn's test. A value of $P < 0.05$ was considered statistically significant.

Results and Discussion

Analysis of components of GLP

The content of naringin, meletin and nitrogen was investigated in Rhizoma Drynariae, Semen Cuscutae, Cornu Cervi Pantotrichum and Deerhorn GlueColla. The content of naringin and meletin was detected by HPLC and TLC, respectively. As shown in Figure 1, GLP contained naringin at a concentration of 57 mg/100 g. GLP, which was spotted onto a TLC plate, could be detected as a fluorescent spot of vivid blue under UV irradiation. At the same position as the positive control (1 μg meletin), strong fluorescence spots were found. The decigram method was used to detected nitrogen in GLP and 6790 mg nitrogen was detected in 100 g GLP.

GLP promotes osteoblasts differentiation and bone formation

The function of osteoblasts correlates closely with their proliferation and differentiated conditions. Statins have been proved to stimulate bone formation in-vitro and in rodents (Mundy et al 1999). SVT, a pro-drug of a potent 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase inhibitor and inhibitor of cholesterol synthesis in humans and animals, has anabolic effects on bone through the promotion of osteoblastic differentiation. It has been suggested that SVT could be used for the treatment of osteoporosis (Maeda et al 2001). We chose the method of serum pharmacology, orally feeding the rats with GLP and treating the MG-63 cells with the rat

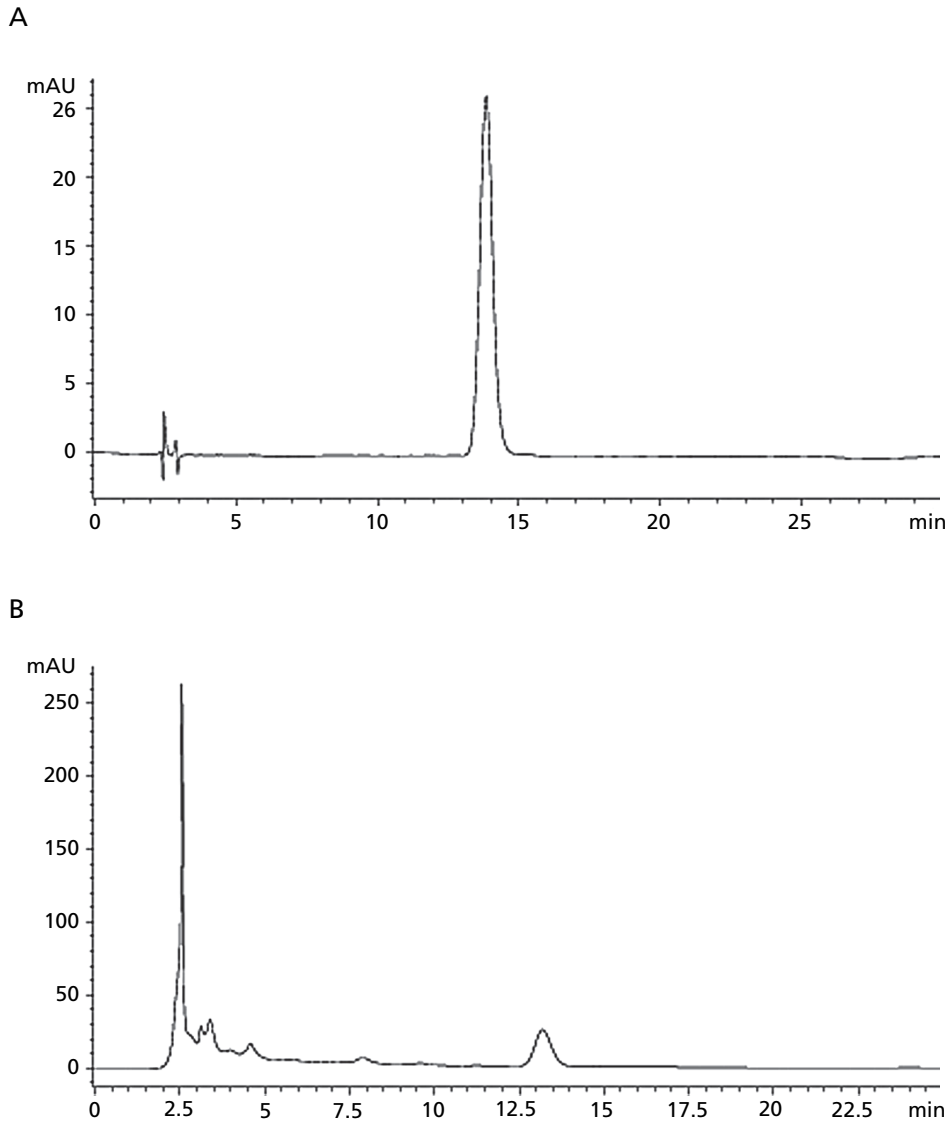

Figure 1 High-performance liquid chromatography was used to separate components of Gu Ling Pian (GLP) and detect the content of naringin A. Positive control; B. GLP sample.

serum, to observe the effect of GLP on the function of osteoblasts. Many researchers believe that serum pharmacology is more scientific and more suited to Chinese traditional medicine than traditional pharmacology in which crude drugs are added directly into the culture system of cells or organs in-vitro. The drugs do not undergo metabolism and biotransformation in the traditional pharmacology study so that there are not the drug interactions, which means that the environment experienced by cells is significantly different compared with that in the serum pharmacological study (Bochu & Liancai 2005). To evaluate the effect of GLP on the function of osteoblasts, we measured proliferation, ALP activity and mineralized bone nodules of MG-63 cells treated with rat serum containing GLP or SVT. In contrast to the control, increasing optical density values, higher ALP activity and more mineralized nodules were observed in the cells exposed to GLP and SVT positive control (Table 1). These results strongly suggest that GLP has the effect of promoting osteoblast differentiation and bone formation in-vitro.

GLP increases OPG and decreases RANKL expression in osteoblasts

The balance between osteoblast and osteoclast function is regulated systemically by a variety of hormones and locally by the production of paracrine factors by osteoblasts or bone marrow stromal cells that regulate osteoclast function (Manolagas & Jilka 1995). The importance of OPG and RANKL, a major discovery of the past decade in bone biology, as regulators of osteoclastogenesis is well known. Previous studies have illustrated that the expression of OPG and RANKL is developmentally regulated by osteoblasts. Expression of OPG increases during osteoblast differentiation, whereas RANKL expression is inversely related to the degree of osteoblast differentiation (Udagawa et al 2000). By secreting OPG and RANKL, osteoblasts regulate the activity of osteoclasts and bone resorption directly. Similarly, drugs and extra stimulations influence osteoclast differentiation and activation by regulating the expression of OPG and RANKL in osteoblasts either partly or totally (Boyle et al 2003). To confirm the effect of GLP on the expression of OPG and RANKL of osteoblasts, we allowed MG-63 cells to be treated with GLP-containing or SVT-containing rat serum for 48 h and detected expression of OPG and RANKL. In contrast to the control, higher OPG mRNA and lower RANKL mRNA levels were observed in MG-63 cells treated with GLP and SVT. The expression of OPG and RANKL were also regulated by GLP and SVT (Table 2). The data provide the evidence that GLP can effectively promote the expression of OPG but has the opposite effect on RANKL in osteoblasts. The findings indicate that GLP might be able to suppress the differentiation and activation of osteoclasts indirectly by regulating the expression of OPG and RANKL in osteoblasts.

GLP-induced osteoblast differentiation and synthesis of OPG/RANKL in MG-63 cells is p38 MAPK dependent

The MAPK pathway plays a critical role in controlling cell proliferation and differentiation. It has been reported that p38 MAPK is involved in the modulation of osteoblasts by varied factors or reagents as well as the expression of OPG and RANKL (Kusumi et al 2005; Pantouli et al 2005; Luo et al 2006). Karsdal et al (2001) showed that p38 MAPK took part in osteoblast elongation induced by TGF-β1, which was evaluated by p38 MAPK inhibitor. A recent study showed that inhibition of p38 MAPK interfered with the differentiation of osteoblasts, and indicates that activation of p38 MAPK is necessary for osteoblasts differentiation (Hu et al 2003). To gain further insight into the mechanism by which GLP regulates the function of osteoblasts, we evaluated cell proliferation and differentiation, and synthesis of OPG/RANKL in MG-63 cells by using an inhibitor specific to p38.

Table 1 Effect of Gu Ling Pian (GLP) on the proliferation and differentiation of MG-63 cells

Group	Cell proliferation (% of control)	Alkaline phosphatase activity (% of control)	No. of mineralization nodules
Control	100.0 ± 27.4	100.0 ± 15.8	86.7 ± 6.7
Low dose GLP	119.5 ± 18.5	142.6 ± 26.5*	119.0 ± 8.7*
High dose GLP	143.2 ± 11.3*	189.8 ± 16.2**	181.3 ± 7.4**
Simvastatin	149.1 ± 15.9*	194.1 ± 16.0**	202.0 ± 12.1**

Each value represents the mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$ compared with control.

Table 2 Effect of Gu Ling Pian (GLP) on expression of osteoprotegerin (OPG) and receptor activator of NF-κB ligand (RANKL) expression in MG-63 cells

Group	OPG (ng mL^{-1})	RANKL (ng mL^{-1})	OPG mRNA (fold increase)	RANKL mRNA (fold increase)
Control	1143.0 ± 402	45.2 ± 4.9	1.01 ± 0.14	1.00 ± 0.09
Low dose GLP	2221.7 ± 602.4*	35.3 ± 6.7	1.80 ± 0.19*	0.89 ± 0.05
High dose GLP	4031.3 ± 430.0**	26.3 ± 4.1*	3.61 ± 0.50**	0.48 ± 0.06**
Simvastatin	4144.0 ± 742.8**	14.7 ± 4.8**	4.14 ± 0.51**	0.23 ± 0.03**

Each value represents the mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$ compared with control.

Table 3 Effect of SB203580 on the proliferation and differentiation in Gu Ling Pian (GLP) treated MG-63 cells

Group	Cell proliferation (% of control)	Alkaline phosphatase activity (% of control)	No. of mineralization nodules
Control	100.0 ± 14.0	100.0 ± 12.1	78.3 ± 9.5
SB203580	99.1 ± 15.2	100.0 ± 9.1	70.7 ± 6.1
GLP	136.8 ± 16.5	157.2 ± 16.2	168.0 ± 17.1
SB203580 + GLP	100.8 ± 16.6*	97.9 ± 16.1**	78.0 ± 14.5**

Each value represents the mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$ compared with GLP treatment alone.

Table 4 Effect of SB203580 on expression of osteoprotegerin (OPG) and receptor activator of NF-κB ligand (RANKL) in Gu Ling Pian (GLP) treated MG-63 cells

Group	OPG (ng mL^{-1})	RANKL (ng mL^{-1})	OPG mRNA (fold increase)	RANKL mRNA (fold increase)
Control	1189.6 ± 310.1	44.7 ± 8.6	1.00 ± 0.11	1.00 ± 0.11
SB203580	1032.1 ± 191.5	44.5 ± 11.9	0.96 ± 0.10	0.93 ± 0.11
GLP	3871.6 ± 317.1	27.9 ± 5.7	2.93 ± 0.42	0.51 ± 0.09
SB203580 + GLP	1265.5 ± 103.0**	38.8 ± 6.1*	1.07 ± 0.08*	1.03 ± 0.09**

Each value represents the mean ± s.d., n = 3. *$P < 0.05$, **$P < 0.01$ compared with GLP treatment alone.

Cell proliferation, ALP activity and mineralization nodules formed were significantly inhibited by SB203580, respectively (Table 3). Higher expression of OPG and lower expression of RANKL were found in cells treated with rat serum containing GLP only, which is in contrast to cells exposed to SB203580. Similar results were also noticed paralleled in mRNA level (Table 4). The results indicate that blocking the p38 MAPK pathway may inhibit GLP-induced osteoblasts proliferation and differentiation, and influence expression of OPG/RANKL regulated by GLP. We also assessed the expression of total p38 and phospho-p38 in MG-63 cells treated with rat serum and/or SB03580. As shown in Figure 2A, total p38 was not altered in MG-63 cells whether they were treated with GLP or not. However, a significant increase was found in the phosphorylation of p38 in MG-63 cells treated with rat serum containing GLP. The expression of p38 induced by GLP was effectively inhibited by SB203580 (Figure 2B). The results indicated that GLP was able to promote the activation of p38 and the effect can be blocked by the p38 inhibitor, SB203580. From the findings, it can be reasonably interpreted that GLP has the effect of promoting osteoblast function and regulating the synthesis of OPG and RANKL.

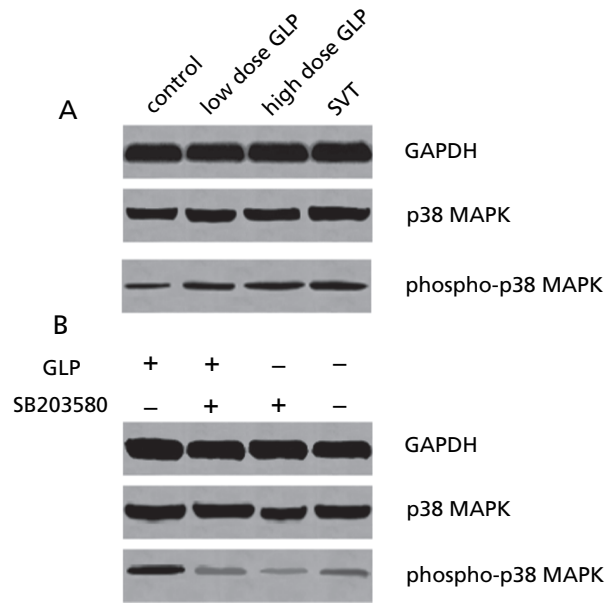

Figure 2 Effect of Gu Ling Pian (GLP) and SB203580 on the expression of p38 in MG-63 cells. A. Effect of GLP on the expression of p38 in MG-63 cells. MG-63 cells (1×10^6 cells) were treated with serum from rats fed with media, low dose GLP, high dose of GLP and simvastatin (SVT) for 48 h, and protein isolated from stimulated MG-63 cells was analysed by Western blot. The level of total p38 and phosphorylated p38 was evaluated. B. Effect of SB203580 on the expression of p38 in MG-63 cells treated with GLP. After treatment with SB203580 for 2 h, MG-63 cells were exposed to serum from rats fed with media, isolated and Western blot was performed to observe the level of total p38 and phosphorylated p38. Results are representative of three independent experiments.

Conclusion

This study provides further evidence that GLP increases bone mineral density and prevents osteoporosis in rat models and in patients. We studied the effects of GLP on the function of osteoblastic-like MG-63 cells. The action of GLP on the expression of OPG/RANKL and p38 MAPK, and the blocking effect of SB203580, were also investigated. We conclude that GLP effectively induces differentiation and maturation of osteoblasts and regulates the expression of OPG and RANKL via the p38 MAPK pathway, and suggest that GLP may be beneficial in stimulating osteoblastic activity, thereby resulting in bone formation. Current

drugs used to treat osteoporosis, including bisphosphonates, calcitonin, estrogen, vitamin D analogues and ipriflavone, mainly inhibit bone resorption, which maintain bone mass by inhibiting the function of osteoclasts. There are few drugs used to treat osteoporosis that are able to increase or recover bone mass. With the potential of promoting the function of osteoblasts directly and inhibiting osteoclasts indirectly, GLP may be an attractive and interesting anti-osteoporosis drug candidate. Further research and possibly clinical trials in patients with osteoporosis is warranted.

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