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RESEARCH ARTICLE

Effect of Ursolic Acid and Oleanolic Acid on Osteoblastic Like Cell-Line MC3T3-E1

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ABSTRACT

To determine whether Ursolic acid and Oleanolic acid could be used as dietary supplements for osteoporosis animals, CCK-8 (cell counting kit -8), ELISA (enzyme-linked immunosorbent assay), FQ-PCR (Real-time Fluorescence Quantitative PCR) and western bolt assays were used on MC3T3-E1 cells. After treatment with Ursolic acid and Oleanolic acid for 48h and 72h, the cell viability showed marked increased. Ursolic acid and Oleanolic acid could up-regulated RANKL protein in supernatant at 48h, and up-regulated both OPG and RANKL protein at 72h, in different pattern. Ursolic acid increased RANKL mRNA at 48h and 72h, OPG mRNA at 72h; Oleanolic acid increased OPG mRNA at 48h and 72h, RANKL mRNA at 72h. Adapt concentrations of Ursolic acid and Oleanolic acid could up-regulated OPG and down-regulated RANKL protein expression. After exposed on Ursolic acid or Oleanolic acid with or without inhibitors for 72h, PD98059, SB203580, SP600125 and LY-294002 could affect Ursolic acid- and Oleanolic acid-induced cell proliferation and the leakage of OPG proteins. Ursolic acid and Oleanolic acid could regulate the phosphorylation of ERK, JNK, p38 and AKT. So Ursolic acid and Oleanolic acid could promote MC3T3-E1 cells proliferation, OPG and RANKL expression dose-dependently and time-dependently, and Ursolic acid had larger range of concentrations and better effect. These osteogenic effects of Ursolic acid and Oleanolic acid related to MAPK and AKT signaling pathways by different patterns.

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INTRODUCTION

Now, osteoporosis is becoming a health concern for elderly animals as well as humans. Traditional estrogen replacement therapy and calcium supplementation appear to have limit effect or obvious side effects (Turn, 1991). Natural compounds have been applied for therapeutic purposes in traditional medicine, traditional veterinary medicine and folk medicine all over the world, especially in Asian countries, as they were presumed to be safer than synthetic compounds due to their presence in the diet, wide availability, and low tolerability. Tulp and Bohlin (2005) have announced that, many natural compounds are currently known and will prove to be more than just 'secondary metabolites' and the chemical diversity of these compounds is tremendous and may offer inspiration for innovations in the fields of medicine, nutrition, agrochemical and life sciences (Dunkel *et al*., 2006). So natural compounds may help the elderly animals ameliorate osteoporosis or improve their quality of life. *Fructus ligustri lucidi*-a traditional herb, containing a great quantity of Oleanolic acid and Ursolic acid showed the effect on kidney protection and nutrition, as well as in bone system (Lin *et al*., 2007). In this case, by using MC3T3-E1 cell line, we investigated the osteogenic effects *in vitro* to assess whether these two triterpenoids could be dietary supplements for osteoporosis animals.

Oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid) and its isomer, Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid), are ubiquitous triterpenoids in plant kingdom, medicinal herbs, and are integral part of the human and animal diet (Liu *et al*., 2005). Oleanolic acid exhibits a wide range of pharmacological and biochemical effects including anti-inflammatory, anti-hyperlipidemic, hypoglycemic effects (Jäger *et al*., 2009) and antibacterial (Shin *et al*., 2012). Ursolic acid has been proved to possess variety of functions such as anticancer (Bashir, 2013), anti-inflammatory, hepato-protective and kidney

protection (Chen *et al*., 2013). In traditional Chinese medicine, bone system is related to liver and kidney.

Altering the relative biological availabilities of OPG, RANKL and RANK has direct consequences for the regulation of both bone resorption and bone remodeling (Blair *et al*., 2006; Tat *et al*., 2006). To this, the expression of OPG and RANKL is considered as an important index to evaluate osteogenic effect. Literaturelly, OPG and RANKL are regulated by many cellular factors and signaling pathways, such as IGF-1 (Rubin *et al*., 2002) and ER (Hofbauer *et al*, 2004), *in vitro*.

MAPKs (mitogen activated protein kinases) control many cellular events, including differentiation, proliferation, and phosphorylate specific sites of target protein substrates to regulate different cellular events. (Ikeda *et al*., 2005; Alemany *et al*., 2010). Medical effects of Ursolic acid and Oleanolic acid were suggested that controlled by MAPK, AKT and other signaling pathways (Bashir, 2013; Xavier *et al*., 2013; Ikeda *et al*., 2005; Zheng *et al*, 2013). In this article, the osteogenic effects of Ursolic acid and Oleanolic acid on cells have been confirmed, and how Ursolic acid and Oleanolic acid functions on osteoblast-like cells via MAPK and AKT signaling pathways have also been explored. Hope the article will be helpful for development of dietary supplements for osteoporosis animal.

MATERIALS AND METHODS

Reagents and Antibodies: Ursolic acid and Oleanolic acid were purchased from National Institutes for Food and Drug Control in Beijing, and all working concentrations were made by diluting in MEM-Alpha culture medium. The final DMSO concentration in the medium was less than 0.1%. Pathway Inhibitors: PD98059, SB 203580, SP 600125 and LY-294,002 were purchased from Sigma-Aldrich (St. Louis, USA). Antibodies were purchased from ThermoFisher scientific (MA, USA), R&D systems (MN, USA), SAB (Signalway Antibody, MD, USA), Abclonal (MA, USA), CST (Cell Signaling Technology, MA, USA) and PTG (Proteintech Group, Wuhan, China).

Cell culture: Cell line MC3T3-E1 was obtained from Chinese Academy of Medical Sciences and the Institute of Basic Medical Sciences Cell Resource Center in Beijing. Cells culture medium was containing 12% FBS, 100U/ml penicillin and 100μg/ml streptomycin (Hyclone, USA).

Cell viability: Cell viability was measured using the Cell Counting Kit-8 (Dojindo China CO., Shanghai, China). Cells in 96-well plate were treated with different concentrations of Ursolic acid and Oleanolic acid for 48h and 72h. Subsequently, different inhibitors (20μM) were added and cells with or without inhibitor were incubated for 72h, visible absorption at 450 nm was measured. The cell proliferation rate = absorbance value of the treated cells/absorbance of the control cells × 100%.

Enzyme-linked immunosorbent assay (ELISA): The treatment of cells was same as cell viability assay. The cell culture supernatant was subjected to ELISA and the concentrations of OPG and RANKL (R&D systems, MN, USA) were measured according to the protocol of the kits. The level of OPG (RANKL) relative to control = absorbance value of the treated cells/absorbance of the control cells × 100%.

Fluorescence quantitative Real-time PCR (FQ-PCR) analysis: Cells were treated with different concentrations of Ursolic acid (10^{-3}-10^{-5}μM) or Oleanolic acid (10^{-2}-10^{-4}μM) for 48h and 72h. Total cellular RNA was extracted Single strand cDNA synthesis was performed using the TransScript Reverse Transcriptase (TransGen Biotech, Beijing, China). Fluorescence quantitative Real-time PCR (FQ-PCR) was performed and individual PCR reactions were carried out with TransStart Green qPCR Super Mix (TransGen Biotech, Beijing) in accordance with the manufacturer's instructions. The expression level of target genes was normalized to the reference gene β-actin.

Western blotting: After treatment with Ursolic acid (10^{-4}μM) or Oleanolic acid (10^{-3}μM) for 72h, cells were lysed in RIPA (Radio Immunoprecipitation Assay) Lysis Buffer (Beyotime, Beijing, China) and protein concentrations were quantified. Protein extractions were separated and then transferred on to nitrocellulose membranes. After 1h blocking in TBST containing 5% skimmed milk, the membrane was incubated with indicated primary antibodies overnight at 4□. The second incubation was performed with horseradish peroxidase-conjugated secondary antibody at room temperature for 1h. The blots were visualized using Electrochemiluminescence Western Blot Kit (Beyotime, Beijing, China) according to the manufacturer's instruction.

Statistic analysis: Each experiment was performed at least three times, and data are shown as the mean±SD. Statistical analysis was analyzed by one-way ANOVA followed by a Least-significant difference (LSD) text for multiple comparisons if necessary. In all cases, $P<0.05$ was considered as significant, $P<0.01$ was considered as remarkable effect.

RESULTS

Effects of Ursolic acid and Oleanolic acid on proliferation of MC3T3-E1 cells: After 48h or 72h Ursolic acid treatments, the cell viability was increased ($P<0.05$), and the growth rate was higher after 72h treatments than 48h. After 72h Ursolic acid treatments, treatments with high concentration gradient showed better effect on cell proliferation (Table 1). Similarly, increased cell proliferation was also found after treatments with Oleanolic acid, only except highest concentration (10μM) ($P<0.01$). After 72h Ursolic acid treatment, treatments with lower concentrations showed more efficient cell proliferation effect than the status in 48h treatment time at the same concentration, and the growth rate have showed time-independent. (Table 1)

Effects of Ursolic acid and Oleanolic acid on the expression level of OPG and RANKL: As shown in Fig.1A and Fig.1B, Ursolic acid mainly up-regulated the expression of RANKL after 48h and 72h ($P<0.05$), up-regulated OPG expression level after 72h treatment time ($P<0.05$), and the up-regulated expression of OPG was in a dose- and time-dependent model. Turning to the treatment

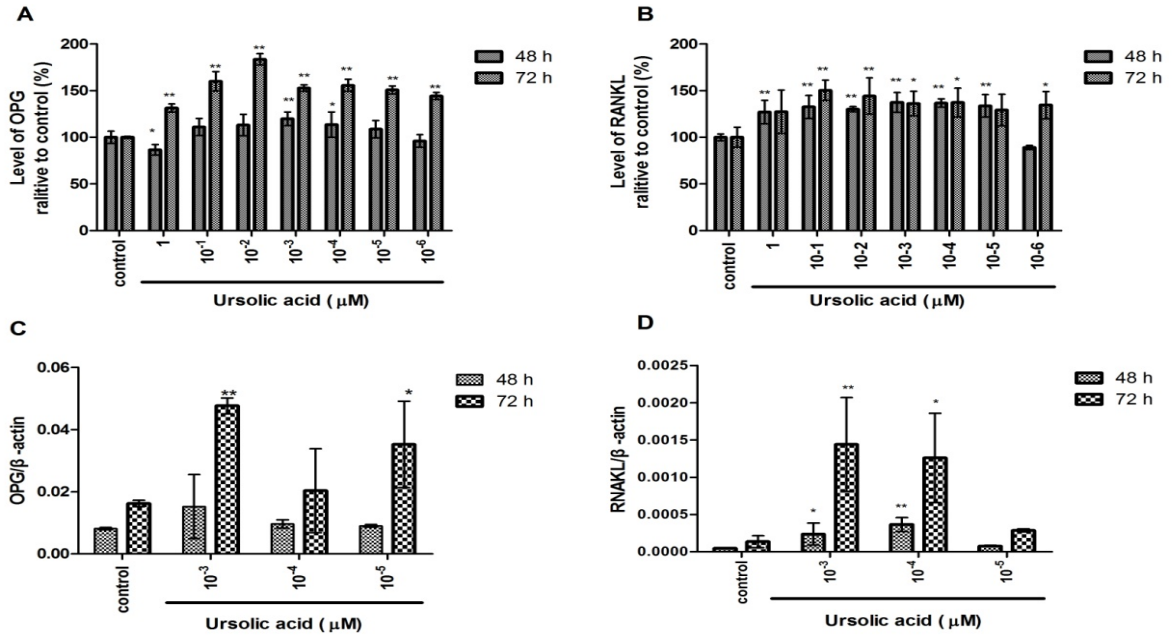

Fig. 1: Effect of Ursolic acid on expression level of OPG and RANKL. ELISA assays to determine the leakage of OPG (A) and RANKL (B) with the concentrations of 1-10^{-6}μM, FQ-PCR assays to examine expression level of OPG (C) and RANKL (D) mRNA with the concentrations of 10^{-3}-10^{-5} μM. Data represent mean±SD. *P<0.05 and **P<0.01 compared to the control group.

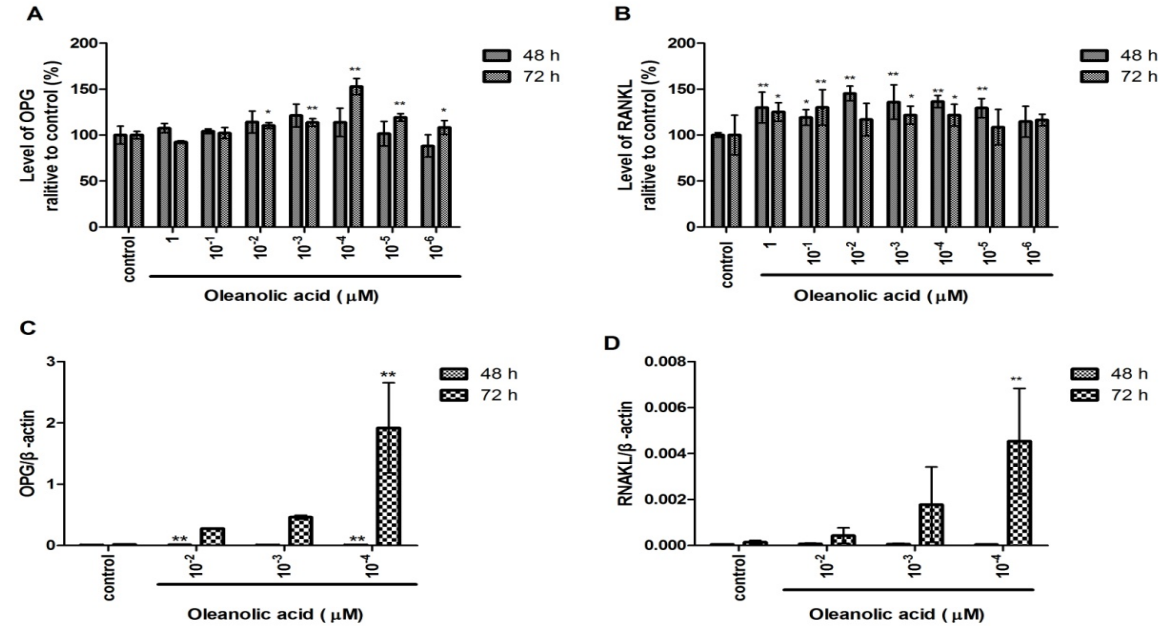

Fig. 2: Effect of Oleanolic acid on expression level of OPG and RANKL. ELISA assays to determine the leakage of OPG (A) and RANKL (B) with the concentrations of 1-10^{-6} μM, FQ-PCR assays to examine expression level of OPG (C) and RANKL mRNA (D) with the concentrations of 10^{-2}-10^{-4} μM. Data represent mean±SD. *P<0.05 and **P<0.01 compared to the control group.

with Oleanolic acid (Fig.2A and Fig.2B), the expression of RANKL was up-regulated after 48h and 72h and OPG levels were only promoted after 72h (P<0.05), and Oleanolic acid that up-regulated the expression of OPG was in a dose- and time-dependent model, but different with the case of Ursolic acid treatments. Cell number is also the variable in the expression of OPG and RANKL, therefore we next compared the relative expression level of OPG (or RANKL) to cell viability (Table 2-3). As shown in Table 2 and 3, the OPG expression level was up-regulated by different concentrations of Ursolic acid and Oleanolic acid treatments.

Turning to the transcription regulation level, FQ-PCR data showed an increased mRNA expression level of RANKL after 48h and/or 72h Ursolic acid treatments as well as the OPG mRNA expression level which increased after 72h treatment (Fig.1C and Fig.1D). Similarly, the OPG mRNA level is increased after treatment with Oleanolic acid, and the increase that after 72h treatment was extremely higher than that of 48h (Fig.2C and Fig.2D). Moreover, the mRNA level of RANKL was increased when a 72h Oleanolic acid treatments has been given. The western blot results (Fig.3) showed that, treatments with 10^{-4}μM of Ursolic acid and 10^{-3}μM of Oleanolic acid up-regulated the

Table 1: Effect of Ursolic acid and Oleanolic acid on proliferation of MC3T3-E1 cells

Ursolic acid (μM)	48 h	72 h	Oleanolic acid (μM)	48 h	72 h
Control	100±5.67	100±2.93	control	100±4.25	100±4.97
10	97.38±3.28	109.50±7.26	10	92.16±2.10**	103.19±4.57
1	113.37±10.3**	117.41±7.44**	1	100.93±2.68	106.25±3.82
10^{-1}	118.02±8.05**	133.25±10.50**	10^{-1}	106.11±1.33*	116.53±9.53**
10^{-2}	109.88±9.48*	121.64±12.22**	10^{-2}	113.81±6.29**	113.47±4.49**
10^{-3}	112.79±9.33*	115.83±7.02**	10^{-3}	112.75±3.64**	114.72±4.57**
10^{-4}	111.92±4.71*	113.19±6.36**	10^{-4}	108.23±5.42**	108.33±10.35*
10^{-5}	109.30±10.55	117.94±5.49**	10^{-5}	108.37±3.29**	102.64±5.65
10^{-6}	106.10±6.57	112.66±6.94*	10^{-6}	102.79±5.07	105.14±8.74
10^{-7}	97.67±3.75	109.50±4.59	10^{-7}	103.32±3.47	100.69±3.68

Data represent mean±SD. *P<0.05 and **P<0.01 compared to the control group in a column.

Table 2: The relative expression level of OPG (or RANKL) to cell viability of Ursolic acid

Treatment of Ursolic acid (μM)	OPG level/ Cell viability (48h)	RANKL level/ Cell viability (48h)	OPG level/ Cell viability (72h)	RANKL level/ Cell viability (72h)
Control	1	1	1	1
1	0.763	1.121	1.119	1.085
10^{-1}	0.941	1.123	1.201	1.129
10^{-2}	1.029	1.184	1.509	1.186
10^{-3}	1.063	1.218	1.320	1.177
10^{-4}	1.014	1.222	1.376	1.212
10^{-5}	0.995	1.224	1.278	1.095
10^{-6}	0.905	0.840	1.281	1.194

Table 3: The relative expression level of OPG (or RANKL) to cell viability of Oleanolic acid

Treatment of Oleanolic acid(μM)	OPG level/Cell viability (48h)	RANKL level/Cell viability (48h)	OPG level/Cell viability (72h)	RANKL level/Cell viability (72h)
Control	1	1	1	1
1	1.066	1.287	0.870	1.179
10^{-1}	0.983	1.123	0.877	1.117
10^{-2}	1.004	1.276	0.973	1.030
10^{-3}	1.076	1.206	0.992	1.061
10^{-4}	1.052	1.261	1.411	1.123
10^{-5}	0.938	1.194	1.162	1.058
10^{-6}	0.857	1.116	1.030	1.107

protein level of OPG while down-regulated the expression of RANKL protein.

Effects of Ursolic acid- and Oleanolic acid-induced cell proliferation and OPG expression via ERK, JNK, p38 and Akt signaling pathways: The using concentration of all inhibitors was 20μM. Treatments were divided into sub-groups: Ursolic acid or Oleanolic acid only, inhibitors only and the mixtures (Ursolic acid or Oleanolic acid plus with inhibitors). Cell growth was inhibited by SB and LY (P<0.05), while PD9 and SP had no effect (Table 4). Ursolic acid- and Oleanolic acid-induced cell proliferation were inhibited by PD9 and SP and Oleanolic acid could partially prevent the inhibition effect by PD9 (Table 4). All four inhibitors inhibit the leakage of OPG (P<0.05) (Table 5). According to Table 5, the inhibition effect by SP was partially prevented by Ursolic acid and Oleanolic acid which altered the OPG expression level to control value. The MEK1/2 inhibitor PD9 acted different in Ursolic acid-and Oleanolic acid-induced cell proliferation, as the inhibition effect by PD9 is promoted by Ursolic acid, while is been partially prevented by Oleanolic acid (Table 4).

Western blots were then used to examine the effects of Ursolic acid and Oleanolic acid on signaling pathways. The protein level of ERK was promoted by 10^{-4}μM Ursolic acid treatment, while was inhibited after 10^{-3}μM Oleanolic acid treatment. Moreover, the phosphorylation of ERK was not affected by Ursolic acid, but inhibited by Oleanolic acid. Ursolic acid inhibited the protein level of JNK, while has no effects on the phosphorylation of JNK (Fig.4). Oleanolic acid promoted both protein level and phosphorylation level of JNK. Ursolic acid could up-regulate p38 and phosphorylation of p38. By contrast, Oleanolic acid down-regulated the phosphorylation of p38 and has no effect on its protein level (Fig. 4). As shown in Fig.4, the phosphorylation of Akt was promoted with either 10^{-4}μM Ursolic acid or 10^{-3}μM Oleanolic acid, while the protein levels of Akt was raised after Oleanolic acid treatment but was not affected by Ursolic acid.

DISCUSSION

Osteoblastic like MC3T3-E1 cell is commonly used in the examination of treatments induced osteogenic effects. Previous researches have proved the induced cell apoptosis by Ursolic acid (Zheng *et al.*, 2012). By the performing of treatments with different concentrations, we observed that both Ursolic acid and Oleanolic acid could promote the cell growth dose-dependently, with which their working concentrations ranged between 10^{-1}-10^{-5}μM. Moreover, after 72h treatment, Ursolic acid appeared a better effect on cell growth than Oleanolic acid do. (Table 1) The effects of natural compounds application is normally under the regulation of usage dose and treatment time. Similar effects were found in our experiments, as the low-dose of Oleanolic acid treatments produced adaptive responses, which similar to "hormesis" (Table 1).

It becomes clear that many clinically relevant metabolic bone diseases in humans or animals, including different forms of osteoporosis are related to, or caused by the alterations of the OPG/RANKL/RANK system (Teitelbaum, 2000). The expression level of OPG and RANKL become an index of medicine treatment effect, like Menaquinone-7 (Katsuyama *et al.*, 2005). Since the effects of two compounds on cell proliferation had been confirmed, we next examined the effect of them on OPG/ RANKL system.

Compared to Oleanolic acid, Ursolic acid appears the better effect on the up-regulation of OPG protein, especially when a 72h treatment with higher concentration was given (Fig.1A and Fig.2A). Unlike some other compounds (Li *et al.*, 2012), the protein level of RANKL has been up-regulated by either Ursolic acid or Oleanolic acid treatments (Fig.1B and Fig. 2B). To investigate whether OPG/RANKL system is participated in the osteogenic effects by the application of Ursolic acid and Oleanolic acid, we performed the comparison between the level of OPG or RANKL and cell viability at same concentration treatment background. As observed in Table 2 and Table 3, the

Table 4: Effect of Ursolic acid and Oleanolic acid induced cell proliferation via ERK, p38, JNK, AKT using inhibitor PD98059, SB203580, SP600125 and LY-294002 (20μM)

Groups	PD98059	SB203580	SP600125	LY-294002
Ursolic acid-(10^{-4}μM)				
Control	100±2.40a	100±1.52b	100±7.22a	100±8.12b
Inhibitor	104.38±6.01a	86.68±4.25a	98.12±9.79a	72.76±4.84a
Inhibitors and Ursolic Acid	104.61±10.99a	90.19±4.23a	102.12±10.49a	71.59±5.08a
Ursolic Acid	122.58±9.63b	109.35±7.45c	120.24±8.87b	110.98±9.94c
Oleanolic acid-(10^{-3} μM)				
Control	100±2.40a	100±1.52b	100±7.22a	100±8.12b
Inhibitor	104.38±6.01a	86.68±4.25a	98.12±9.79a	72.76±4.84a
Inhibitor and Oleanolic Acid	113.13±1.64b	81.07±4.39a	99.29±6.09a	72.15±3.56a
Oleanolic Acid	126.96±12.35c	112.85±9.02c	119.29±3.18b	117.07±12.28c

Data represent mean±SD. Different letters in a column under specific acid indicate a significant (P<0.05) difference.

Table 5: Effect of Ursolic acid and Oleanolic acid induced OPG expression via ERK, p38, JNK, AKT by using inhibitor PD98059, SB203580, SP600125, LY-294002 (20μM)

	PD98059	SB203580	SP600125	LY-294002
Ursolic acid-(10^{-4}μM)				
Control	100±1.21b	100±3.14b	100±2.27b	100±1.51b
Inhibitor	74.60±2.14a	89.36±4.36a	84.32±2.05a	67.81±1.68a
Inhibitors and Ursolic Acid	73.59±4.27a	89.36±2.29a	100.54±3.35b	69.52±1.40a
Ursolic Acid	126.77±3.31c	126.60±3.09c	112.43±4.81c	115.41±1.51c
Oleanolic acid-(10^{-3} μM)				
Control	100±1.21c	100±3.14b	100±2.27b	100±1.51b
Inhibitor	74.60±2.14a	89.36±4.36a	84.32±2.05a	67.81±1.68a
Inhibitor and Oleanolic Acid	81.85±7.82b	82.98±5.43a	102.16±4.77b	66.44±1.44a
Oleanolic Acid	129.44±1.53d	112.23±6.71c	117.30±3.5c	119.25±1.34c

Data represent mean±SD. Different letters in a column under specific acid indicate a significant (P<0.05) difference.

leakage of RANKL was promoted after 48h treatments, and fallen of when a 72h treatment was given, while the leakage of OPG protein was raised after 72h treatment. In contrast, the mRNA level of RANKL was highly increased after 72h treatment, which suggested a blockage in RANKL protein translation (Fig.1C-D and 2C-D). Western Blot (Fig.3) confirmed the up-regulation of OPG expression and down-regulation of RANKL expression after the medium concentration treatments of two compounds was given. Taken together, the effects of two compounds on the up-regulation of OPG, RANKL and OPG/RANKL ratio could be treated as the potential usage of osteoporosis cures. So the two compounds have potential of dietary supplement for osteoporosis animals at appropriate doses. While, the molecular mechanisms of them to active MC3T3-E1 cells and release OPG were remained to be discovered. Subsequently, we examined relationship between two compounds treatments and the MAPKs, AKT signaling pathways.

Cell proliferation and protein expression are controlled by MAPKs and AKT signaling pathways (Ikeda *et al.*, 2005; Osaki and Gama, 2013). Previous researches have presented that Ursolic acid had effects on MAPK and AKT pathways (Ikeda *et al.*, 2005; Alemany *et al.*, 2010; Bashir, 2013). Here, we have explored that, the effects of two compounds on cell proliferation and expression of OPG through MAPKs and AKT signaling pathways. As shown in Table 4 and Table 5, SB203580 and LY-294,002 inhibited MC3T3-E1 cells growth, PD98059 and SP600125 have no effect on cell proliferation, and all these inhibitors we used affected the leakage of OPG.

PD98059 is a specific inhibitor for MEK, which blocks the activation of downstream ERK-1/2 (Li *et al.*, 2014). The effects of Ursolic acid and Oleanolic acid on cell growth and OPG expression were controlled by ERK pathway (Table 4 and Table 5). MEK1 services as the most essential kinase that functions in Ursolic acid- and Oleanolic acid- induced cell proliferation and OPG expression via ERK, was also

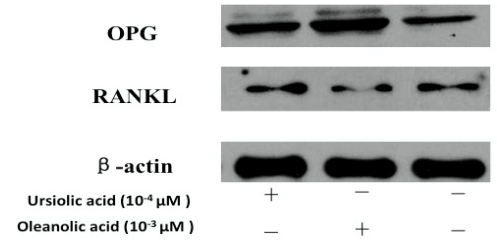

Fig. 3: Western Blot assay was used to show the expression level of OPG and RANKL with Ursolic acid and Oleanolic acid.

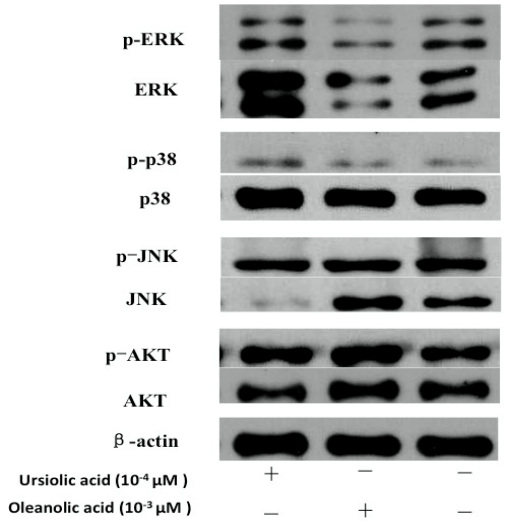

Fig. 4: Western Blot assay was used to show the expression level of ERK, p-ERK, p-p38, p38, p-JNK, JNK, p-AKT and AKT with Ursolic acid and Oleanolic acid.

up-regulated by Ursolic acid, since the activity of p-ERK has not been increased (Fig. 4).

SB203580 has been proved to inhibit the phosphorylation of p38 (p-p38), and the up-regulation and duration of ERK phosphorylation has also been delayed

(Zaringhalam *et al.*, 2013). Moreover, SB203580 could inhibit the Oleanolic acid promoted activation of p-p38 in MC3T3-E1 cells (Fig.4). Ursolic acid - and Oleanolic acid-induced cell proliferation and OPG expression hence was related to p-p38 (Table 4 and Table 5).

SP600125 is a reversible ATP-competitive inhibitor. It serves with significant inhibition of JNK1, -2, and -3, and it blocks the activation of the JNK pathway by inhibiting the mitogen-activated protein kinase (MAPKKK) (Doan *et al.*, 2012). As shown in Fig.4, Ursolic acid inhibited the expression of JNK, while up-regulated the p-JNK level to its normal value. We therefore suggested that, Ursolic acid- and Oleanolic acid- induced cell proliferation and OPG expression were related to the phosphorylation of JNK (Table 4 and Table 5). Besides, the OPG level detected in cells that treated with SP600125 only and SP600125 combined with Ursolic acid or Oleanolic acid, a significant difference was observed, which might due to the effect of Ursolic acid- and Oleanolic acid- induced OPG expression is JNK activity in-dependently.

The most important role of LY-294002 is serve as a PI3-k inhibitor, which significantly inhibits the growth and ascites formation of ovarian carcinoma *in vivo* (Hu *et al.*, 2000). In addition, LY 294002 partially inhibits the glucose uptake, amino acid uptake and protein synthesis that stimulated by insulin (Sanchezmargalet *et al.*, 1994). The up-regulation effect on p-AKT was observed in cells with two compounds treatments (Fig.4). LY 294002 blocked all the cell growth and OPG promoted effects produced by Ursolic acid and Oleanolic acid treatments through the inhibition of AKT up-steam kinase PI3-K (Table 4 and Table 5). Taken together, the effects of two compounds on cell proliferation and OPG expression are related to PI3K/AKT pathways.

Conclusion: Ursolic acid and Oleanolic acid dose- and time-dependently promoted cell proliferation, OPG and RANKL expression in MC3T3-E1 cells, and Ursolic acid worked better than Oleanolic acid. Effects of Ursolic acid and Oleanolic acid are related to ERK, p38, JNK and AKT signaling pathways in different patterns.

Author's contribution: Qin Li contribute to MP, DL, MC, IL, and IJ; Ying-Sai Fan and Zong-qin Gao contribute to DL and IL; Kai Fan contribute to IJ; Zhong-jie Liu contribute to JV and provide experimental conditions.

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