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Full Length Research Paper

Effects of advanced glycation end products on expressions of EMMPRIN and MMP-2 in mouse osteoblasts

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This study investigated the effects of advanced glycation end products (AGEs) on expressions of extracellular matrix metalloproteinase inducer (EMMPRIN) and matrix metalloproteinase-2 (MMP-2) in mouse osteoblasts. MC3T3-E1 cells were incubated with AGEs of different concentrations (50, 100, 200 and 400 mg/L) for various durations (12, 24 and 48 h). The expressions of EMMPRIN mRNA and protein as well as MMP-2 expression and activity were detected. Cells were treated with aminoguanidine (AG) of 0, 100, 200 and 400 µmol/L) plus 200 mg/L AGEs for 24 h, then expressions of EMMPRIN mRNA and protein were measured. Cells were treated with EMMPRIN antibody (5 µg/ml), and MMP-2 activity was determined with zymography. After AGEs treatment, the expressions of EMMPRIN mRNA and protein as well as MMP-2 expression and activity were significantly increased in a time and concentration dependent manner; AGEs plus AG of different concentrations could markedly decrease the expressions of EMMPRIN mRNA and protein when compared with controls in a concentration dependent manner; administration of EMMPRIN antibody dramatically suppressed the activity of MMP-2. AGEs could increase the expressions of EMMPRIN and MMP-2 and elevate MMP-2 activity; AG could suppress the increased expression of EMMPRIN induced by AGEs; EMMPRIN antibody conferred suppressive effects on the expression of MMP-2 induced by AGEs. Therefore, AGEs may be involved in the pathogenesis of osteoporosis via increasing the expression of EMMPRIN and MMP-2 and promoting MMP-2 activity.

Key words: Advanced glycation end products, osteoblast, extracellular matrix metalloproteinase inducer, matrix metalloproteinase -2, osteoporosis,

INTRODUCTION

Membrane-type matrix metalloproteinases (MT-MMPs) refer to members of the membrane-type MMP subfamily that includes MT1-MMP(MMP-14), MT2-MMP(MMP-15), MT3-MMP(MMP-16), MT4-MMP(MMP-17), MT5-MMP(MMP-24) and MT6-MMP; each member of this subfamily contains a potential transmembrane domain suggesting that these proteins are expressed at the cell surface. Not only most of MT-MMPs play a major role on receptors of MMPs, but also they are capable of activating other MMPs. Active forms of MT-MMPs reach membrane bound areas at the cell surface, identify them and activate other MMPs. For instance, MT1-MMP activates MMP2 protein. The imbalance between the synthesis and degradation of extracellular matrix (ECM) is one of the important mechanisms in the pathogenesis of osteoporosis (OP). Some studies have indicated the expressions of matrix metalloproteinases (MMPs) including MMP-1, MMP-2, MMP-3, MT1-MMP and MT2-MMP could be induced by extracellular matrix metalloproteinase inducer (EMMPRIN) in fibroblasts, finally affecting the fate of ECM. Extracellular matrix metalloproteinase inducer (EMMPRIN) is capable of stimulating the production of MMPs, enhancing their activity and Involving in extracellular matrix degradation by transcriptional regulation of MMPs and their activators (MT1-MMP, MT2-MMP). It stimulates the production of MMPs of fibroblasts, monocyte-macrophages and endothelial

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cells. EMMPRIN stimulates collagenase transcription through MAPK P38, increases MMP-1 mRNA, of which active P38 is essential. EMMPRIN is an adhesion protein involved in cell-cell, cell-matrix adhesion. EMMPRIN is also involved in inflammation.

Evidence has indicated that advanced glycation end products (AGEs) could regulate the expression of MMPs and their activity in some cell types, thus affecting the balance between the synthesis and degradation of ECM. In the present study, mouse osteoblasts (MC3T3-E1) were employed to investigate the effects of AGEs, EMMPRIN antibody and AG on expressions of EMMPRIN and MMP-2 and explore the potential roles of AGEs in the pathogenesis of OP.

MATERIALS AND METHODS

Reagents

Bovine serum albumin (BSA; AMRESCO, USA), gelatin, aminoguanidine (AG; Sigma, USA), α-MEM medium, agarose (Gibco, USA), Trizol, RT-PCR kit (Takara, Japan), primers for EMMPRIN and GAPDH (Sangong, China), BCA-100 (Bradford, China), rabbit anti-mouse EMMPRIN and MMP-2 monoclonal antibody (Santa Cruz, USA), rabbit anti-mouse GAPDH monoclonal antibody (Kangcheng, China), goat anti-rabbit antibody conjugated to horseradish peroxidase (Boster, China), DNA Marker D2000 and HRP-DAB (TIANGEN, China) were used in the present study.

BSA and glucose were dissolved in the phosphate buffered saline (PBS, pH 7.4) at a final concentration of 5.0 g/L and 50 mmol/L, respectively. Then, the solution was sterilized by filtration and incubated in dark at 37℃ for 90 days. The unbound glucose was removed through dialysis. The BSA without glucose served as negative control. AGEs was identified with fluorescence scanning analysis (excitation wavelength: 370 nm; emission wavelength: 440 nm; slit: 3 nm).

Methods

Cell culture and treatments

The mouse osteoblasts (MC3T3-E1) with normal morphology and biological characteristics were kindly provided by Professor Bachem of Institute of Clinical Chemistry, University of Ulm, Germany. The cell cultures were maintained in α-MEM medium containing 10% fetal calf serum (FCS) at 37℃ in a humidified atmosphere of 5% CO_2 - 95% air.

Cells in the logarithmic phase were seeded at a density of 3×10^4 cells/mL in a 6-well plate. When 80 ~ 90% confluence was observed, medium was refreshed and glucose deprivation was conducted for 24 h. Then, the treatment of AGEs-BSA with different concentrations (0, 50, 100, 200 and 400 mg/L) was performed and the BSA (400 mg/L) without glucose served as negative control. After 24 h of treatment, the cells and supernatant were harvested independently.

In addition, cells were incubated with AGEs-BSA (200 mg/L) for 0, 12, 24 and 48 h and cells and supernatant were harvested independently. In the negative control group, cells were cultured in BSA solution (200 mg/L) without glucose for 48 h. Experiment was performed in triplicates.

In respect of AG treatment, cells in the logarithmic phase were seeded at a density of 3×10^4 cells/mL in a 6-well plate. When 80 ~ 90% confluence was observed, cells were maintained in α-MEM medium without serum and glucose for 24 h. Then, the cells were incubated with AGEs-BSA (200 mg/L) plus AG of different concentrations (0, 100, 200, 400 μmol/L) for 24 h. In the control groups, AG of 400 μmol/L and α-MEM without serum were independently administrated.

Additionally, the cells were incubated with EMMPRIN antibody (5 μg/ml) for 24 h. As the control group, cells were cultured in the α-MEM without serum.

Detection of EMMPRIN mRNA with RT-PCR

Total RNA was extracted with trizol. The concentration of extracted RNA was measured with ultraviolet spectrophotometer at 260 and 280 nm (A_{260nm}/A_{280nm}: 1.60 ~ 1.80). The integrity of RNA was confirmed by formaldehyde gel electrophoresis and the ratio of A_{28s} to A_{18s} was greater than 2.

The primers of EMMPRIN and GAPDH were synthesized by Songong, Shanghai. For EMMPRIN, forward: 5'-GTCTAGAGGAT CCATCCAAACCTCGGTCCA-3', reverse: 5'-TCTAGAGAATTC CACGCAGTGAGATGGTTT-3'.

The expected size of amplified products was 542 bp. For GAPDH, forward: 5'-ACCACAGTCCATGCCATCAC-3', reverse: 5'-TCCAC CACCCTGTTGCT GTA-3'.

The expected size was 452 bp. Then, 2 μl of RNA was used for amplification. The DNA was denatured at 94℃ for 2 min, followed by 32 cycles of amplification. Each cycle consisted of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min, and extension at 72℃ for 2 min with an additional extension at 72℃ for 8 min. Then, 5 μl of PCR products was loaded onto 2% agarose gel (containing 0.5 mg/L ethidium bromide) (100 V; 40 min). The RNA was visulized with GelDoc-It imaging system and the optical density (OD) was analyzed with Smartview software and was normalized by the OD of GAPDH.

Detection of EMMPRIN and MMP-2 protein with western blot

The cells were incubated with MC-CelLytics LY006 on the ice for 30 min for lysis, followed by centrifugation (12000 r/min, 30 min) at 4℃. The supernatant was obtained and protein concentration was determined with Bradford method. Protein samples were denatured in a gel-loading buffer at 95℃ for 5 min. Then, these samples and standard proteins (20 μl) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (100 V, 2 h) and then transferred onto polyvinylodene difluoride (PVDF) membrane which was then blocked in 5% non-fat milk at room temperature for 1 h.

Subsequently, the membrane was incubated with rabbit anti-mouse monoclonal antibody (1:300) at 4℃ overnight. The membrane was rinsed with PBS and incubated with goat anti-rabbit EMMPRIN or MMP-2 monoclonal antibody conjugated to horseradish peroxidase (1:2000) at room temperature for 1 h, followed by washing with PBS. Enhanced chemiluminescence detection was performed with DAB method. The OD was analyzed with Smartview software and normalized by that of GAPDH.

Measurement of MMP-2 activity with zymography

The supernatant was mixed with loading buffer of equal volume which was then loaded onto the separating gel for electrophoresis (40 mA, 180 min) at 4℃. Following electrophoresis, the SDS was removed from the gel (or zymogram) by incubation in unbuffered Triton X-100, followed by incubation in an appropriate digestion buffer, for 20 h at 37℃. The zymogram was subsequently stained with Coomassie Brilliant Blue and areas of digestion appear as clear bands against a darkly stained background where the

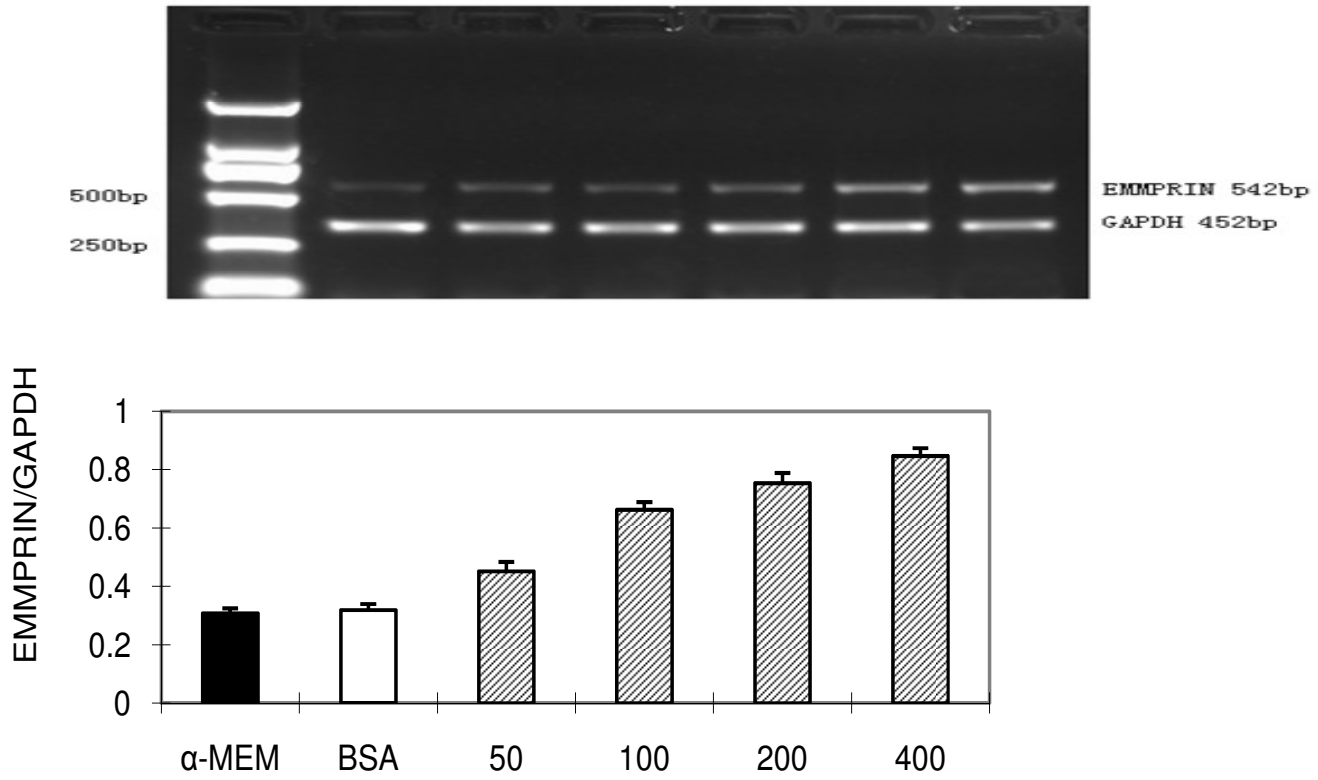

Figure 1. Effects of AGEs with different concentrations on expression of EMMPRIN mRNA in MC3T3-E1 cells.

substrate had been degraded by the enzyme. Gels were then photographed. The photographs were scanned (BandScan) and relative densities were calculated as follows:

$$\text{Activity} = \text{Area}_{\text{band}} \times (\text{Density}_{\text{band}} - \text{Density}_{\text{background}}).$$

Statistical analysis

Data were presented as means ± standard deviation ($\bar{x}$ ± SD) and SPASS 13.0 was used for statistical analysis. Analysis of variance was performed for comparisons between multiple groups. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of AGEs-BSA at different concentrations on the expressions of EMMPRIN mRNA and protein in MC3T3-E1 cells

Effects of AGEs-BSA at different concentrations on the expression of EMMPRIN mRNA

The mRNA expression of EMMPRIN was indicated in the MC3T3-E1 cells maintained in the α-MEM without serum, and the normalized density was 0.307 ± 0.018. In the group with BSA of 400 mg/L (24 h), the normalized density was 0.319 ± 0.021 which was not different from that in the α-MEM group ($P > 0.05$). After treatment with AGEs of 50, 100, 200 and 400 mg/L for 24 h, the normalized density of EMMPRIN mRNA was 0.452 ± 0.031, 0.613 ± 0.027, 0.754 ± 0.029 and 0.867 ± 0.025, respectively, which were significantly higher than that in the BSA group and α-MEM group ($P < 0.01$). The increased expression of EMMPRIN mRNA was in a concentration dependent manner (Figure 1).

Effects of AGEs at different concentrations on the expression of EMMPRIN protein

The protein expression of EMMPRIN was noted in the MC3T3-E1 cells maintained in the α-MEM without serum and the normalized density was 0.214 ± 0.025. In the group with BSA of 400 mg/L (24 h), the normalized density was 0.220 ± 0.013 which was not different from that in the α-MEM group ($P > 0.05$). After incubation with AGEs-BSA of 50, 100, 200 and 400 mg/L for 24 h, the normalized density of EMMPRIN protein was 0.328 ± 0.032, 0.457 ± 0.021, 0.629 ± 0.018 and 0.783 ± 0.024, respectively, which were dramatically higher than that in the BSA group and α-MEM group ($P < 0.01$). The increased expression of EMMPRIN protein was also in a concentration dependent manner (Figure 2).

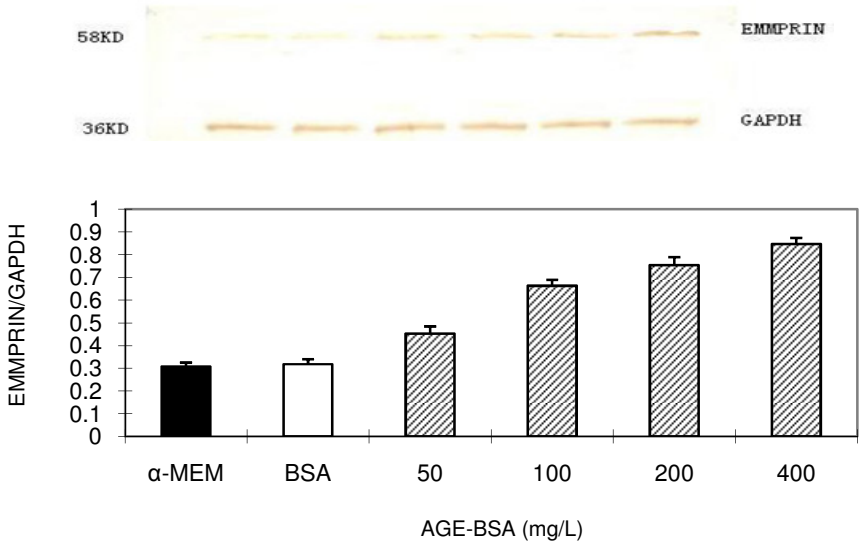

Figure 2. Effects of AGEs with different concentrations on expression of EMMPRIN protein in MC3T3-E1 cells.

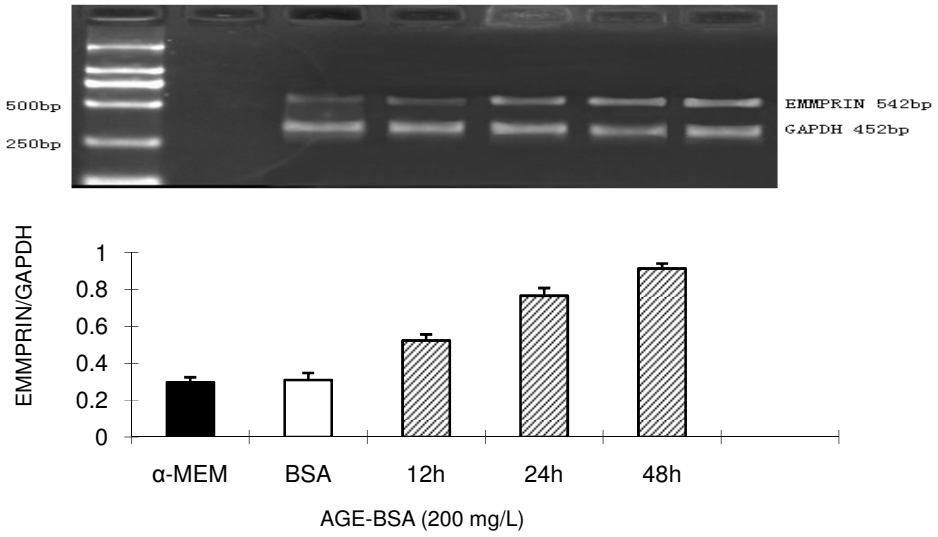

Figure 3. Effects of incubation with AGEs for different durations on expression of EMMPRIN mRNA in MC3T3-E1 cells.

Effects of AGEs-BSA treatment for different durations on the expressions of EMMPRIN mRNA and protein in the MC3T3-E1 cells

Effects of AGEs treatment for different durations on the expression of EMMPRIN mRNA

Immediately after AGEs treatment (0 h), the expression of EMMPRIN mRNA was found in the MC3T3-E1 cells maintained in the α-MEM and the normalized density was 0.298 ± 0.026. In the group with BSA of 200 mg/L (48 h), the normalized density was 0.311 ± 0.037 which was not different from that in the α-MEM group ($P > 0.05$). After incubation with AGEs of 200 mg/L for 12, 24 and 48 h, the normalized density of EMMPRIN mRNA was 0.524 ± 0.033, 0.707 ± 0.042 and 0.865 ± 0.027, respectively, which were dramatically higher than that in the BSA group and α-MEM group ($P < 0.01$). The increased expression of EMMPRIN mRNA was in a time dependent manner (Figure 3).

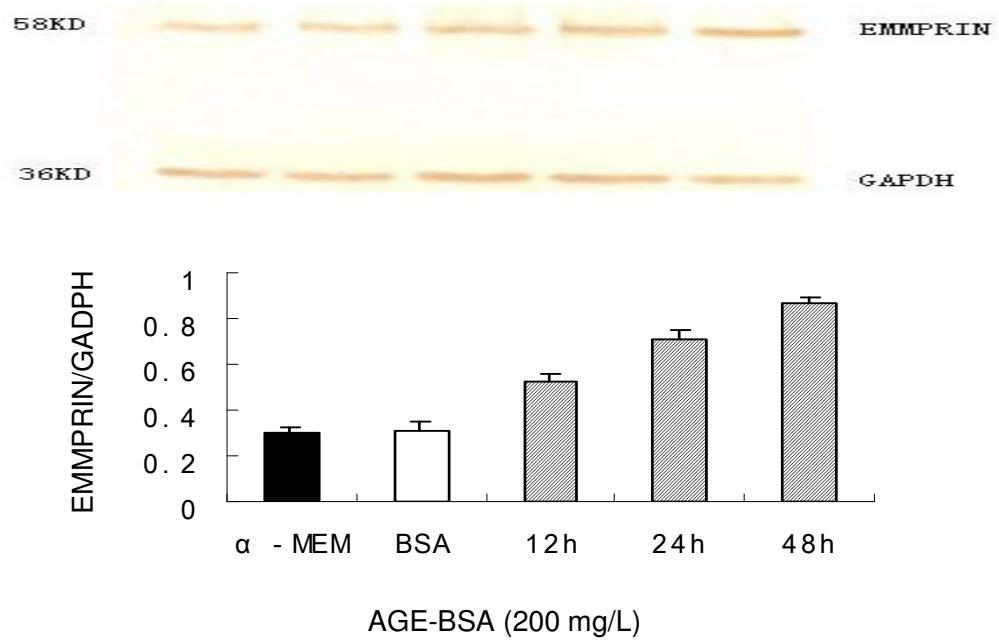

Figure 4. Effects of AGEs incubation for different durations on expression of EMMPRIN protein in MC3T3-E1 cells.

Effects of AGEs treatment for different durations on the expression of EMMPRIN protein

Immediately after AGEs treatment (0 h), the expression of EMMPRIN protein was found in the MC3T3-E1 maintained in the α-MEM and the normalized density was 0.298 ± 0.026. In the group with BSA of 200 mg/L (48 h), the normalized density was 0.206 ± 0.029 which was not different from that in the α-MEM group ($P > 0.05$). After incubation with AGEs-BSA of 200 mg/L for 12, 24 and 48 h, the normalized density of EMMPRIN mRNA was 0.325 ± 0.023, 0.489 ± 0.034 and 0.615 ± 0.027, respectively, which were dramatically higher than that in the BSA group and α-MEM group ($P < 0.01$). The increased expression of EMMPRIN protein was in a time dependent manner (Figure 4).

Effects of AGEs with different concentrations on the MMP-2 expression and activity in the supernatants

Effects of AGEs with different concentrations on the MMP-2 expression

The normalized density of MMP-2 was 78.823 ± 4.712 in the α-MEM group and 80.254 ± 3.015 in the BSA group, indicating no significant difference ($P > 0.05$). After incubation with AGEs of 50, 100, 200 and 400 mg/L for 24 h, the normalized density of MMP-2 was 107.661 ± 5.287, 132.304 ± 2.946, 169.725 ± 3.368 and 194.126 ± 4.604, respectively, which were markedly increased when compared with that in the α-MEM group and BSA group ($P < 0.05$). The increased expression of MMP-2 was in a concentration dependent manner ($P < 0.05$) (Figure 5).

Effects of AGEs with different concentrations on the MMP-2 activity

MMP-2 activity was indicated in the MC3T3-E1 cells maintained in the α-MEM without serum and its activity was 157.27 ± 2.81 INT·mm. After incubation with BSA of 400 mg/L for 24 h, the MMP-2 activity was 180.20 ± 5.62 INT·mm which was not different from that in the α-MEM group. After incubation with AGEs of 50, 100, 200 and 400 mg/L for 24 h, the MMP-2 activity was 225.12 ± 5.01, 305.83 ± 5.21, 363.04 ± 8.04 and 410.63 ± 16.84, respectively, which were dramatically increased when compared with that in the α-MEM group and BSA group ($P < 0.05$). The increased MMP-2 activity was in a concentration dependent manner ($P < 0.05$) (Figure 6).

Effects of AGEs incubation for different durations on MMP-2 expression and activity in the supernatants

Effects of AGEs treatment for different durations on the MMP-2 expression

In the α-MEM group, the normalized density of MMP-2 was 50.432 ± 3.171. In the BSA group, the cells were incubated with BSA of 200 mg/L for 48 h and the

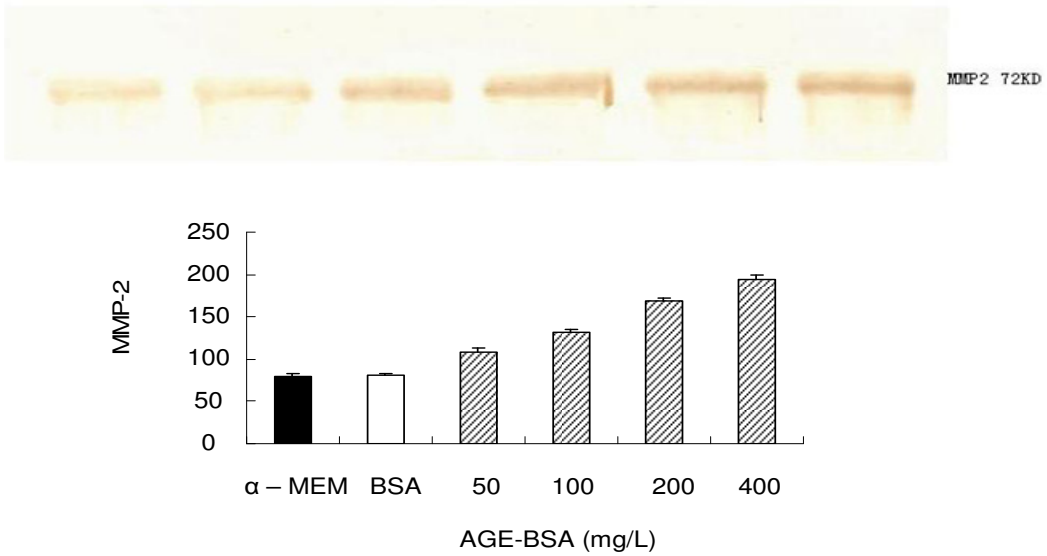

Figure 5. Effects of AGEs with different concentrations on the MMP-2 expression in the supernatants.

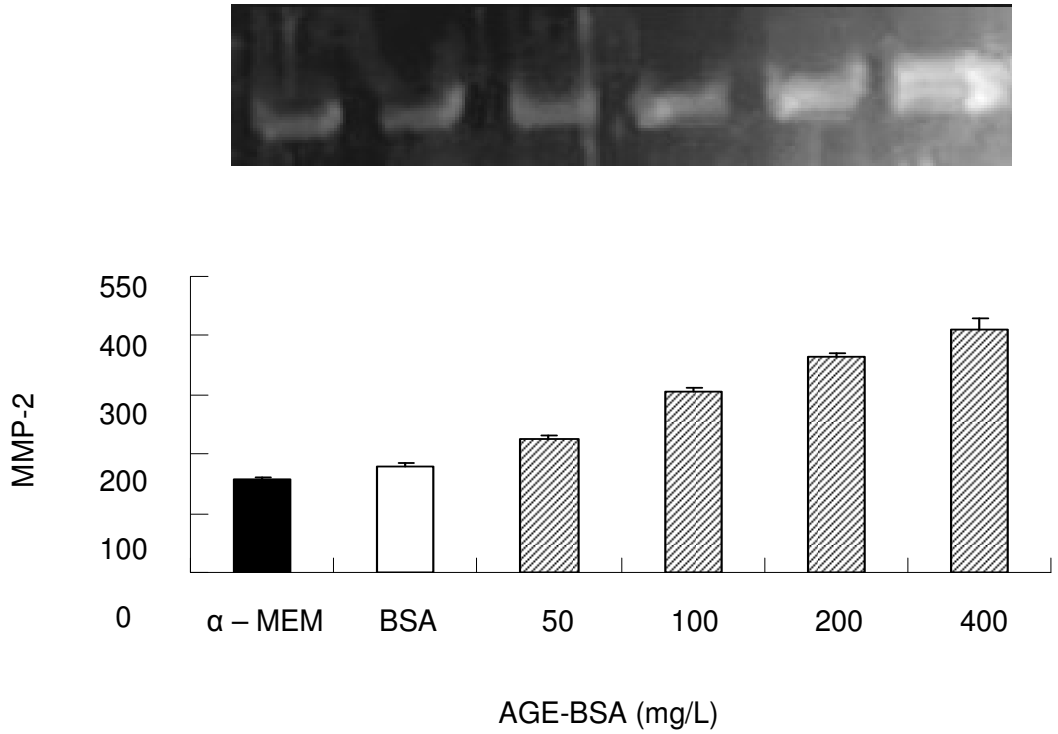

Figure 6. Effects of AGEs with different concentrations on the MMP-2 activity in the supernatants.

normalized density of MMP-2 was 51.670 ± 1.929 which was not different from that in the α-MEM group. After incubation with AGEs (200 mg/L) for 12, 24 and 48 h, the normalized density of MMP-2 was 48.725 ± 2.436, 7575.196 ± 3.078 and 98.524 ± 4.103, respectively. When compared with α-MEM group and BSA group, profound increase was only found in the 24 and 48 h AGEs-BSA treated groups ($P < 0.05$) displaying a time

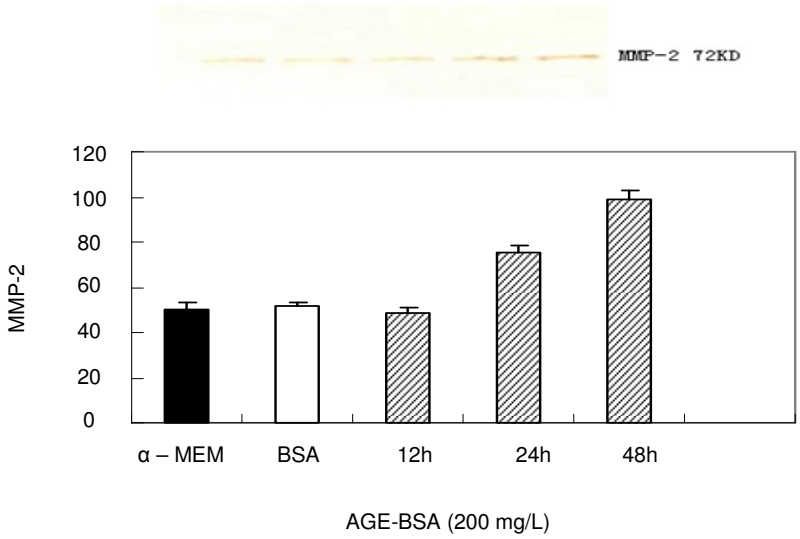

Figure 7. Effects of AGEs treatment for different durations on the MMP-2 expression in the supernatants.

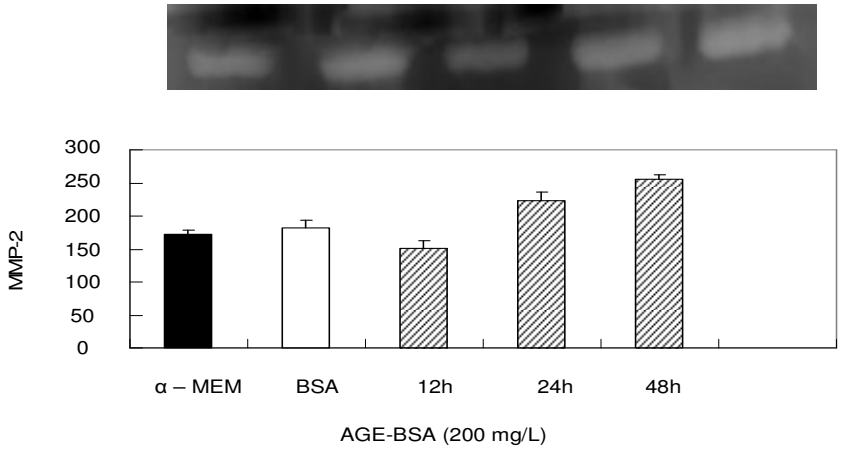

Figure 8. Effects of AGEs treatment for different durations on the MMP-2 activity in the supernatants.

dependent manner (Figure 7).

Effects of AGEs treatment for different durations on MMP-2 activity

In the α-MEM group, the MMP-2 activity was 172.10 ± 6.76 INT·mm. In the BSA group, the cells were incubated with BSA of 200 mg/L for 48 h and the MMP-2 activity was 181.35 ± 11.65 INT·mm which was not different from that in the α-MEM group. After incubation with AGEs of 200 mg/L for 12, 24 and 48 h, the MMP-2 activity was 150.03 ± 12.63, 222.18 ± 14.53 and 256.53 ± 5.96, respectively. When compared with that in the α-MEM group and BSA group, the MMP-2 activity was markedly elevated in the 24 and 48 h AGEs-BSA treated groups ($P < 0.05$) which were in a time dependent manner (Figure 8).

Effects of AG plus AGEs on the expressions of EMMPRIN mRNA and protein in MC3T3-E1 cells

Effects of AG plus AGEs on the expression of EMMPRIN mRNA

The normalized density in the α-MEM and AGEs group was 0.474 ± 0.025 and 0.942 ± 0.028, respectively. After incubation with AGEs and AG of 100, 200 and 400

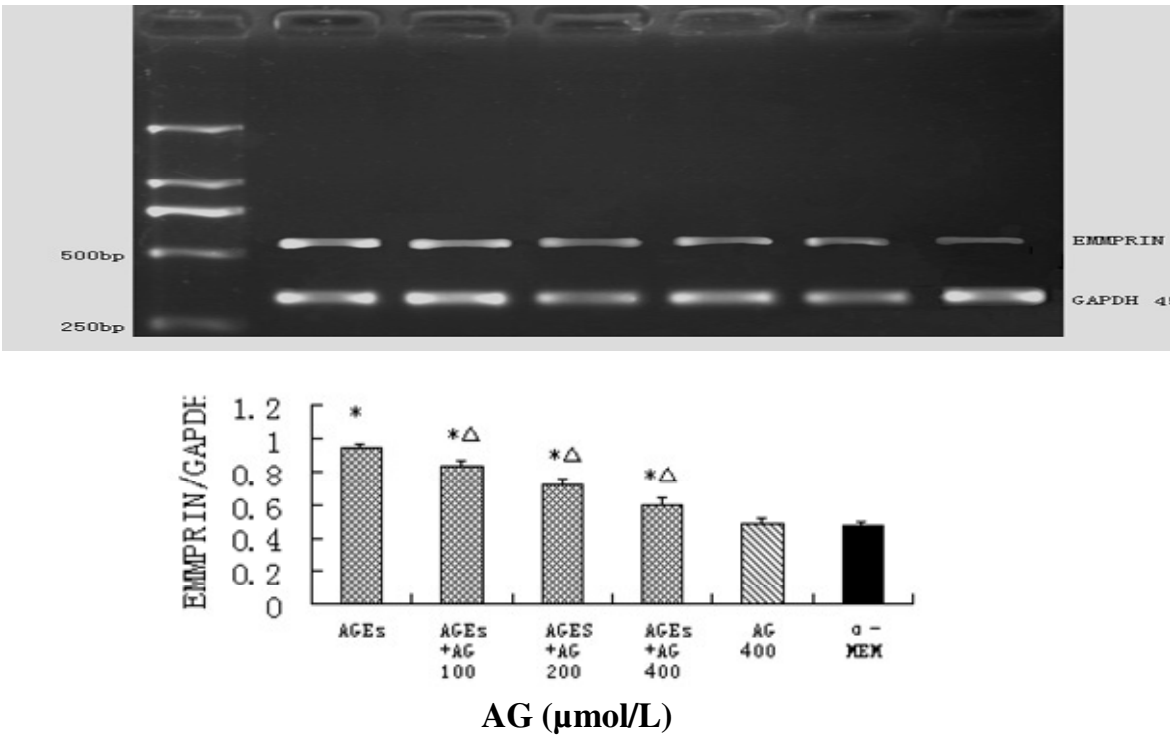

Figure 9. Effects of AG in combination of AGEs on expression of EMMPRIN mRNA in MC3T3-E1 cells. *P < 0.05 vs. α-MEM, AG, $^{\triangle}P$ < 0.05 vs. α-MEM, AG, AGEs-BSA

μmol/L, the normalized density was 0.835 ± 0.032, 0.728 ± 0.029 and 0.596 ± 0.041, respectively. In the cells with AG treatment alone, the normalized density was 0.481 ± 0.034. The AGEs treatment markedly induced the expression of EMMPRIN mRNA when compared with that in the α-MEM group (P < 0.01). However, the administration of AG with different concentrations reduced the increased expression of EMMPRIN mRNA induced by AGEs-BSA which was in a concentration dependent manner. But in the combined treatment groups, the expression of EMMPRIN mRNA in the 400 μmol/L AG treated group was still higher than that in the α-MEM group. Nevertheless, the expression of EMMPRIN mRNA in cells receiving AG (400 μmol/L) alone was not different from that in the α-MEM group (Figure 9).

Effects of AG plus AGEs on the expression of EMMPRIN protein

The normalized density in the α-MEM and AGEs group was 0.472 ± 0.036 and 1.016 ± 0.032, respectively. After incubation with AGEs and AG of 100, 200 and 400 μmol/L, the normalized density was 0.851 ± 0.019, 0.732 ± 0.045 and 0.617 ± 0.021, respectively. In the cells with AG treatment alone, the normalized density was 0.485 ± 0.024. The AGEs-BSA treatment markedly induced the expression of EMMPRIN protein when compared with that in the α-MEM group (P < 0.01). However, the administration of AG with different concentrations reduced the increased expression of EMMPRIN protein induced by AGEs, which was in a concentration dependent manner. But in the combined treatment groups, the expression of EMMPRIN protein in the 400 μmol/L AG treated group was still higher than that in the α-MEM group. Nevertheless, the expression of EMMPRIN protein in cells receiving AG (400 μmol/L) alone was not different from that in the α-MEM group (Figure 10).

Effects of EMMPRIN antibody on the MMP-2 activity in the supernatants

The MMP-2 activity was noted in the MC3T3-E1 cells maintained in the α-MEM without serum, and its activity was 867.95 ± 113.46 INT·mm. After incubation with EMMPRIN antibody of 5 μg/ml for 24 h, the MMP-2 activity was reduced to 554.71 ± 86.90 INT·mm which was dramatically decreased when compared with that in the α-MEM group (P < 0.05) (Figure 11).

Effects of EMMPRIN antibody on the MMP-2 activity in the MC3T3-E1 supernatants interfered with AGEs-BSA

The MMP-2 activity was noted by MC3T3-E1 in the

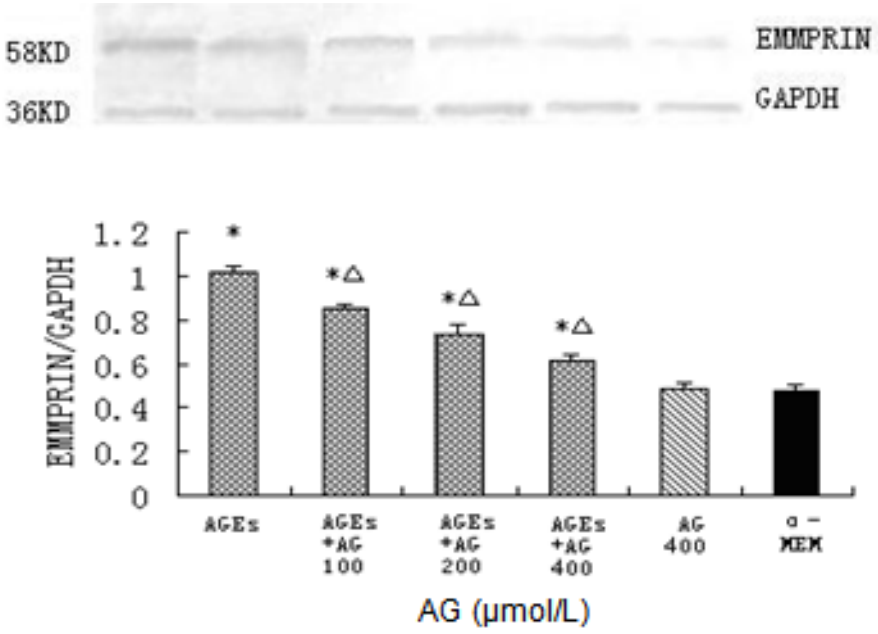

Figure 10. Effects of AG in combination of AGEs-BSA on expression of EMMPRIN protein in MC3T3-E1 cells.
*P < 0.05 *vs* α-MEM, AG, △P < 0.05 *vs* α-MEM, AG, AGEs-BSA

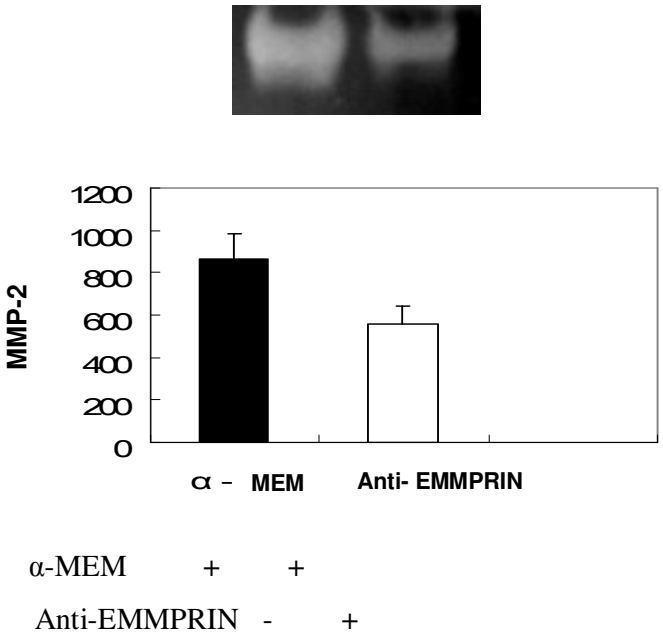

Figure 11. Effects of EMMPRIN antibody on MMP-2 activity in the supernatant of MC3T3-E1 cell cultures.

DMEM group without serum and the amount of enzymatic hydrolysis in the strap was 106.71 ± 18.95(INT•mm). After interfered with AGEs-BSA of 50 mg•L^{-1} for 24 h, the MMP-2 secretion increased and the amount of enzymatic hydrolysis in the strap was 160.76 ± 17.45(INT•mm), which was significantly different from that in the blank group (P < 0.05). After incubation with EMMPRIN antibody of 2 ug•ml^{-1} for 24 h, the amount of enzymatic hydrolysis in the MMP-2 strip was 127.63 ± 11.36(INT•mm), which was significantly decreased compared with that in the AGEs-BSA group (P < 0.05) (Figure 12).

DISCUSSION

Osteoporosis (OP) is one of chronic diseases with complicated pathogenesis. Advanced age, menopause and diabetes are the precipitating factors of OP. These factors may lead to glucose metabolism disorders, thus leading to increased production and accumulation of AGEs. Increasing evidence has indicated that AGEs play an important role in the pathogenesis of OP. Previous studies focused on the effects of AGEs on the osteogenesis in patients with OP. These studies demonstrated that AGEs at a high level could reduce the number of osteoblasts and their motility, thus resulting in decreased osteogenesis (Katayama et al., 1996; Antonio, 1997). Some studies found AGEs could facilitate the bone absorption leading to OP (Vlassara et al., 1988; Miyata et al., 1996). Therefore, AGEs may be involved in the pathogenesis of OP via exerting effects on osteogenesis and bone absorption. Nowadays, the exact mechanisms of AGEs implicated in the pathogenesis of OP are still not well understood and more studies are required to further explore the effects of AGEs on the bone absorption.

MMP-2 is a kind of gelatinase belonging to metal-dependent proteolytic enzyme super-gene family. It is one of the main enzymes involving in the breakdown of ECM including collagen, elastin, fibronectin, etc. In addition, it specialized in the degradation of denatured type I, II and III collagens and natural type, I, II, III and IV collagens. The MMP-2 is secreted as an inactive proprotein which is soluble and activated when cleaved by extracellular proteinases. MMP-2 not only involves in the bone absorption by osteoclasts but the activation of osteoclasts. Furthermore, it plays a critical role in the migration and adhesion of osteoclasts to mineralized bone [reference]. Although MMP-2 participates in the physiological bone remodeling, its expression will be significantly increased in the pathological bone remodeling which may be an important mechanism implicating in the occurrence and development of OP. In the present study, we investigated the effects of AGEs on the expression of MMP-2 in the MC3T3-E1 cells, providing basis for exploring the role of MMP-2 in the pathogenesis of OP.

The results indicated expression of MMP-2 in the MC3T3-E1 cells without treatment. Administration of AGEs significantly increased the MMP-2 expression which was in a concentration dependent manner. In addition, under the stimulation of AGEs, the expression of MMP-2 increased gradually over time, indicating a time dependent manner. The expression and activity of MMP-2 are regulated by a variety of cytokines and mediators including IL-1, IL-6 and TNF-α. Evidence has indicated the expression and activity of MMP-2 could be enhanced by EMMPRIN. In addition, collagen is one of long-lived proteins and susceptible to non-enzymatic cross-linking induced by AGEs and denatured type I may facilitate the specific degradation of gelatinase. Therefore, the following pathways may be implicated in the increased expression of MMP-2 in MC3T3-E1 cells:

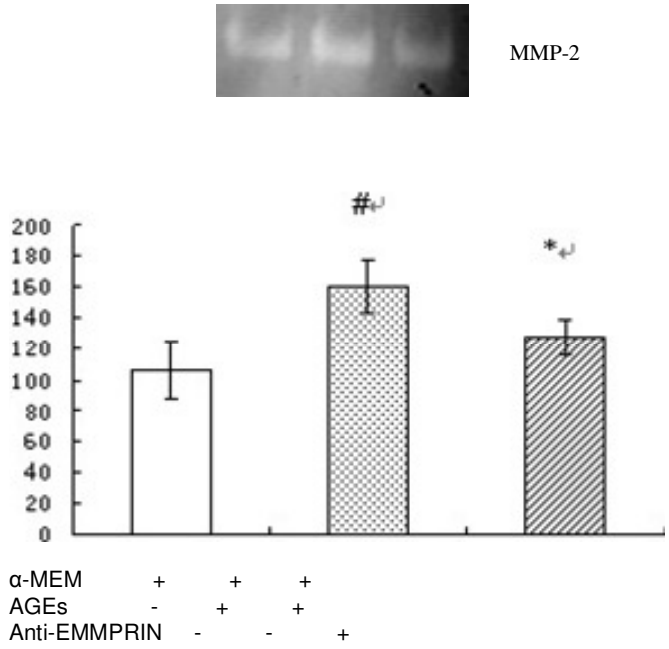

Figure 12. Effects of Anti-EMMPRIN on the MMP-2 activity secreted by MC3T3-E1 intervened with AGEs (50 mg·L^{-1}. $^{\#}P < 0.05$ compared with α-MEM. $^{*}P < 0.05$ compared with AGEs.

(1) AGEs up-regulate the EMMPRIN expression leading to increased synthesis and secretion of MMP-2;
(2) AGEs promote the release of cytokines including IL-1, IL-6 and TNF-α leading to the increase of MMP-2;
(3)Glycosylation of type I collagen results in the formation of AGEs-type I collagen cross-linking which promotes the secretion of MMP-2.

Thus, the expression of MMP-2 in osteoblasts can be up-regulated by AGEs, thus leading to the activation (Holliday et al., 2003), migration and adhesion (Sato et al., 1998) of osteoclasts. This process increases the bone absorption mediated by osteoclasts, finally resulting in OP.

EMMPRIN, also known as basigin or CD147, is a glycoprotein and a member of the immunoglobulin superfamily of adhesion molecules. Post-translational modification has been frequently found in the EMMPRIN which is characterized by glycosylation. The degree of glycosylation is tissue specific and the molecule weight of purified EMMPRIN in different tissues varies from 40 to 68 kD. In addition, different distribution and glycosylation of EMMPRIN have been observed in different species and EMMPRIN with different glycosylation exerts various effects in different tissues. EMMPRIN is one of adhesion molecules and involves in the adhesion between cells or cells and matrix. Study also indicated EMMPRIN was involved in the inflammatory response. The EMMPRIN, as an upstream inducer of MMPs, mainly induces the expression and secretion of various MMPs (MMP-1,

MMP-2, MMP-3, MT1-MMP, MT2-MMP) in fibroblasts, monocytes and macrophages leading to degradation of ECM. These processes play critical roles not only in the invasion and metastasis of cancers, but in physiological and pathophysiological remodeling. Study indicated increased expression of EMMPRIN in tissues undergoing remodeling such as injured joints and bones in the rheumatoid arthritis. Evidence has demonstrated that EMMPRIN played an important role in the destruction of joints, cartilages and bones in the rheumatoid arthritis through stimulating synthesis and secretion of MMP-2 and MMP-9. In the present study, the *in vitro* expression of EMMPRIN was significantly increased under the stimuli-tion of AGEs. We postulated that the increased expre-ssion and secretion of MMP-2 by AGEs was mediated by the EMMPRIN which was confirmed by the fact that EMMPRIN antibody dramatically decreased the expression of MMP-2. Kyoshi et al. (2007) demonstrated administration of EMMPRIN antibody markedly decreased the activity of MMP-2 in human laryngeal squamous cell carcinoma cells, which further confirmed our hypothesis.

Nowadays, few studies have been conducted to explore the relationship between the AGEs and EMMPRIN. The mechanisms involved in the increased expression of EMMPRIN induced by AGEs in our study were unclear. It has been confirmed the existence of AGEs receptors in the MC3T3-E1 cells. Therefore, the effects of AGEs may be mediated by the interaction between AGEs and their receptors. Yuan et al. 2002 found that the initiation of intracellular signal could be mediated by the interaction between AGEs and their corresponding receptors leading to increased expression of cytokines (IL-1, IL-6, TNF-α, GM-CSF), growth factors and adhesion molecules including EMMPRIN. Studies also found EMMPRIN expression could be induced by IL-1, IL-6 or GM-CSF (Liang et al., 2002; Kasinrerk et al., 1992). Additionally, a majority of EMMPRIN are required to be glycosylated which enables EMMPRIN to function. The treatment of AGEs with a high concentration for a prolonged duration facilitates the glycosylation of EMMPRIN, thus leading to its activation.

The mechanisms, implicated in the increased synthesis and secretion of MMPs by EMMPRIN, were unclear. Guo et al. (2000) indicated significant increase of MMPs expression in the fibroblasts of coculture system (tumor cells and fibroblasts), but tumor cells and fibroblasts did not interact each other. These results indicated the effects of EMMPRIN on fibroblasts might be conferred through solubility and fibroblasts might have receptors for EMMPRIN. The up-regulation of MMPs may be mediated by the interaction between EMMPRIN and its receptors. It has been confirmed that the activation of osteoclasts and the regulation and induction of bone absorption were mediated by the integrins on the cell surface. Osteoclasts bind to corresponding receptors on the bone matrix via integrins leading to migration and adhesion of osteoblasts. Studies demonstrated that EMMPRIN could interact with $\alpha_3\beta_1$ or $\alpha_6\beta_1$ leading to the induction of MMPs expression (Berditcheriski et al., 1997; Sun and Hemler, 2001). Therefore, $\alpha_3\beta_1$ and $\alpha_6\beta_1$ may be the surface receptors for EMMPRIN. Evidence indicated EMMPRIN could function via paracrine or autocrine. There are two possible pathways involved in the increased expression of MMPs induced by EMMPRIN:

(1) The secreted EMMPRIN interacts with the corresponding receptors on the adjacent cells via paracine leading to increased expression of MMPs;
(2) Secreted EMMPRIN interacts with receptors on the same cells that produced it leading to up-regulation of MMPs expression.

Sun and Hemler (2001) found EMMPRIN implicated in the homophilic interaction in which EMMPRIN exerted effects via autocrine. Once EMMPRIN interacts with receptors on the cell surface, intracellular signal is initiated and the expression of MMPs is up-regulated at transcription level. Lim et al. (1998) indicated EMMPRIN could stimulate the expression of MMP-1 in the fibroblasts via MAPK p38 signaling pathway. However, Taylor et al. (2002) demonstrated that EMMPRIN could increase the expression of MMP-2 in the fibroblasts through phospholipase A2 and 5-lipoxygenase signaling pathway.

AG is an inhibitor for AGEs and their protein cross-linking. Studies indicated AG could down-regulate the production and activity of AGEs, activated the osteoblast and promoted the adhesion of osteoblast to bone matrix leading to osteogenesis (Cortizo et al., 2000). Munch et al (Munch et al., 1997) indicated sustained suppression of bone absorption under the treatment of AG with certain concentrations. Experiment (Kong et al., 2003) also showed the production of AGEs in the bone was markedly increased in the ovariectomied rats accompanied by low bone mineral density and decreased blood calcium. Administration of AG to these rats, however, could reduce the amount of AGEs and increase the bone mineral density and blood calcium. These results suggested that AG exerted suppressive effects on AGEs through facilitating osteogenesis and reducing bone absorption.

Conclusion

In our experiment, AGEs plus AG with different concentrations decreased the expression of EMMPRIN mRNA and protein in MC3T3-E1 cells in a concentration dependent manner. The administration of AG could not reduce the expression of EMMPRIN to a normal level and the expression of EMMPRIN was still in a high level under AG treatment with the highest concentration. However, administration of AG alone could not confer suppressive effects on the expression of EMMPRIN. These results

suggested AG could down-regulate the increased expression of EMMPRIN in the fibroblasts induced by AGEs, which led to reduction of MMPs synthesis, resulting in decrease of bone absorption. But the mechanisms implicated in the suppressive effects of AG on AGEs were still unclear. On one hand, AG may inhibit the collagen glycosylation and their cross-linking between collagens leading to reduction of collagen degradation and improvement of collagen's biochemical properties. On the other hand, the inhibition of collagen glycosylation leads to increased degradation of MMPs (Mclennanet al., 2002). Furthermore, AG may bind with receptors of AGEs which disrupt the interaction between AGEs and their receptors. These processes affect the release of cytokines, leading to the reduced expression of EMMPRIN and decreased release of MMPs.

Taken together, our study indicated that AGEs could increase the secretion of MMP-2 via elevating the expression of EMMPRIN in vitro, which might play an important role the pathogenesis of OP. Therefore, inhibition of the effects conferred by AGEs or selective inhibition of EMMPRIN synthesis may be alternative ways in the treatment of OP.

REFERENCES

Antonio D (1997). Effects of advanced glycation end-products on the proliferation and differentiation of osteoblast-like cells. Mol. Cell Biochem., 170: 43-45.

Berditcheriski F, Chang S, Bodorova J, Martin E (1997). Generation of monoclonal antibodies to integrin-associated proteins: evidence that $\alpha_3\beta_1$ complexes with EMMPRIN/basigin/OX47 /M6[J]. Biol. Chem., 272: 29174-29180.

Cortizo AM, Caporossi M, Lettieri G, Susana B (2000). Vanadate induced nitric oxide production: role in osteoblast growth and differentiation. Eur. J. Pharmaacaol., 400(2-3): 279-285.

Guo H, Li R, Zucker S, Bryan B (2000). EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface. Cancer Res. 60: 888-891.

Holliday LS, Vakani A, Archer L, Dolce C (2003). Effecta of matrix metalloproteinase inhibitors on bone resorption and orthodontic tooth movement[J]. Dent. Res., 82(9): 687-691.

Kasinrerk W, Fiebiger E, Stefanova I, Baumruker T, Knapp W, Stockinger H (1992). Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin and chicken HT7 molecule[J]. Immunol., 149: 847-854.

Katayama Y, Akatsy T, Yamamoto M, Kugai N, Nagata N (1996). Role of nonenzymatic glycosylation of type I collagen in diabetic osteopenia[J]. Bone Miner Res., 11: 931-937.

Kong DJ, Li E, Tong XX, Li ZH, Li B (2003). Effects of late advanced glycation end products on the osteoporosis induced by ovariectomy and therapeutic effects of aminoguanidine. [Zhong Guo Lao Nian Za Zhi] (Chinese), 1(23): 40-42.

Kyoshi H, Noriko Y, Kiwamu Y, Yoshihiro M, Mitsutaka M, Shinsuke S, Haruki S, Kazuo I (2007). Soluble EMMPRIN stimulates the migration of HEP-2 human laryngeal carcinoma cells, accompanied by increased MMP-2 production in fibroblasts. Arch. Histol. Cytol., 70: 267-277.

Liang L, Major T, Bocan T (2002). Characterization of the promoter of human extracellular matrix metalloproteinase inducer (EMMPRIN)[J]. Gene., 282(122): 75-86

Lim M, Martinez T, Jablous D, Cameron R, Guo H, Toole B, Li J, Basbaum C (1998). Tumor-derived EMMPRIN (extracellular matrix metalloproteinase inducer) stimulates collagenase transcription through MAPK p 38[J]. FEBS Lett., 441(1): 88-92.

Mclennan SV, Martell SK, Yue DK (2002). Effects of Mesangium Glycation on matrix metalloproteinase activities: possible role in diabetic nephropathy[J]. Diabetes, 51(8): 2612-2618.

Miyata T, Kawai R, Taketomi S, Sprague SM (1996). Possible involvement of advanced glycation end-products in bone resorption. Nephrol Dial Transplant, 11(Suppl5): 54-57.

Munch G, Thome J, Foley P, Schinzel R, Riederer P (1997). Advanced glycation end products in ageing and Alzheimer's disease[J]. Brain Res Brain Res. Rev., 23(2): 134-143.

Sato T, Foged NT, Delaisse JM (1998). The migration of purified osteoclasts through collagen is inhibited by matrix metalloproteinase inhibitors[J]. Bone Min.er Res., 13(1): 59-66.

Sun J, Hemler ME (2001). Regulation of MMP1 and MMP2 production through CD147/ interactions [J]. Cancer Res., 61: 2276-2281.

Taylor PM, Woodfield RJ, Hodgkin MN, Pettitt TR, Martin A, Kerr DJ, Wakelam MJ (2002). Breast cancer cell-derived EMMPRIN stimulates fibroblast MMP2 release through a phospholipase A2 and 5-lipoxygenase catalyzed pathway [J]. Oncogene. 21: 5765-5772.

Vlassara H, Brownlee M, Manogub K, Manogub KR, Dinarello CA, Pasagian A (1988). Cachectin TNF and IL-1 induced by glucose-modified protein: role in normal tissues remodeling. Science, 240: 1546-1548.

Yuan ZQ, Feldman RI, Sun M, Olashaw NE, Coppola D, Sussman GE, Shelley SA, Nicosia SV, Cheng JQ (2002). Inhibition of JNK by cellular stress- and tumor necrosis factor alpha-induced AKT2 through activation of the NF kappa B pathway in human epithelial Cells, J. Biol. Chem., 277(33): 29973-29982.