学 位 論 文 の 要 旨

氏名　　岡﨑　恭子

学 位 論 文 名　Advanced Glycation End Products (AGEs), but Not High Glucose, Inhibit the Osteoblastic Differentiation of Mouse Stromal ST2 Cells Through the Suppression of Osterix Expression, and Inhibit Cell Growth and Increasing Cell Apoptosis

発 表 雑 誌 名　Calcified Tissue International

（巻: 初頁～終頁等, 年）　(**91**: 286-296, 2012)

著 者 名　Kyoko Okazaki, Toru Yamaguchi, Ken-ichiro Tanaka, Masakazu Notsu, Noriko Ogawa, Shozo Yano, Toshitsugu Sugimoto

論 文 内 容 の 要 旨

INTRODUCTION

Diabetes mellitus is known to be associated with osteoporotic fractures through a decrease in osteoblastic bone formation rather than an increase in osteoclastic bone resorption.

Recently, it has been documented that advanced glycation end products (AGEs), proteins modified post-translationally by high glucose conditions, play important roles in the complications of the diabetes mellitus. AGEs also seem to be associated with osteoporosis in diabetes. Previous studies have shown that the receptor for AGEs (RAGE) was expressed in human bone-derived cells, and that AGEs impaired the proliferation, bone matrix production and mineralization of osteoblasts, which may lead to osteopenia seen in diabetic patients.

Osteoblasts are derived from bone marrow stromal cells. In diabetic patients, it is possible that high glucose or AGEs may affect the proliferation and differentiation of stromal stem cells as well as osteoblasts. However, only few studies were conducted on this issue.

In this study, to further clarify this issue, we used ST2 cells, which are clonal stromal cells isolated from bone marrow of BC8 mice. When cultured with ascorbic acid and BMP-2, ST2 cells exhibit the typical characteristics of osteoblasts. We examined the effects of high glucose as well as AGE2 and AGE3, which have potent biological activities and are strongly implicated in diabetic complications, on the osteoblastic differentiation, growth, and apoptosis of the cells.

MATERIALS AND METHODS

Mouse ST2 cells were cultured in α-MEM with 10% fetal bovine serum and antibiotics in 5% CO_2 at 37°C. AGE-BSA were prepared by incubating albumin with 0.1 M DL-glyceraldehyde (AGE2) or glycolaldehyde (AGE3) and 5 mM diethylenetriaminepentaacetic acid in 0.2 M phosphate buffer (pH7.4) at 37℃ for 7 days. For induction of osteoblastic differentiation, 5mM β-glycerophosphate, 100 μg/mL ascorbic acid, and 100 ng/mL BMP-2 were added in medium after reaching confluence. The cells were treated with AGE-BSA or high glucose. The mineralization of the cells was determined using von Kossa staining and Alizarin red staining on days 21. ALP activity was assayed on days 3. Total RNA was collected on days 1 to 3, 7, and 14, and Osteocalcin (OCN), collagen-1, Osterix (OSX), Runx2, RAGE mRNA expressions were measured by real-time PCR. Total protein was extracted on days 0, 7, and 14, and Western blot analysis with Runx2, Osterix, and β-actin antibodies was performed. Cell viability was evaluated by cell count on days 1 to 5 and by a WST-8 assay at 24 to 72 hours and the apoptotic cell death was analyzed in an ELISA for DNA fragments by an absorbance at 405nm at 24 to 72 hours following the manufacturer's protocols.

RESULTS AND DISCUSSION

We first examined whether or not AGEs could inhibit the osteoblastic differentiation of ST2 cells. Ten to 200 μg/mL AGE2 or AGE3 alone dose-dependently inhibited the mineralization. AGE2 or AGE3 alone (200 μg/mL) significantly inhibited ALP activities as well as the mineralization of the cells ($p<0.01$). In contrast, 22 mM glucose alone or in combination with 200 μg/mL AGE2 or AGE3 did not affect these cellular phenotypes.

Next, we examined mRNA expressions of OCN and collagen-1. Either AGE2 or AGE3 alone (200 μg/mL) significantly decreased OCN mRNA expression on days 14 ($p<0.01$), while AGE3 significantly increased collagen-1 mRNA expression on days 14 ($p<0.01$). We also examined mRNA and protein expression of OSX and Runx2 in the cells. Two hundred μg/mL each of AGE2 and AGE3 significantly decreased OSX mRNA expression through days 7 to 14 and through days 3 to 14, respectively ($p<0.01$). Either 200 μg/mL AGE2 or AGE3 decreased the levels of OSX and Runx2 protein expressions on days 7 and 14.

Then, we examined the effects of high glucose, AGE2, or AGE3 on the cell growth and apoptosis of the cells. AGE2 or AGE3 significantly suppressed cell growth and increased apoptotic cell death in time- and dose-dependent manners ($p<0.01$).

We also examined the effects of high glucose or AGEs on RAGE mRNA expression in the cells by real-time PCR. Twenty-two mM glucose as well as 200 μg/mL AGE3 significantly increased RAGE mRNA expression in the cells on days 2 and on days 2 and 3, respectively ($p<0.01$).

In this study, we found that AGE2 or AGE3 alone significantly inhibited the osteoblastic differentiation and mineralization of mouse stromal ST2 cells. In contrast, we have previously shown that AGE2 or AGE3 alone had no effect on the mineralization of mouse osteoblastic MC3T3-E1 cells. Because osteoblasts are derived from bone marrow stromal cells, AGEs seem to act on cells of the osteoblast lineage more potently in an immature stage than in a differentiated stage.

AGEs are known to influence the development of osteoblasts by binding to the specific receptor, RAGE. We have previously shown that the combination of high glucose and AGE2 or AGE3 inhibits the mineralization of osteoblastic MC3T3-E1 cells through a glucose-induced increase in RAGE expression. In contrast, high glucose in combination with AGE2 or AGE3 had no effect on osteoblastic differentiation of ST2 cells in this study. It may be because the extent and duration of the increase in RAGE mRNA expression by high glucose were more potent and longer in MC3T3-E1 cells than in ST2 cells.

Runx2 and OSX are essential transcription factors for osteoblastic differentiation. In this study, AGE2 and AGE3 significantly decreased OSX mRNA expression in ST2 cells on days 3. The levels of Runx2 and OSX protein expressions were also decreased by AGE2 and AGE3 on days 7. These findings suggest that AGE2 and AGE3 impair the osteoblastic differentiation of the cells by suppressing the expressions of Runx2 and OSX at the early stage, which results in diminishing OCN expression and mineralization at the later stage. On the other hand, AGE3 increased collagen-1 mRNA expression, a marker for immature osteoblasts, on days 14, suggesting that prolonged exposure of ST2 cells with AGE3 kept cellular phenotypes immature.

As pluripotent stem cells, bone marrow stromal cells can differentiate to osteoblasts, cartilage, muscle, adipocytes, and are involved in the formation, remodeling, and repair of musculoskeletal tissues. In this study, we found that AGE2 or AGE3, but not high glucose, significantly inhibited cell growth and increased apoptosis of mouse stromal ST2 cells. The current findings suggest that defects in the number of osteoblasts as well as inhibition of osteoblastic differentiation, both induced by AGEs, may underlie diabetes-related bone loss.

CONCLUSION

We found that physiologically important AGE2 and AGE3, but not high glucose, could inhibit the osteoblastic differentiation and mineralization of mouse stromal ST2 cells by decreasing OSX expression and partly by increasing RAGE expression. These AGEs also inhibited cell growth and increased apoptosis of the cells. These findings suggest that AGEs may act on cells of the osteoblast lineage more potently in an immature stage than in a differentiated stage, and inhibit their osteoblastic differentiation as well as decreasing the number of mature osteoblasts. These mechanisms may reduce bone formation and induce osteopenia in diabetes.