学 位 論 文 の 要 旨

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学 位 論 文 名	The Combination of High Glucose and Advanced Glycation End-Products(AGEs) Inhibits the Mineralization of Osteoblastic MC3T3-E1 Cells Through Glucose-Induced Increase in the Receptor for AGEs
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論 文 内 容 の 要 旨

Introduction

Type 1 diabetes mellitus is known to be associated with reduced bone mass and increased bone fractures. This is thought to be due to a decrease in osteoblastic bone formation rather than an increase in osteoclastic bone resorption. Serum from diabetic patients was reported to inhibit both proliferation of and collagen biosynthesis in osteoblasts. In vitro studies have shown that high glucose concentrations inhibited the proliferation of osteoblastic cells.

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. Their subtypes, AGE2 and AGE3, are known to deteriorate retinopathy, disturb glomerular homeostasis and participate in onset and development of neuropathy. AGEs also seem to be associated with diabetic osteopenia. These actions are known to be mediated by the receptor for AGEs (RAGE). Previous studies have shown that RAGE was expressed in human bone-derived cells, and that AGEs inhibited the synthesis of type I collagen and osteocalcin (OCN) in the cells, thereby suppressing mature bone nodule formation.

Although either high glucose or AGEs were shown to impair the proliferation and mineralization of osteoblasts, little is known about the effect of high glucose on RAGE mRNA expression in osteoblasts or the effects of the combined treatment of high glucose with AGEs on the differentiation of the cells. In diabetic patients, it is possible that osteoblasts might be exposed to both high glucose and AGEs, because high glucose-containing blood prevails throughout the body and modify a variety of proteins in the circulation and microenvironment of tissue.

In this study, we examined the effects of high glucose or AGEs alone, or their combination on RAGE mRNA expression and the mineralization of MC3T3-E1 cells.

Materials and Methods

Cell culture

MC3T3-E1 cells were cultured in α-MEM supplemented with 10% FBS and 1% penicillin streptomycin in 5% CO_2 at 37℃.

Preparation of AGEs

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.

Cell mineralization assay

Mineralization of the cells was determined using von Kossa staining and Alizarin red staining after cells were grown in α-MEM supplemented with 10mM β-glycerophosphate for 2 weeks after reaching confluency.

Real-time PCR quantification of gene expression

Total RNA was isolated using Trisol reagent, and first-strand cDNA was synthesized using oligo-dT primer and SuperScript Ⅲ cDNA synthesis kit. The cDNA was amplified using an ABI PRISM 7000 sequence detection system.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical evaluation was carried out with one-way analysis of variance (ANOVA). For all statistical tests, a value of $P<0.05$ was considered to indicate a statistically significant difference.

Results

Cultured MC3T3-E1 cells were incubated with each of 22 mM glucose (The concentration is comparable to that in untreated diabetic patients), 22 mM mannitol, 300 μg/ml AGE2, and 300 μg/ml AGE3 after the cells reached confluency. On days 21, von Kossa staining and the quantification of Alizarin red staining were performed. Mineralization of MC3T3-E1 cells was unchanged by a single treatment with each of these agents.

Then, we examined the effects of high glucose or AGEs on RAGE mRNA expressions in the cells. Real-time PCR revealed that 22 mM glucose caused 10.3- and 6.5-fold increase in RAGE mRNA expressions on days 7 and 14, respectively. However, neither of 22 mM mannitol, 300 μg/ml AGE2, nor 300 μg/ml AGE3 affected the expression.

Next, we examined the effects of concomitant addition of high glucose and AGEs on the mineralization of MC3T3-E1 cells. Twenty-two mM glucose in combination with 300 μg/ml AGE2, but not with 300 μg/ml AGE3, significantly inhibited the mineralization of the cells by von Kossa staining. The quantification of Alizarin red staining showed that the mineralization was reduced to approximately 3/4 that of the control.

Next, we examined the effects of concomitant addition of high glucose and AGEs on OCN mRNA expressions in MC3T3-E1 cells. Real-time PCR revealed that combination of 22 mM glucose with 300 μg/ml AGE2 caused 0.29-, 0.67-, and 0.41-fold decrease in OCN mRNA expressions on days 7, 14, and 21, respectively, and combination of 22 mM glucose with 300 μg/ml AGE3 caused 0.21-,

0.47-, and 0.32-fold decrease in the OCN expressions on days 7, 14, and 21, respectively.

Discussion

Previous studies showed that high glucose suppressed the proliferation of cultured osteoblasts and bone marrow stem cells. On the other hand, we found that 22 mM glucose alone had no influence on the mineralization of osteoblastic MC3T3-E1 cells in this study, suggesting that factors other than high glucose would be necessary to account for the suppression of osteoblastic mineralization and bone formation in diabetic patients.

Several studies have shown that AGEs impaired both bone matrix production and mineralization of osteoblasts. Thus, AGEs rather than high glucose seem to be causative factors for osteopenia in type 1 diabetes. In this study, combination of 22 mM glucose with 300 μg/ml AGE2 significantly inhibited the mineralization of MC3T3-E1 cells, and combination of 22 mM glucose with either 300 μg/ml AGE2 or AGE3 apparently decreased OCN mRNA expression, although high glucose or each of AGEs alone had no effect on osteoblastic mineralization. In diabetic patients, both blood circulation and microenvironment of each tissue are ubiquitously exposed to high glucose concentrations. Thus, it is highly likely that AGEs act in concert with high glucose upon osteoblasts in the microenvironment of bone marrow. The present data suggest that treatments of MC3T3-E1 cells with high glucose and AGEs in combination might exert an additional or synergistic inhibitory effect on the mineralization of the cells, which is not seen in the single treatment of each agent.

AGEs are known to influence the development of osteoblasts through their specific receptor, RAGE. We found that high glucose, but not mannitol or AGEs, markedly increased mRNA expression of RAGE by real-time PCR. Thus, the inhibitory effect of combination of high glucose with AGEs on osteoblastic mineralization in the present study might be augmented by glucose-induced increase in RAGE expression. Previous studies showed that AGEs did not modify expression of RAGE in osteoblastic MC3T3-E1 cells but increased its expression in mesenchymal stem cells, which are progenitors of osteoblasts. This finding suggests that the effects of AGEs on RAGE expressions might depend on differential stages of the cells of osteoblastic lineage and could be miscellaneous. On the other hand, our literature search failed to detect any studies that showed a glucose-induced increase in RAGE expression in osteoblasts, and our current study firstly suggest that high glucose, but not AGEs, is important for the receptor modulation in the cells.

Although we found no significant effects of a single treatment of either high glucose, AGE2, or AGE3 on the mineralization of MC3T3-E1 cells, each of these agents was shown to suppress the mineralization of undifferentiated cells such as rat bone-marrow stromal cells or human mesenchymal stem cells. Thus, high glucose or AGEs per se may more readily act on the immature stages of the cells of osteoblastic lineage than on differentiated osteoblasts.

Conclusion

We found that combination of high glucose with AGEs could additively or synergistically inhibit osteoblastic mineralization through glucose-induced increase in RAGE expression. Our findings suggest that high glucose and AGEs, both of which are important for the pathogenesis and complication of diabetes and co-exist in the microenvironment of bone marrow, might interract with each other and exacerbate their suppressive effects on osteoblastic mineralization.