学 位 論 文 の 要 旨

氏名　　足立　奈緒子

学 位 論 文 名　Insulin-Like Growth Factor-I Protects Against the Detrimental Effects of Advanced Glycation End Products and High Glucose in Myoblastic C2C12 Cells

発 表 雑 誌 名（巻，初頁～終頁，年）　Calcified Tissue International（**105**: 89-96, 2019）

著 者 名　Naoko Adachi, Ippei Kanazawa, Ken-ichiro Tanaka, Ayumu Takeno, Masakazu Notsu, Sayuri Tanaka, Toshitsugu Sugimoto

論 文 内 容 の 要 旨

INTRODUCTION

Sarcopenia is a progressive disease with decreases in skeletal muscle mass and function, resulting in deterioration of activities of daily living and quality of life as well as increases in fall risk and mortality in elderly people. Previous studies have shown that a risk of sarcopenia is increased in patients with diabetes mellitus.

Advanced glycation end products (AGEs) are generated by sequential non-enzymatic chemical glycoxidation of protein amino group. AGEs formation is increased when patients have DM. Several studies have shown that serum AGEs levels are higher in patients with DM than those in healthy subjects. AGEs have physiological activities and directly impact cell functions through the receptor for AGEs (RAGE). Therefore, it is suggested that AGEs may be involved in the mechanism of various diabetic complications. Several studies showed that serum AGEs levels are associated with decreased grip power and gait speed in elderly women. We previously showed that serum levels of pentosidine, which is an advanced glycation end products (AGEs), were independently associated with muscle mass reduction in patients with type 2 diabetes. However, it is unclear whether AGEs affect myoblastic cells. Thus, we examined the effects of AGEs on differentiation and apoptosis in myoblasts. Moreover, whether insulin-like growth factor-I (IGF-I), the hormone with anabolic effects on muscle, rescued the effects of AGEs on myoblasts was investigated.

MATERIALS AND METHODS

We used a mouse myoblast cell line, C2C12 cells. The cells were cultured in Dulbecco's

modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Invitrogen). The medium was changed twice a week. After the cells reached 80% confluency, the cells were cultured in DMEM with 2% horse serum for 2 days to differentiate into myotube. Then, they were incubated with BSA-free DMEM with vehicle (Cont), bovine serum albumin (BSA), AGE2, AGE3, IGF-I, and/or high glucose. Real-time PCR and western blot analyses were performed to examine the expression of MyoD and Myogenin, both of which are important molecules regulating muscle differentiation. For Apoptosis was examined using a DNA fragment detection ELISA kit.

RESULTS AND DISCUSSION

We examined the effects of AGEs and IGF-I on the mRNA expressions of *MyoD* and *Myogenin*, both of which were important molecules regulating muscle differentiation, in C2C12 cells by real-time PCR. Treatment with AGE3 significantly inhibited the mRNA expressions of *MyoD* and *Myogenin* compared to BSA. IGF-I significantly increased the mRNA expressions of *MyoD* and *Myogenin* compared to Cont. Moreover, Co-treatment of IGF-I with AGE2 (AGE2+IGF-I) or AGE3 (AGE3+IGF-I) significantly increased the mRNA expressions of *MyoD* and *Myogenin* compared to treatment with AGE2 or AGE3. We then examined the effects of AGEs and IGF-I on the protein expressions of MyoD and Myogenin by Western blot. Treatment with AGE2 or AGE3 significantly decreased MyoD and Myogenin protein expressions compared to BSA, whereas IGF-I significantly increased MyoD and Myogenin protein expressions compared to Cont. Co-treatment of IGF-I with AGE2 (AGE2+IGF-I) or AGE3 (AGE3+IGF-I) significantly increased MyoD and Myogenin protein expressions compared to treatment with AGE2 or AGE3. Real-time PCR showed that AGE2 and AGE3 significantly decreased the mRNA expression of *Igf-I* compared to BSA. IGF-I significantly decreased the mRNA expression of *Igf-I* compared to Cont, whereas co-treatment of IGF-I with AGE2 (AGE2+IGF-I) or AGE3 (AGE3+IGF-I) significantly increased the mRNA expression of *Igf-I* compared to AGE2 or AGE3.

To examine the effects of AGEs and IGF-I on Akt activation, C2C12 cells were treated with AGEs and/or IGF-I for 24 h, and protein was collected. Western blot showed that IGF-I significantly stimulated pAkt, tAkt, and ratio of pAkt/tAkt compared to Cont. Moreover, AGE2 and AGE3 significantly suppressed tAkt expression compared to BSA. IGF-I (BSA+IGF-I) significantly increased pAkt compared to AGE2 or AGE3.

We then examined the effects of HG, AGE3, and IGF-I on the mRNA expression of *Rage* in C2C12 cells by real-time PCR. HG significantly increased the mRNA expression of *Rage*, while AGE3 tended to decrease it although the difference was not significant. In contrast, IGF-I

significantly decreased the mRNA expression of *Rage*.

We examined the contribution of HG to the effects of AGE3 on MyoD and Myogenin expressions of C2C12 cells. HG alone did not affect the mRNA expression of *MyoD* or *Myogenin*. AGE3 significantly decreased the mRNA expressions of *MyoD* and *Myogenin* regardless of the presence of HG. Co-incubation of IGF-I with HG and AGE3 (HG+AGE3+IGF-I) significantly increased the mRNA expressions of *MyoD* and *Myogenin* compared to HG+AGE3.

The effects of HG, AGEs, and IGF-I on apoptosis of C2C12 cells were examined by using a DNA fragment detection ELISA kit. Treatment with AGE2 and AGE3 for 24 h significantly increased the apoptosis of C2C12 cells compared to BSA. Although HG alone did not affect the apoptosis, co-treatment of HG with AGE3 (HG+AGE3) significantly increased it compared to Cont, HG, and AGE3. Co-incubation of IGF-I with AGE2 or AGE3 (AGE2+IGF-I or AGE3+IGF-I) significantly attenuated the apoptotic effects of AGE2 and AGE3. Moreover, co-incubation of IGF-I with HG and AGE3 (HG+AGE3+IGF-I) significantly attenuated the apoptotic affects of HG and AGE3 (HG+AGE3).

We examined the effects of AGEs and IGF-I on the mRNA expressions of *MyoD* and *Myogenin*, both of which were important molecules regulating muscle differentiation, in C2C12 cells by real-time PCR. AGE3 significantly decreased the mRNA of MyoD and Myogenin in C2C12 cells. Moreover, AGEs significantly decreased endogenous IGF-I expression. We examined the contribution of HG to the effects of AGE3 on MyoD and Myogenin expressions of C2C12 cells. High glucose alone did not affect the differentiation and apoptosis of the cells; however, high glucose significantly increased the expression of receptor for AGEs (RAGE) and enhanced the AGE3-induced apoptosis. In contrast, IGF-I significantly decreased RAGE expression and attenuated the detrimental effects of AGEs on the expressions of MyoD and Myogenin as well as apoptosis. Furthermore, IGF-I significantly attenuated the effects of high glucose plus AGE3 on the myoblastic differentiation and apoptosis. Moreover, AGE2 and AGE3 significantly increased the apoptosis.

CONCLUSION

These findings indicate that AGEs inhibit myogenic differentiation and increase apoptosis in C2C12 cells, and that high glucose increases RAGE and enhances the AGE3-induced apoptosis, suggesting that AGEs and high glucose might contribute to the reduction of muscle mass and function. Moreover, IGF-I attenuated the detrimental effects of AGEs and high glucose in myoblastic cells; thus, IGF-I-Akt signal could be a therapeutic target of DM-induced sarcopenia.