学 位 論 文 の 要 旨

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学　位　論　文　名　Advanced Glycation End Products-Induced Reactive Oxygen Species Generation Is Partly Through NF-kappa B Activation in Human Aortic Endothelial Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Tumor necrosis factor (TNF)-α and reactive oxygen species (ROS) are involved in the endothelial dysfunction and the progression of atherosclerosis. In the pathogenesis of diabetic micro- and macro-vascular complications, advanced glycation end products (AGEs) and their receptor signaling are thought to play pivotal roles. Previous study demonstrated that AGEs-induced vascular dysfunction is associated with NF-κB signaling. In addition, the AGE and its receptor (RAGE) signaling plays an important role in regulating the TNF-α expression and oxidative stress, which interact with each other in diabetic mice.

However, it is yet to be proved whether this theory is true in the case of human arteries, and how NF-κB is involved in that interaction. Thus, we examined the interaction among AGEs, TNF-α secretion and ROS production using human aortic endothelial cells (HAoEC), and clarified the significance of NF-κB in AGEs-induced vascular dysfunction.

MATERIALS AND METHODS

Primary human aortic endothelial cells were purchased from Cell Applications and cultured in endothelial cell growth medium. AGE3, one of common AGEs, was prepared under

sterile conditions with 0.1M glycolaldehyde as described previously. Cells on a 96-well plate were incubated in 300μl of culture medium with BSA as vehicle or AGE3 (30–300μg/ml) in the presence or absence of TNF-α (0.1ng/ml) after the cells had reached confluency. In some experiments, cells were pretreated with free radical scavenger TEMPOL, NF-κB inhibitor PDTC, TNF-α inhibitor 654256, or an anti-TNF-α neutralizing antibody 30 min before adding AGE3 or the vehicle. The concentrations of TNF-α and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the culture medium were measured by ELISA.

RESULTS AND DISCUSSION

Treatment with AGE3 at 300μg/ml for 24 and 48h significantly increased TNF-α secretion in HAoEC. Next, we treated HAoEC with vehicle or 30–300μg/ml AGE3 for 24h and found that AGE3 increased TNF-α secretion in a dose dependent manner. The 8-OHdG level in the culture medium was significantly increased in the cells incubated with 300μg/ml of AGE3 for 24 and 48h. Next, we treated HAoEC with vehicle, TNF-α or 30–300μg/ml AGE3 for 24h, and found that the 8-OHdG level was significantly increased by around 2 fold in the treatment with AGE3 at 100μg/ml or more. Addition of TNF-α (100pg/ml) also induced cellular oxidative response. Treatment with TEMPOL resulted in a significant decrease of AGE3-induced TNF-α secretion by almost 90%, suggesting that AGE3-induced TNF-α secretion is mediated by ROS. On the other hand, NF-κB inhibition by PDTC pretreatment significantly suppressed AGE3-induced TNF-α secretion by about 10%. These results suggest that AGE3-induced TNF-α secretion might be mediated through NF-κB dependent and independent mechanisms. PDTC inhibited AGE3-induced 8-OHdG generation by 30%, indicating that AGE3-induced oxidative stress is partly mediated by NF-κB. Moreover, either anti-TNF-α neutralizing antibody or TNF-α inhibitor suppressed AGE3-induced ROS generation to 30%, indicating that AGE3-induced ROS generation is partly mediated by TNF-α. Significant difference in ROS generation between NF-κB and TNF-α inhibition may be explained by the NF-κB dependent and independent mechanisms of ROS and TNF-α interaction.

In this study, our results indicate that AGE3 stimulates TNF-α secretion and ROS production in human aortic endothelial cells and that NF-κB is involved in this signaling. Vascular endothelial dysfunction is considered an early event of atherosclerosis, and recent growing evidences suggest an important role of ROS and TNF-α in the endothelial dysfunction.

We and others have previously shown in *in vitro* studies that uremic toxins such as indoxyl sulfate and phenylacetic acid induce ROS production and TNF-α secretion in vascular endothelial cells. These findings indicate an involvement of uremic toxins in the vascular dysfunction in renal failure. Likewise, AGEs can be involved in the pathogenesis of vascular diseases especially in diabetes mellitus, because AGEs accumulate more than normal in the blood and arteries of patients with diabetes.

The present findings indicate that AGEs-induced ROS production might be mediated through mechanisms dependent and independent of NF-κB. The interaction of AGEs with RAGE in endothelial cells leads to the stimulation of cytosolic ROS production mediated by NAD(P)H oxidase and the mitochondrial electron transport system, as well as by impairment of antioxidant defense. On the other hand, the activated NF-κB leads to the expression of TNF-α, which binds to the receptor TNFR-1 to stimulate ROS production and NF-κB activation. Thus, AGEs-activated NF-κB may play a critical role as an accelerator in a vicious cycle of ROS and TNF-α. Concerning ROS-induced TNF-α secretion, we speculate that the activation of not only NF-κB but also a series of mitogen-activated protein kinases (MAPK), such as p38, stress-activated protein kinase/c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) 1/2 in response to ROS stimulates TNF-α production. Since the dual involvement of MAPK and NF-κB in TNF-α production has been reported, MAPK pathways can be involved in the AGEs and ROS-induced TNF-α production in human endothelial cells.

Inhibition of sustained NF-κB activity and TNF-α production, in other words, inhibiting chronic low grade inflammation in the vessel wall is a new therapeutic strategy for vascular disease in diabetes like cancers and immune disorders. So far, several molecules such as high molecular weight hyaluronic acid, resveratrol, viscolin, and betulinic acid have been reported as the candidate. Further study is necessary for their clinical application.

CONCLUSION

AGEs-induced ROS generation is, at least in part, mediated through NF-κB activation and TNF-α secretion in human aortic endothelial cells.