Abstract

Relationship between *Porphromonas gingivalis* and MnSOD

in periodontitis patients with type 2 diabetes mellitus

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【Introduction】

Periodontitis is a chronic inflammatory disease, and the onset and progression of periodontitis is associated with the genetic and environmental risk factors. In particular, diabetes is a risk factor for periodontitis, and poor glycemic control increases the risk for periodontitis. The periodontitis was reported to worsen the glycemic control, and mutual relationship between periodontitis and diabetes attracted attention.

Control of Reactive oxygen species (ROS) is important to maintain homeostasis of living bodies, and elevation of ROS is known to shorten the lifespan. In physiological conditions, ROS is produced in the process that cells take up glucose and use it as energy. However, in infectious diseases, a large amount of ROS is produced and used for sterilization. In periodontitis patients with diabetes, large amount of ROS is suggested to generate through the abnormal glucose metabolism and periodontopathic bacterial infection, but it is difficult to measure the amount of ROS in vivo. The effects of ROS in periodontitis patients with diabetes mellitus have not been sufficiently clarified.

Manganese superoxide dismutase (MnSOD) is a major antioxidant enzyme that detoxifies ROS generated in the cell. It has been reported that serum MnSOD increases in the elderly, and it is suggested that serum MnSOD increases with increase in active oxygen due to aging.

In this study, to elucidate the relationship between serum MnSOD and periodontitis in diabetes patients, serum MnSOD was examined in diabetic patients and control subjects. In addition, to clarify the effects of glucose and *P. gingivalis* on the production of MnSOD in monocyte lineage, which are the source of serum MnSOD, the effects of glucose and *P. gingivalis* LPS on MnSOD production THP-1 cells were examined.

【Materials and methods】

1. Relationship between serum MnSOD and periodontitis in diabetic patients

Participants were type 2 diabetes patients (n = 76) and non-diabetic control subjects (n = 78), who visited dental hospital in Health Sciences University of Hokkaido. Dental examination, including number of the teeth, Probing pocket depth (PPD), Bleeding on probing (BoP) were measured. Saliva was collected from the participants, and DNA in the saliva was extracted. Quantitative polymerase chain reaction (qPCR) was performed using primers specific to 16srRNA of *P. gingivalis*. Known concentration of *P. gingivalis* was used as positive control. LDL cholesterol, HDL cholesterol, neutral fat, fasting blood glucose and HbA1c were available from the data of blood test. Part of the serum from the subjects was used for the measurement of serum MnSOD.

2. The effects of glucose concentrations and *P. gingivalis* on MnSOD production in monocyte lineage of cells

Human monocyte cell line THP-1 cells were cultured in RPMI supplemented with 10% FBS and different concentrations of glucose (100, 200, 300 mg/dl), and stimulated with *P. gingivalis* lipopolysaccharide (LPS). After the cultivation, the supernatant and the cells were collected. RNA was extracted from the cells, and cDNA was synthesized by reverse transcription. The qPCR was performed using primers specific for MnSOD, TNF-α, and β-actin. The mRNA expression in un-stimulated THP-1 cells was used as a positive control. The expression of TNF-α and MnSOD mRNAwere expressed as TNF-α/β-actin ratio and MnSOD/β-actin ratio, respectively. Tumor necrosis factor (TNF) -α concentration in the culture supernatant was measured by ELISA method.

IBM SPSS Statistics 23 was used for statistical analysis. Two groups were compared by Mann-Whitney U test or Pearsons's chi-square test. Multiple groups were compared by ANOVA with Tukey's HSD Post Hoc test. Multiple regression

analysis was performed in diabetic group, and serum MnSOD was set as dependent variable, while the data from blood test/dental examination and bacteria test were set as independent variables. The significance level was set at $p < 0.05$.

【Results and Discussion】

1. Relationship between serum MnSOD and periodontitis in diabetic patients

The serum MnSOD in the diabetic group was significantly higher than those in the control group. In the diabetic group, fasting blood glucose, BoP and the number of teeth having PPD ≧ 4 mm or more were significantly higher than those in the control group. HDL cholesterol and the current number of teeth were significantly lower in diabetes group than control. The significant positive correlation between serum MnSOD and the number of salivary *P. gingivalis* was detected by the multiple regression analysis. These results suggested that serum MnSOD production increased in response to the infection with *P. gingivalis* infection in periodontitis patients with diabetes.

2. The effects of glucose concentrations and *P. gingivalis* on MnSOD production in monocyte lineage of cells

When THP-1 cells was stimulated with *P. gingivalis* LPS, mRNA of MnSOD increased up to 12 hours after stimulation, and then decreased. When MnSOD mRNA was examined on 12 hours after stimulation with *P. gingivalis* LPS at different glucose concentrations, and the expression of MnSOD mRNA was highest in THP-1 cells at glucose concentration of 300 mg/dl. TNF-α was detected in the culture supernatants of THP-1 cells stimulated with *P. gingivalis* LPS. TNF-α stimulated cells to express MnSOD mRNA. TACE inhibitor abrogates the production of TNF-α in culture supernatants of THP-1 cells stimulated with *P. gingivalis* LPS. However, TACE inhibitor did not abrogate MnSOD mRNA expression in THP-1 cells stimulated with *P. gingivalis* LPS. These results

suggested that *P. gingivalis* infection might stimulate monocytes directly to produce serum MnSOD, or it stimulate indirectly via TNF-α induction in periodontitis patients with diabetes.

【Conclusion】

The present study suggested that serum MnSOD was augmented by the infection with *P. gingivalis* in periodontitis patients with T2DM.