The Role of Reactive Oxygen Species(ROS) in oral KB cells with *Porphyromonas Gingivalis*

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*Porphyromonas Gingivalis*가 구강내 세포에서 미치는 Reactive Oxygen Species(ROS) 효과

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Abstract

Porphyromonas gingivalis(*P. gingivalis*) is a Gram-negative oral anaerobe that is involved in the pathogenesis of periodontitis and is a member of more than 500 bacterial species that live in the oral cavity. Oxidative stress is characterized by an accumulation of reactive oxygen species (ROS) and plays a key role in the progression of inflammatory diseases. This study was to investigate the effects of TNF-α on cell viability in *P. gingivalis*-infected oral KB cells. And TNF-α seems to play an important role in early phase of *P.gingivalis* infection of periodontitis.

1. Introduction

Porphyromonas gingivalis is a Gram-negative oral anaerobe that is involved in the pathogenesis of periodontitis, an inflammatory disease that destroys the tissues supporting the tooth which eventually may lead to tooth loss. *Porphyromonas gingivalis* can locally invade periodontal tissues and evade the host defense mechanisms[1,2]. In doing so, it utilizes a panel of virulence factors that cause deregulation of the innate immune and inflammatory responses. *Porphyromonas gingivalis* is strongly correlated with chronic periodontitis. Its chronic persistence in the periodontium depends on its ability to evade host immunity without inhibiting the overall inflammatory response, which is actually beneficial for this and other periodontal bacteria[3].

Oxidative stress describes a metabolic state where the amount of reactive oxygen species (ROS) is strikingly increased above physiological levels. ROS originate from the incomplete reduction of molecular oxygen resulting in the accumulation of oxidants like hydrogen peroxide (H_2O_2) or free radicals like the hyperoxide (superoxide) anion[4].

We hypothesized that ROS are regulated by inflammatory stimuli in oral KB cells due to an imbalance of redox system activation. The current study will enhance the knowledge of the role played by oxidative stress in periodontal tissues which might improve diagnostic and therapeutic strategies in oral diseases.

2. Methods

The cellular effects of *P. gingivalis* were assessed using flow cytometry analyzing reactive oxygen species (ROS) production.

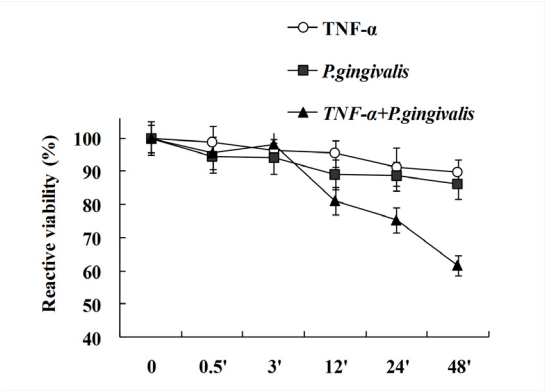
All experiments were performed in triplicate is given as means SD. Results were compared by ANOVA; Dunnett's and Tukey-Kramer tests were performed as posttest to calculate whether differences between treated and control groups were significant. A value of 0.05 was considered as significant.

3. Results

3.1 Cell viability of oral KB cells

Cell viability of oral KB cells treated with TNF-α, *P. gingivalis* MOI 10, or combination of TNF-α and *P. gingivalis* by MTS assay[Fig. 1]. KB cells (1.0×10^6 cells) in 100 mm culture dishes with serum free MEM were incubated with TNF-α (10 ng/ml) alone or *P. gingivalis* (MOI 100) alone or combination TNF-α (1 ng/ml) and *P. gingivalis* (MOI 10) for the indicated time periods. Cell viability of oral KB cells treated with TNF-α (10 ng/ml; open circle) or *P. gingivalis* (MOI 10; closed square) or combination [TNF-α (1 ng/ml) and *P. gingivalis* (MOI 10); closed triangle].

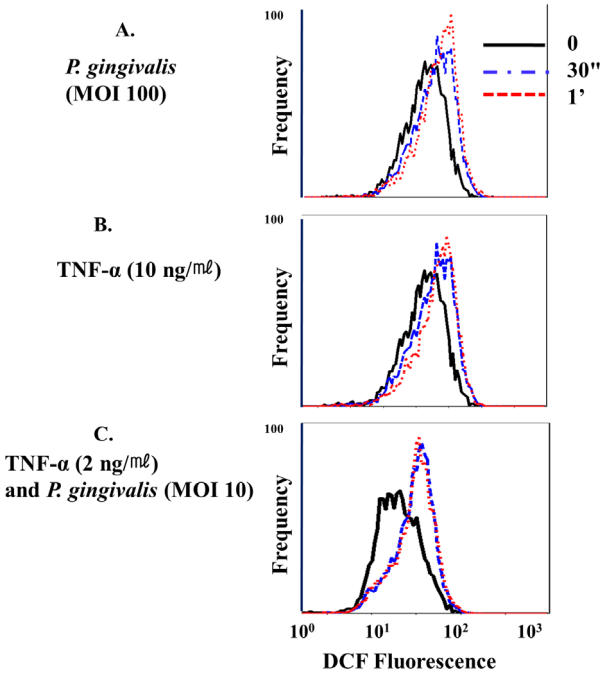
[Fig. 1] Cell viability of KB cells treated with TNF-α, *P. gingivalis* MOI 10, or combination of TNF-α and *P. gingivalis* by MTS assay.

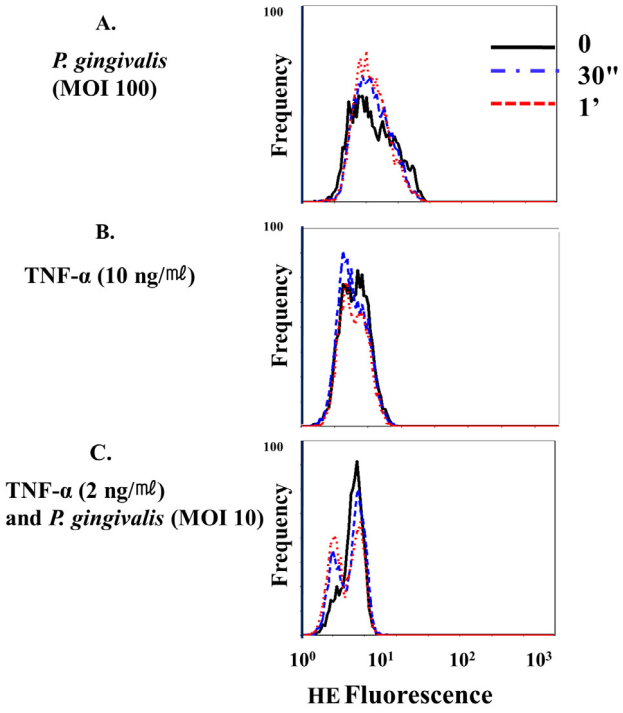


3.2 Detection of ROS in oral KB cells

Detection of ROS in oral KB cells treated with *P. gingivalis* (MOI 100), TNF-α (10 ng/㎖) and combination treatment of [TNF-α (2 ng/㎖)and *P. gingivalis* (MOI 10)]. Oral KB epithelial cells were stained with $DCFH_2$-DA, and analyzed via flow cytometry[Fig. 2]. The y axes of panels A, B and C reflect the frequency of events on a linear scale, while the x axes indicate increasing levels of ROS as estimated by the DCF fluorescence on a logarithmic scale. (A) A representative experiment in which ROS levels were measured in KB cells from a subject infected with P. gingivalis (MOI 100), TNF-α (10 ng/㎖) and combination treatment of [TNF-α (2 ng/㎖)and P. gingivalis (MOI 10)] for 0, 30min and 1 hr.

[Fig. 2] Detection of oral KB cells treated with TNF-α, *P. gingivalis* MOI 10, or combination of TNF-α and *P. gingivalis*





4. Conclusion

Oxidative stress is discussed as an important cofactor in the etiology and pathogenesis of several oral and dental diseases, for example, in inflammatory processes like periodontitis. In this study suggested that superoxide radical production

through NADPH oxidase were effects of oral epithelial cells treated with *P. gingivalis* (MOI 100) and TNF-α (10 ng/ml) which plays a pivotal role during oral diseases. H_2O_2 production might be involved in cell death. TNF-α seems to play an important role in early phase of *P. gingivalis* infection of periodontitis.

References

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