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ABSTRACT OF DISSERTATION

Title	**"Biological impacts of resveratrol, quercetin, and N-acetylcysteine on oxidative stress in human gingival fibroblasts"** **「ヒト歯肉線維芽細胞における酸化ストレスに対するレスベラトロール、ケルセチン、及び N-アセチルシステインの生物学的影響」**
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Background:

Chronic periodontitis is an infectious disease; which after bacterial infection, the initial inflammatory response activates the immune system through cytokines and chemokines. Neutrophils are the first line of defense and release a variety of toxic products including reactive oxygen species (ROS). ROS have been proposed as second messengers to mediate cellular responses in many normal biologic processes, but at higher concentrations they induce tissue damage by initiating free radical chain reactions. Under normal conditions, protective antioxidants (AOs; enzymes and redox molecules) rapidly repair the damage induced by ROS. An imbalance between ROS and AOs may be a key factor in the initiation and development of periodontal disease. The enhanced and continuous production of ROS and/or impaired function of AOs leads to oxidative stress (OS), which progressively induces the destruction of periodontal structure, alveolar bone, and connective tissue.

Polyphenols are now attracting attention as potential sources of agents capable of inhibiting, reversing, or retarding the progression of diseases caused by OS and inflammatory processes.

Among the wide range of polyphenols, resveratrol is widely available and found in red wine. Resveratrol is thought to have cardio-protective, anti-inflammatory, and anti-aging properties in animal model systems. Quercetin is the major polyphenol in the human diet found in fruits and vegetables. Quercetin inhibits the activation of protein kinase C (PKC) and the release of histamine. Another AO is the thiol N-acetylcysteine (NAC), a glutathione (GSH) precursor. NAC has a wide range of protective effects against DNA damage and carcinogenesis.

Human gingival fibroblasts (HGFs) are the major constituents of gingival tissues and are responsible to maintain homeostasis by regulating collagen metabolism. Therefore, the promotion of collagen synthesis and suppression of ROS may contribute to the integrity of gingival tissues and prevention of periodontitis.

Objective:

It has been hypothesized that the use of AOs will lessen the damage caused by ROS in response to OS and retard the initiation of periodontitis. Therefore, the aim of this study is to compare the biological effects of three AOs: resveratrol, quercetin, and NAC on cultured HGFs under OS induced by exposure to H_2O_2.

Materials and Methods:

1) Cell viability: HGFs were stimulated with H_2O_2 to induce OS and treated with resveratrol, quercetin and NAC. The cell viability was monitored in real-time and expressed as Cell Index. 2) ***ROS production:*** HGFs, stimulated with H_2O_2 and treated with AOs were dyed, to evidence ROS within cells and viewed under confocal laser microscope. 3) ***Type I Collagen gene expression:*** HGFs under OS were treated with AOs for 3 hrs. Later, cells were incubated for 3 to 72 hrs. Gene expression was quantified by qRT-PCR. 4) ***Mitochondrial respiration:*** the direct readout of cellular respiration (oxygen consumption and extra-cellular acidification rate) in HGFs under OS treated with AOs was determined with Extracellular Flux Analyzer.

Results:

Real-time cytotoxicity analyses revealed that resveratrol and quercetin enhanced cell proliferation even under OS. Of the antioxidants tested, resveratrol was the most effective at inhibiting ROS production. HGFs incubated with resveratrol and quercetin up-regulated the transcription of type I collagen gene after 3 h, but only resveratrol sustained this up-regulation for 24 h. A measurement of the oxygen consumption rate (OCR=mitochondrial respiration) showed that resveratrol generated the highest maximal respiratory capacity, followed by quercetin and NAC. Simultaneous measurement of OCR and the extracellular acidification rate (non-mitochondrial respiration) revealed that resveratrol and quercetin induced an increase in mitochondrial respiration when compared with untreated cells. NAC treatment consumed less oxygen and enhanced more non-mitochondrial respiration.

Conclusion:

Resveratrol was the most effective antioxidant in terms of real-time cytotoxicity analysis, reduction of ROS production, and enhancement of type I collagen synthesis and mitochondrial respiration in HGFs. Hence, resveratrol could be used as a beneficial supplement during the treatment of OS disorders. However, further studies using *in vivo* models are necessary to support the clinical use of AOs as a supplement to reduce oxidative stress and prevent periodontitis in humans.