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Original Contribution

Mass spectrometry-based metabolomic profiling identifies alterations in salivary redox status and fatty acid metabolism in response to inflammation and oxidative stress in periodontal disease

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ABSTRACT

Periodontal diseases represent the most common chronic inflammatory diseases in humans and a major cause of tooth loss. Combining mass spectrometry-based ionomics and targeted lipidomics on fatty acid metabolites, we identified significant alterations in redox status and fatty acid metabolism in saliva in response to chronic inflammation and oxidative stress in periodontal disease in a cohort of nonsmoker subjects with chronic periodontitis. For the first time, ionomic profiling of around 30 ions in saliva revealed significantly decreased levels of redox-active metal ions including Mn, Cu, and Zn in the periodontal group, which is consistent with decreased levels of superoxide dismutases in saliva and serum. A targeted lipidomic approach was employed to monitor the major metabolites of arachidonic acid and linoleic acid in saliva. We observed increased levels of cyclooxygenase products including PGE_2, PGD_2, and $PGF_{2\alpha}$ and TXB_2, but decreased level of PGI_2 in the periodontal group. A unique pattern of the lipoxygenase products of arachidonic acid and linoleic acid was observed with increased level of 5-HETE but decreased levels of 13-HODE and 9-HODE. Levels of salivary F_2-isoprostanes, free radical lipid peroxidation products, and a gold standard for oxidative stress in vivo were also significantly elevated. Taking these data together, our study using multiple powerful omics techniques demonstrates that local redox alteration contributes significantly to periodontitis through the modulation of fatty acid metabolism in response to inflammation and oxidative stress. This study highlights the importance of redox status in periodontitis and provides a rationale for preventing periodontal disease by dietary interventions aiming to restore redox balance.

Periodontal diseases including gingivitis and periodontitis are inflammatory conditions of gingivae, periodontal ligament, and alveolar bone [1]. According to a recent report on the global burden of oral conditions, periodontal diseases are among the

Abbreviations: AA, arachidonic acid; COX, cyclooxygenase; EET, epoxyeicosatrienoic acid; DHETE, dihydroxyeicosatrienoic acid; FFQ, food frequency questionnaire; FAME, fatty acid methyl ester; 5-F_{2t}-IsoP, 5-series F_{2t}-isoprostane; 15-F_{2t}-IsoP, 15-series F_{2t}-isoprostane, GC–MS, gas chromatography–mass spectrometry; HETE, hydroxyeicosatetraenoic acid; HODE, hydroxyoctadecadienoic acid; HPLC, high-performance liquid chromatography; ICP–MS, inductively coupled plasma mass spectrometry; IsoP, isoprostane; LC–MS, liquid chromatography–mass spectrometry; LOX, lipoxygenase; NEFA, nonesterified fatty acid; ROS, reactive oxygen species; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostacyclin I_2; PUFA, polyunsaturated fatty acid; SOD, superoxide dismutase; TXB_2, thromboxane B_2; UPLC, ultrapressure liquid chromatography

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most widespread human inflammatory disorders, affecting around 10.79% of the worldwide population [2]. Periodontitis is a complex multifactorial disease [3] and accumulating evidence indicates that chronic inflammation [4], redox imbalance [1], and oxidative stress [5,6] contribute to the disease onset and progression. However, the exact mechanism that leads to local and systemic redox alterations in periodontal diseases remains poorly defined.

Metabolomics is an emerging discipline that studies all the metabolites and their metabolic pathways in a given biological system. It offers advantages over conventional techniques to interrogate the system of interest in an unbiased way and at a systems biology level [7]. An increasing number of studies have emerged in recent years to investigate the metabolic changes in periodontal diseases and some novel mechanistic insights have been obtained [8–10]. Lipidomics is a subfield of metabolomics that investigates all the lipid species and their downstream metabolites (termed the lipidome) in a given system [11,12]. For example, a targeted lipidomics approach identified unique patterns of diacylglycerol species in neutrophils from localized aggressive periodontitis [13]. Ions, including metals and nonmetal nutrients, play an important role in multiple biological processes and the collection of all ions in a given biological system is termed the ionome [14]. Ionomics or metalomics is an emerging subdivision of metabolomics that studies all the ions in a system (ionome). Using the state-of-the-art inductively coupled plasma mass spectrometry (ICP–MS)[2] technique, a recent study found strong associations between ionomic profiles and metabolic abnormalities including overweight/obesity, metabolic syndrome, and type 2 diabetes [15]. Even though substantial evidence suggests that metal ions are important modulators of cellular redox status and oxidative stress, the levels of various metal and nonmetal ions and their roles in the pathogenesis of periodontal disease have not been studied at systems biology levels.

Overwhelming evidence points to the roles of chronic inflammation and oxidative stress caused by local and systematic alterations in redox status in the pathogenesis of periodontal diseases. The relationship between the local redox imbalance and oxidative stress remains elusive because the factors that are involved in oxidative stress and inflammatory responses are not readily dissected in vivo [1]. Arachidonic acid (AA) is one of the most important polyunsaturated fatty acids (PUFAs) in mammalian cells. Its metabolites, collectively termed eicosanoids, play an important role in numerous physiological and pathophysiological processes [16,17]. Under normal conditions, most AA is esterified on phospholipids in cellular membranes. Under inflammatory stimuli, AA is hydrolyzed by phospholipase A_2 and presented to several enzymes including lipoxygenases (LOXs), cyclooxygenases (COXs), and P450s in a cell and tissue-dependent manner (Fig. 1). LOXs oxidize AA to generate hydroxyeicosatetraenoic acid (HETEs) and leukotrienes including LTA_4, LTB_4, and LTC_4. This pathway has been shown to play an important role in allergic reactions. COXs including COX-1 and COX-2 oxidize AA to prostaglandin H_2 and other downstream prostaglandins, such as PGD_2, E_2, $F_{2\alpha}$, prostacyclin (PGI_2), and thromboxanes by their respective synthases. COX activation is a hallmark of inflammation, and inhibition of COX activity by nonsteroidal anti-inflammatory drug or selective COX-2 inhibitors has been widely used clinically. Cytochrome P450 enzymes oxidize AA to generate epoxyeicosatrienoic acid (EET) and dihydroxyeicosatrienoic acid (DHETE). Under oxidative stress conditions, AA can be oxidized to a number of free radical oxidation products, among which isoprostanes, isomers of prostaglandins, have evolved as the gold standard to assess oxidative stress status in vivo [18]. Thus, metabolites derived from arachidonic acid can serve as an important indicator for inflammatory responses and oxidative stress status. Limited studies have reported the levels of selected AA metabolites in the context of periodontal diseases but none of these studies involves metabolic profiling of all the major pathways of AA at a systems biology level [19].

Combining ionomic and targeted lipidomic techniques, we herein report that local imbalance of redox-sensitive ions in saliva of chronic periodontal patients contributes significantly to inflammatory responses and oxidative stress reflected by the altered metabolism of arachidonic acid and antioxidant enzymes including superoxide dismutase (SOD). The low levels of redox-active trace metals such as Cu, Zn, and Mn in saliva of the periodontal group are consistent with the dietary intake analysis based on a food frequency questionnaire (FFQ). Thus restoration of the redox balance via dietary interventions may be an effective strategy to prevent or slow down the progression of periodontal diseases through restoration of the local and systemic redox balance.

Material and methods

Reagents

SOD, xanthine oxidase, luminol, xanthine, and vitamins A, E, and C were purchased from Sigma–Aldrich Chemical (Milwaukee, WI, USA). HPLC-quality solvents, such as methanol, water, 2-propanol,

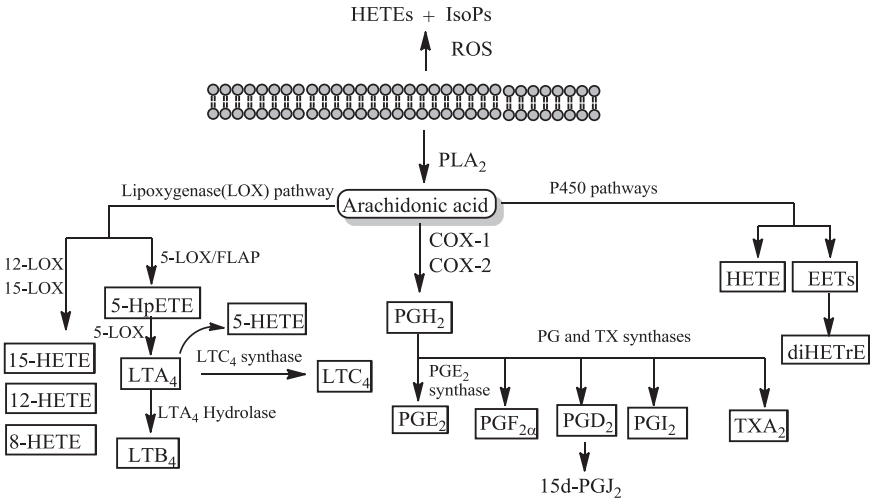

Fig. 1. Major metabolic pathways of arachidonic acid.