4-hydroxy-2-nonenal (HNE) is a Viable Salivary Biomarker for Lipid Peroxidation in Stimulated and Unstimulated Saliva Procured from Healthy Volunteers

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Abstract

Background: Oxidative stress is well-known to be a pathological factor underlying several chronic inflammatory diseases. 4-hydroxy-2-nonenal (HNE) is a reactive aldehyde formed from oxidation of n-6 polyunsaturated fatty acids, the most common of which in human are delta-5 oleic acid (corn/soybean oil) and arachidonic acid. Once formed, these reactive aldehydes modify proteins, RNA, and other lipids and trigger inflammatory signaling cascades that if allowed to persist, can cause disease. Traditionally, patients endure discomfort from invasive procedures that aim to measure oxidative biomarkers. Saliva offers an alternative, noninvasive method to test oxidative stress.

Objective: We hypothesized that the oxidative biomarker HNE can be determined in stimulated and unstimulated saliva procured from healthy volunteers, and be used as a non-invasive surrogate of oxidative/carbonyl stress.

Methods: Whole stimulated and unstimulated saliva was procured through the process of drooling from confirmed healthy volunteers. Unstimulated saliva was obtained over a time period of 3 minutes, whereas stimulated saliva was induced by paraffin (gum-like substance) and obtained over 2 minutes. Samples were prepared through centrifugation and placed on dry ice and stored in −80°C freezer. The sandwich or indirect Enzyme linked Immunoassorbent Assay (ELISA) method was used to evaluate the levels of HNE.

Results: GraphPad analysis was performed on data obtained. All samples procured from subjects contained levels of the oxidative biomarker HNE. There was higher HNE levels in unstimulated saliva in comparison to stimulated saliva in most subjects. Stimulated saliva had much less variability.

Conclusion: Saliva has the potential to be a convenient, noninvasive, and comprehensive tool in evaluating the overall health of the body. As a result, further studies are needed to identify and develop a wide array of methods to detect salivary biomarkers. These findings suggest that HNE is a viable salivary biomarker for lipid peroxidation in stimulated and unstimulated saliva of healthy volunteers.

Introduction

Linoleic Pathway to HNE

Fig. 1. Formations of some temporary, unstable intermediate conformations (Green & Gold) on the pathways from linoleic acid (C18:2) to HNE.

Objectives: HNE can be exploited to detect lipid peroxidation in stimulated and unstimulated saliva obtained from healthy volunteers.

Abstract

Results

Methods

Patients were recruited verbally and through flyer as healthy volunteers and informed consent obtained. Dr. Gordon performed oral evaluation.

Unstimulated and Stimulated saliva were procured per protocol from 11 total subjects.

Samples prepared through centrifugation at 14,000 rpm for 10 minutes, placed on dry ice, then stored in −80°C freezer.

Amount of HNE-modified protein adducts in saliva quantified by ELISA approach developed and validated in the laboratory of Dr. Anderson.

Outcome: A group statistical analysis was performed on obtained data. The levels of HNE in stimulated saliva may be lower than unstimulated because there is an increase in the total saliva volume per subject, thereby diluting the concentration of HNE in saliva samples.

Conclusion: Unstimulated saliva may have greater variability because it reflects a more accurate oxidative state of each subject. Saliva is a noninvasive diagnostic fluid because it is noninvasive and easily repeatable. It offers a broad spectrum of biologically relevant compounds that can be measured:

- Oxidative Stress
- Antioxidant status
- Lipid stress
- Salivary markers of oxidative stress can be used for screening and monitoring of oral diseases such as periodontitis or caries.

Future Directions

- Analyze larger population size of healthy and unhealthy volunteers to further assess whether HNE-adducts can serve as surrogate biomarkers of chronic disease.
- Compare HNE-adducts in saliva and blood to perform the determination whether these markers of oxidative/carbonyl stress in saliva are indicative of systemic oxidative stress, or if localized to the oral compartments.
- Stimulated method of drawing paraffin may potentially mediate the issue of Xerostomia opposed to unstimulated.

Origins of Salivary markers of Oxidative Stress

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References