in vivo *23*: 343-346 (2009)

Does *Enterococcus faecalis* Contribute to Salivary Thiobarbituric Acid-reacting Substances?

BARBORA VLKOVÁ[1,2] and PETER CELEC[1,2,3]

[1]*Department of Molecular Biology, Faculty of Natural Sciences,* [2]*Geneton, and*
[3]*Institute of Pathophysiology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic*

Abstract. *Thiobarbituric acid-reacting substances (TBARS) are widely used as markers of oxidative stress and lipoperoxidation. Salivary TBARS are affected by age, smoking and periodontal status. The origin of these compounds in saliva is unknown, but microbial factors and inflammation have been previously postulated as potential candidates. Virulence factors of Enterococcus faecalis include resistance to oxidative stress and the production of extracellular free radicals. E. faecalis has been linked to the pathogenesis of caries and periodontitis, both associated with oxidative stress. It is hypothesized that E. faecalis produces an extracellular superoxide causing oxidative damage to membranes of host cells. This lipoperoxidation leads to increased salivary TBARS levels. The hypothesis should be tested in future studies.*

In endocrinology, saliva has become a widely recognized source of information, both in clinical studies and basic research. Several steroid hormones can be measured in saliva (1). The sampling is really non-invasive making it especially useful in studies involving children or in those with repeated sampling. However, the most important advantage is that the salivary levels correlate tightly with the free bio-available fraction in plasma, which is difficult to measure.

Malondialdehyde is a marker of oxidative stress. It is used for the estimation of lipoperoxidation as it is a degradation product of fatty acids. The most widely used technique for the determination of malondialdehyde is the colorimetric method using the reaction with thiobarbituric acid (2). The specificity of this reaction is discussed in the literature, as other compounds react with thiobarbituric acid (3). This group of compounds is called thiobarbituric acid-reacting substances (TBARS). Although the group is heterogeneous, it is still used as a marker of oxidative stress, as the vast majority of TBARS are products of oxidative damage of lipids.

TBARS are detectable in saliva. The concentrations are much lower than in plasma, but still measurable with sufficiently sensitive methods. The intraindividual and interindividual variability of salivary TBARS is under investigation. Factors already known to increase the levels of salivary TBARS include age, smoking and periodontal status (4-6). Although significant, the effect of all these factors is rather small. Thus, other factors determining the levels of salivary TBARS have to be identified to understand the potential of using salivary TBARS in research or even in a clinical setting.

The origin of TBARS detected in saliva is unknown. It seems that in contrast to other biomarkers measured in saliva, the source of salivary TBARS is not plasma. However, this finding comes from a small underpowered study that showed no correlation between salivary and plasma levels of TBARS (4). Nevertheless, much more probable is that salivary TBARS are produced locally in the oral cavity. An important source of free radicals that cause oxidative stress is inflammation (7). The papillary bleeding index, as a marker of the periodontal status, correlates with salivary TBARS significantly (4).

Therefore, inflammation seems to contribute to the local production, yet only a small fraction of the variability of salivary TBARS can be explained by inflammation of the gums (8).

Enterococci, Gram-positive bacteria, are commensal microorganisms naturally present in humans and are often found in the environment. Enterococci are facultative anaerobic organisms; they prefer using oxygen, but can also persist in the enviromnent without oxygen. These bacteria normally constitute human intestinal flora, however the endogenous sources are attributed mostly to enterococcal infections.

Correspondence to: Peter Celec, MD, Dipl. Ing., MSc, Ph.D., MPH, Institute of Pathophysiology and Department of Molecular Biology, Comenius University, Sasinkova 4, 811 08 Bratislava, Slovak Republic. Tel: +421259357296, Fax: +421259357601, e-mail: petercelec@gmail.com

Key Words: TBARS, malondialdehyde, salivary biomarkers, oxidative stress.

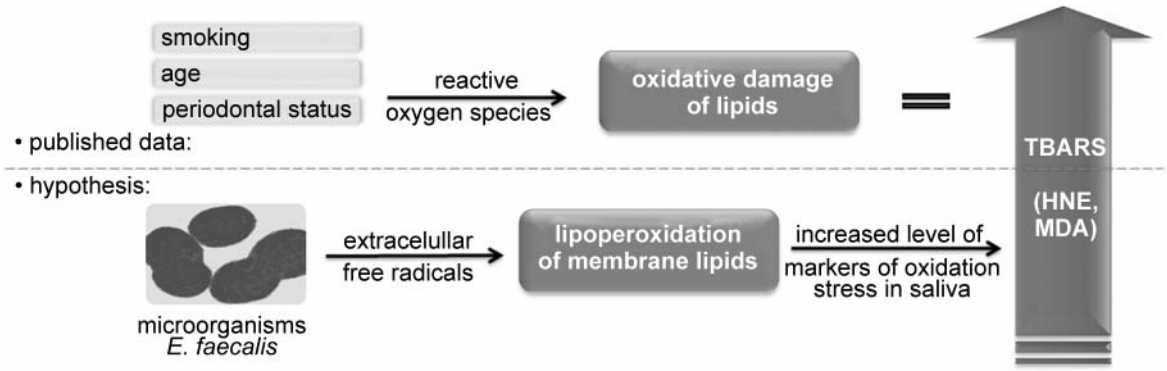

Figure 1. *The hypothesis: E. faecalis strains present in the oral cavity produce extracellular superoxide, free radicals which cause oxidative damage of the membrane lipids of host cells leading to an increasing level of salivary TBARS, a marker of oxidative stress and lipoperoxidation (TBARS: thiobarbituric acid-reacting substances, HNE: hydroxynonenal, MDA: malondialdehyde).*

Enterococcus faecalis is one of the two most common species of the genus Enterococcus. Strains isolated from different clinical and environmetal sources possess a large number of genes that encode potential virulence factors among isolates. This organism can be found in various niches in the gastrointestinal tract including the oral cavity. *E. faecalis* is highly resistant to both the host immune system and environmental conditions such as pH, temperature and osmolarity. *E. faecalis* virulence factors enable its survival and even the colonization of previously sterile loci such as the dental root canal (adhesion production, high exchange rate of plasmids, production of metalloproteinase and hyaluronidase) (9). The virulence is in some aspects even stronger than the virulence of the caries-causing *Streptococcus mutans* (10). Indeed, the presence of *E. faecalis* among other bacteria is associated with root caries (11).

One of the main characteristics of pathogenic strains of *E. faecalis* is the extracellular production of superoxide anion. *E. faecalis* produces extracellular superoxidase and is increasingly associated with nosocomial and opportunistic infections in humans. The vast majority of clinical isolates are able to produce this free radical, indicating the importance of superoxide production for its virulence (12). The mechanism of superoxide production was the reduction of oxygen by dimethylmenaquinone – a molecule important in anaerobic respiration (13). Recently, the production of extracellular superoxide by *E. faecalis* has been linked to carcinogenesis, at least in the colon. Wang and Huycke showed *in vitro* that the produced superoxide induced cyclooxygenase-2, promoting chromosomal instability in mammalian cells (14).

E. faecalis not only produces free radicals but it has also several antioxidant mechanisms for surviving oxidative conditions including peroxidases, catalase and superoxide dismutase (15, 16). The oxidative stress response is mediated by the transcription factors hypR and PerR (17, 18). The mechanisms of antioxidative metabolism of *E. faecalis* have been recently reviewed (19).

Based on the presented data in the literature, it is hypothesized that *E. faecalis* producing extracellular superoxide might be an important contributor to the levels of oxidative stress markers in the oral cavity (Figure 1). Superoxide anion radicals cause lipoperoxidation of the lipids in the plasma membrane of surrounding host cells leading to the production of hydroxynonenal and malondialdehyde, the main components of TBARS. The hypothesis is testable by association studies analyzing the correlation between levels of salivary TBARS and the quantitative presence of *E. faecalis* using currently available molecular techniques. It also can be proven experimentally by analyzing the effects of adding *E. faecalis* to the oral microflora of gnotobiotic experimental animals on the salivary markers of oxidative stress. Proving this hypothesis is important to uncover the origin of salivary TBARS, but also to understand the role of *E. faecalis*-induced oxidative stress in oral diseases such as caries or periodontitis.

Acknowledgements

The authors are supported by grants from the Slovak Research and Development Agency (LPP-0133-06), the Ministry of Education (VEGA 1/4316/07 and VEGA 1/0307/08) and the Ministry of Health (Grant 2006/23-UK-02) of the Slovak republic.

References

1 Vining RF and McGinley RA: The measurement of hormones in saliva: possibilities and pitfalls. J Steroid Biochem *27*: 81-94, 1987.
2 Yagi K: Simple assay for the level of total lipid peroxides in serum or plasma. Methods Mol Biol *108*: 101-106, 1998.
3 Lykkesfeldt J: Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta *380*: 50-58, 2007.

4 Celec P, Hodosy J, Celecova V, Vodrazka J, Cervenka T, Halcak L, Bozek P, Kopani M and Kudela M: Salivary thiobarbituric acid-reacting substances and malondialdehyde – their relationship to reported smoking and to parodontal status described by the papillary bleeding index. Dis Markers *21*: 133-137, 2005.
5 Guentsch A, Preshaw PM, Bremer-Streck S, Klinger G, Glockmann E and Sigusch BW: Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. Clin Oral Investig *12*: 345-352, 2008.
6 Mashayekhi F, Aghahoseini F, Rezaie A, Zamani MJ, Khorasani R and Abdollahi M: Alteration of cyclic nucleotides levels and oxidative stress in saliva of human subjects with periodontitis. J Contemp Dent Pract *6*: 46-53, 2005.
7 Akalin FA, Baltacioglu E, Alver A and Karabulut E: Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. J Clin Periodontol *34*: 558-565, 2007.
8 Tsai CC, Chen HS, Chen SL, Ho YP, Ho KY, Wu YM and Hung CC: Lipid peroxidation: a possible role in the induction and progression of chronic periodontitis. J Periodontal Res *40*: 378-384, 2005.
9 Kayaoglu G and Orstavik D: Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. Crit Rev Oral Biol Med *15*: 308-320, 2004.
10 Nakajo K, Komori R, Ishikawa S, Ueno T, Suzuki Y, Iwami Y and Takahashi N: Resistance to acidic and alkaline environments in the endodontic pathogen *Enterococcus faecalis*. Oral Microbiol Immunol *21*: 283-288, 2006.
11 Preza D, Olsen I, Aas JA, Willumsen T, Grinde B and Paster BJ: Bacterial profiles of root caries in elderly patients. J Clin Microbiol *46*: 2015-2021, 2008.
12 Huycke MM, Joyce W and Wack MF: Augmented production of extracellular superoxide by blood isolates of *Enterococcus faecalis*. J Infect Dis *173*: 743-746, 1996.
13 Huycke MM, Moore D, Joyce W, Wise P, Shepard L, Kotake Y and Gilmore MS: Extracellular superoxide production by *Enterococcus faecalis* requires demethylmenaquinone and is attenuated by functional terminal quinol oxidases. Mol Microbiol *42*: 729-740, 2001.
14 Wang X and Huycke MM: Extracellular superoxide production by *Enterococcus faecalis* promotes chromosomal instability in mammalian cells. Gastroenterology *132*: 551-561, 2007.
15 La Carbona S, Sauvageot N, Giard JC, Benachour A, Posteraro B, Auffray Y, Sanquinetti M and Hartke A: Comparative study of the physiological roles of three peroxidases (NADH peroxidase, alkyl hydroperoxide reductase and thiol peroxidase) in oxidative stress response, survival inside macrophages and virulence of *Enterococcus faecalis*. Mol Microbiol *66*: 1148-1163, 2007.
16 Verneuil N, Maze A, Sanguinetti M, Laplace JM, Benachour A, Auffray Y, Giard JC and Hartke A: Implication of (Mn)superoxide dismutase of *Enterococcus faecalis* in oxidative stress responses and survival inside macrophages. Microbiology *152*: 2579-2589, 2006.
17 Verneuil N, Rince A, Sanguinetti M, Auffray Y, Hartke A and Giard JC: Implication of hypR in the virulence and oxidative stress response of *Enterococcus faecalis*. FEMS Microbiol Lett *252*: 137-141, 2005.
18 Verneuil N, Rince A, Sanguinetti M, Posteraro B, Fadda G, Auffray Y, Hartke A and Giard JC: Contribution of a PerR-like regulator to the oxidative stress response and virulence of *Enterococcus faecalis*. Microbiology *151*: 3997-4004, 2005.
19 Riboulet E, Verneuil N, La Carbona S, Sauvageot N, Auffray Y, Hartke A and Giard JC: Relationships between oxidative stress response and virulence in *Enterococcus faecalis*. J Mol Microbiol Biotechnol *13*: 140-146, 2007.

Received October 13, 2008
Revised November 21, 2008
Accepted December 1, 2008