

OXIDATIVE CONSUMPTION OF ROOT CARIES BIOMOLECULES BY A NOVEL ANTI-BACTERIAL OZONE DELIVERY SYSTEM

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Introduction

In addition to lowered pH values, the profile and concentrations of microbial-derived short-chain organic acids is an important demineralisation parameter regarding the induction, development and progression of dental caries.

The nature, rate and extent of salivary reductant consumption [e.g., that of volatile sulphur compounds (VSCs) responsible for halitosis (predominantly methyl mercaptan and hydrogen sulphide), their amino acid precursors (L-cysteine and L-methionine), thiocyanate, urate and pyruvate] by ozone (O₃) reflects the oxidising capacity of this microbicidal agent, a parameter of much relevance to its therapeutic and aesthetic actions.

High resolution proton (¹H) nuclear magnetic resonance (NMR) spectroscopic analysis of human saliva, gingival crevicular fluid (GCF) or appropriate chemical model systems serves as a very useful technique for the *in vitro* evaluation of the O₃-mediated oxidation of such biomolecules, and we have recently found that it also provides much valuable regarding the molecular mechanisms associated with the potential therapeutic actions of this reactive oxygen species (ROS).

Aim

The aim of this study was to assess a multicomponent evaluation of the oxidative consumption of root caries biomolecules by O₃ using a high resolution ¹H NMR spectroscopy.

Materials and Methods

Ozone delivery system

The ozone-delivery system employed in this study was the HealOzone Unit (CurOzone USA). This system includes a source of oxidizing gas from a dental handpiece with a cup for delivering the gas to a lesion.

¹H NMR spectroscopy

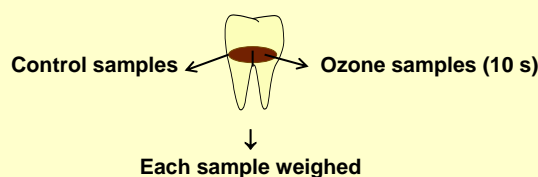
Nuclear magnetic resonance (NMR) spectroscopy uses energy from the radiofrequency region of the electromagnetic spectrum to detect changes in the alignment of nuclear magnets during exposure to a powerful external magnetic field. The absorption frequency is dependent on the magnetic (and, therefore, chemical) environment of nuclei (Silwood *et al.*, 1999)

Patients

All participants (n=16 for ¹H NMR analysis) were attending the Dental Hospital for routine oral health care (mean ± s.e. age 56.2 ± 3.1 yr. ; range 50-69 yr.). Four of the patients were smokers. Only patients who had refrained from oral activities (i.e., eating, drinking, tooth-brushing, oral rinsing, smoking, etc.) for at least 2 hr. prior to sample collection were selected.

Study design

16 primary root carious lesions (PRCLs) samples



¹H NMR analysis at an operating frequency of 600 MHz

Results

The expanded 0.50 - 4.50 and 5.50 - 9.00 ppm regions of a typical 600 MHz ¹H NMR spectrum of a post-neutralised HClO₄ extract of caries collected from a patient are shown in Figure 1. The spectra acquired contain many resonances assignable to a wide variety of low-molecular-mass metabolites, and illustrate the multicomponent analytical ability of the technique employed. Indeed, these spectra contain well-resolved, sharp signals assignable to bacterial- or yeast-derived organic acid anions [including formate (Form), acetate (Ace), propionate (Pro), *n*-butyrate (Bu), lactate (Lac), and pyruvate (Pyr)], amino acids (predominantly alanine and glycine) and carbohydrates such as glucose.

Ozone gave rise

- to the oxidative decarboxylation of the electron-donor pyruvate (generating acetate and CO₂ as products),
- the oxidation of the volatile sulphur compound (VSC) precursor methionine to its corresponding sulphoxide (Met-S-CH₃).
- evidence for the O₃-mediated oxidation of 3-D-hydroxybutyrate was also obtained.

References

C. J. L. Silwood, E. Lynch, S. Seddon, A. Sheerin, A. Claxson and M. Grootveld. H NMR analysis of microbial- derived organic acid in primary root carious lesions and saliva. *NMR in Biomedicine* 1999; 12; 345-356.

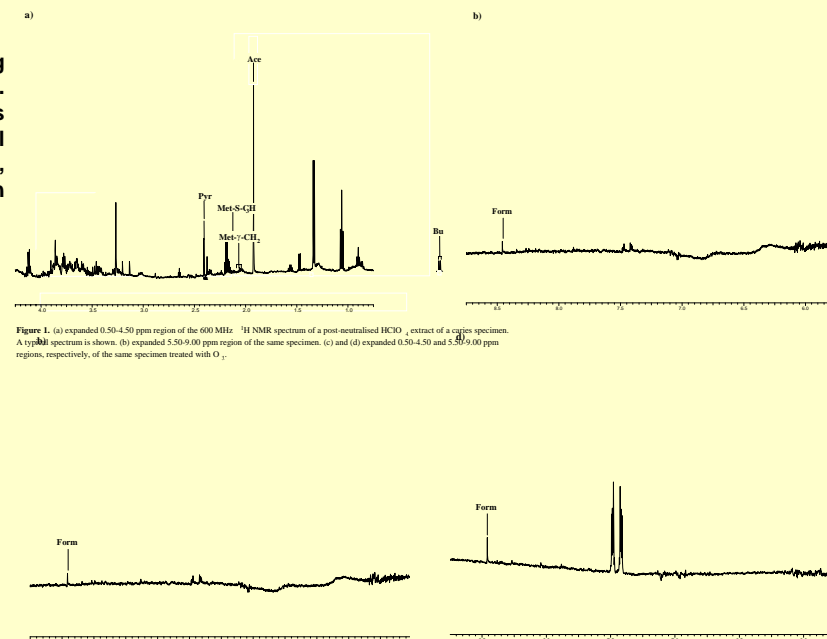


Figure 1. (a) expanded 0.50-4.50 ppm region of the 600 MHz ¹H NMR spectrum of a post-neutralised HClO₄ extract of a caries specimen. A typical spectrum is shown. (b) expanded 5.50-9.00 ppm region of the same specimen. (c) and (d) expanded 0.50-4.50 and 5.50-9.00 ppm regions, respectively, of the same specimen treated with O₃.

Discussion

Consumption of methionine by O₃ is of great importance to oral hygiene and clinical periodontology since both CH₃SH and H₂S are generated from this amino acid via metabolic pathways operational in gram-negative microorganisms. Hence, our data indicate that O₃ has the capacity to clinically alleviate oral malodour via the direct oxidative inactivation of VSCs and their amino acid precursors..

As demonstrated here, the technique is of much value concerning multicomponent assessments of the interactions of O₃ with human caries biomolecules, and the oxidative decarboxylation of pyruvate by this oxidant evaluated in this study serves as an important fundamental example of this which may be of some relevance to its mechanisms of action. Indeed, pyruvic acid is a very powerful proton donor (K_a = 3.20 mM) being much stronger in this capacity than lactic acid (K_a = 0.14 mM), and hence may play an important role in promoting tooth demineralisation processes.

Conclusion

In conclusion, the removal of caries pyruvate by O₃ may suppress the development and progression of tooth demineralisation. In view of these considerations, O₃ may offer caries-preventative and management actions and experiments to investigate this further are currently in progress.