ABSTRACT

Objectives: To demonstrate the ability of salivary metabolite analysis to quantify the level of bacterial inhibition delivered by a chemo-therapeutic product.

Methods: This was an 8 week randomized, double-blind parallel study carried out at the University of Texas, San Antonio. Study participation was on a voluntary basis with 153 subjects meeting the criteria and completing the study. All subjects were generally healthy adults presenting some level of visible anterior gingivitis as determined by a clinical bleeding examination. Subjects were stratified according to gender and gingivitis status and assigned to equal treatment groups. At baseline all subjects were supplied with a manual toothbrush and Crest Cavity Protection Dentifrice which they continued to use during the 8 week intervention in addition to one of four mouthrinses containing 0%, 0.01%, 0.035% or 0.07% cetylpyridinium chloride (CPC). At baseline and 4 and 8 weeks of treatment all subjects supplied wake-up lavage saliva samples which were frozen and brought to the clinical site. Saliva samples were analyzed at a later date by 1H NMR and multivariate data analysis was carried out on the saliva spectra. Results: Partial Least Square (PLS) modelling was carried out blinded to quantify the change from baseline to intervention for each treatment leg separately (p<<0.01). When the effect of each treatment was compared to that of the 0.07% rinse a dose response was observed ($R^2 = 0.986$). Only the two highest strength rinses demonstrated a statistical drop in propionate levels, associated with an anaerobic salivary microbiological environment and the strongest rinse showed a significant rise in lactate proportions also shown to be linked with a low plaque state. Conclusions: There is a linear dose response between CPC concentration and the inhibition of bacterial metabolism, characterised by a shift from anaerobic metabolite products to aerobic. Placebo and 0.01% CPC rinses showed weak product effects with no significant positive health aspects.

INTRODUCTION

The field of objective analysis in Oral Care is ever expanding and so too is our understanding of high-throughput "omics" techniques. Here we present more on the application of relatively simple analytical tools in quantifying the nature and magnitude of an oral anti-microbial using metabolomics. To date we have presented the effect of a positive control vs a negative control (Crest Pro-Health Dentifrice vs Crest Cavity Protection)[1] and related salivary measurement to objective plaque measurement (Digital Plaque Image Analysis)[2]. In this we present information on the sensitivity and consistency of measuring antibacterial efficacy by salivary analysis.

MATERIALS AND METHODS

Wake-up saliva samples were collected for two weeks at baseline, at weeks 3-4 and weeks 7-8. These samples were frozen and collected on clinic visits for analysis.

Procedure for salivary analysis:
- Solids were removed from samples by centrifugation immediately following defrost.
- Pairs of samples were pooled according to study day (baseline day 1 with baseline day 2 etc) in equal volumes (0.44ml each)
- A deuterated buffer solution was added to each sample to stabilise pH before analysis and enable analysis (0.08 ml)
- Each sample was run on high-resolution 1H Nuclear Magnetic Resonance (NMR) Spectroscopy (700 MHz) to obtain a saliva spectrum

Procedure for data analysis:
- A median value was calculated for each metabolite for each subject in each treatment phase (baseline, week 4, week 8).
- The medians from each treatment leg (A-D) were collected together and used to generate partial least squares models of treatment effect
- These treatment effect models included the rise or fall of metabolites from baseline and accountability of statistical significance.
- For a summary including all metabolites a vector analysis was carried out to show the % agreement between each of the treatment effects using all major salivary metabolites

All sample and data analysis were carried out blinded. Subjects were blinded as to the treatment they were using also.

RESULTS

Lactate and propionate are important markers in oral ecology, rising proportions of propionate are indicative of a rise in gram negative metabolism and this is usually accompanied by a fall in the proportion of lactate.[3] This trend is borne out with increasing levels of CPC in this study increasing lactate proportions by 37% above baseline levels and propionate falling 11% (left).

This illustrates CPC’s mode of action of killing and suppressing bacteria to achieve a less mature plaque. It also demonstrates this method is applicable to measure antibacterial activity by an active other than Stannous Fluoride.

CONCLUSIONS

CPC shows a linear dose effect with consistent and increasing lactate rises and propionate falls which are known to result from a shift away from pathogenic gram-negative anaerobe metabolism. The salivary analysis method presented (salivary metabolomics) shows it is sensitive to the difference of 0.07% and 0.035% strength CPC rinses vs placebo and all three can be differentiated; 0.01% CPC rinse cannot be differentiated from placebo.

It has now been presented that this method can measure treatment effects for Stannous Fluoride and CPC and that the effect being measured is indicative of plaque reductions, known to be an important mechanism in gum protection.


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