Assessment of Oral Bacteria Contributing to Blood Pressure Regulation via the Symbiotic Nitrate-Nitrite-Nitric Oxide Pathway

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Objectives: The objective of this study is to determine if select oral bacteria are able to produce nitric oxide (NO) or its derivatives. Previously, we demonstrated that members of the genera Neisseria, Actinomyces, Veillonella, Fusobacterium, and Prevotella have the potential to influence host blood pressure, and we hypothesized that production of NO underlies this physiologic observation. Here, we use gas-chromatography-mass spectroscopy (GC-MS) to detect NO in bacterial cultures, creating a screening assay for use with oral samples.

Experimental Methods: Bacterial strains used in this study are: *Neisseria oral taxon* 15, *N. flava*, *N. mucosa*, *V. dispar*, *A. oris*, *F. polymorphum*, and *P. nigrescens*. Bacteria were grown anaerobically in capped test tubes in SHI media supplemented with hemin, menadione, +/- nitrate (+/- N). Aliquots of headspace gas were injected into an HP 5973A mass spectrometer interfaced with an HP 6890 gas chromatograph. Peak areas were determined in auto-integration mode, and ChemStation was used to analyze the ion chromatogram.

Results: NO, a highly reactive ion, was not directly detected, however the reaction byproduct nitrogen dioxide was present in samples from *Neisseria oral taxon* 15 +N, *N. flava* +N, *V. dispar* +N, and *P. nigrescens +N.* Interestingly, *N. mucosa* produced agmatine and arecoline, which are known to regulate host production of NO, and also produced phenylephrine and metaraminol, known blood pressure regulators in humans.

Conclusions: We found GC-MS to be a feasible and sensitive method for detection of NO and its derivatives. Further, we discovered that other mechanisms of host blood pressure regulation may exist.

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