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Dietary nitrate improves endothelial function in ligature induced periodontitis in mice

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Introduction: Evidence supports an association between periodontal disease and cardiovascular disease (CVD) risk; the latter characterised by endothelial dysfunction. Recent evidence suggests that elevation of circulating nitrite levels, accomplished via dietary inorganic nitrate (NO_3^-) intake, may offer a mode of NO delivery that could restore endothelial function in CVD. Thus, we investigated the effect of dietary nitrate on a ligature-induced model of periodontitis associated with endothelial dysfunction.

Methods: Adult male C57BL/6J mice were fed potassium nitrate (KNO_3, 15mM in the drinking water) or equimolar KCL for 1 week prior to sham or ligature placement, as previously described[1]. At 2 weeks following ligature placement dental bone loss was determined by measurement of the distance from the mesial buccal cement-enamel junction to the alveolar bone crest[1]. Gingival mRNA expression using qRT-PCR, circulating inflammatory cell identification using flow cytometry and aortic ring reactivity using organ bath were determined. Data are given as mean±SEM and analysed using an unpaired student t-test or Two-Way ANOVA.

Results: Ligature placement resulted in alveolar bone loss (sham, 1.09±0.04mm, n=11; ligature, 2.23±0.05mm, n=12, P<0.0001) that was modestly attenuated by KNO_3 treatment (2.02±0.05mm, n=15, P<0.05) and associated with reduced gingival expression of IL-1β mRNA (KCl, 1±0.19, KNO_3, 0.66±0.17, n=5). KNO_3 treatment also reduced circulating neutrophil numbers (KCl, $3.85\pm0.64\times10^5$ cells/ml, n=15; KNO_3, $2.27\pm0.36\times10^5$ cells/ml, n=13, P<0.05), with no change in circulating monocytes or the cell adhesion molecules CD11b, CD62L or CD162 on either cell type. Whilst Spermine NoNoate and phenylephrine concentration curves were unaffected, acetylcholine-induced relaxation was reduced following ligature placement (Sham, max: 93.8±2.5%; ligature 74.7±5.1%, n=5, P<0.05) and restored by KNO_3 treatment (103.8±6.5%, n=5, P<0.001). In experiments where KNO_3 was given following induction of periodontal disease, whilst there was no improvement of bone loss, KNO_3 treatment did reverse the endothelial dysfunction (acetylcholine-induced relaxation, Sham, max: 92.4±2.33%; ligature 79.93±1.39%; ligature plus KNO_3 92.86±3.52%, n=5, P<0.01).

Conclusions: Dietary nitrate treatment resulted in a reduction in periodontal bone loss associated with a reduction in the local inflammatory response reflected by reduced IL-1β, a pro-inflammatory cytokine. This reduction in oral inflammation was associated with reductions in systemic inflammation particularly in terms of neutrophil numbers and protected against vascular dysfunction. We suggest that these findings highlight the potential of dietary nitrate therapy in prevention and treatment of periodontal disease.

References: 1. Toshiharu A and Hajishengallis G. (2013). *J Immunol Methods* **394**:49-54