Endotoxin Changes during Natural Periodontitis Progression in Dogs

R. Donovan-Brand*, A.G. Snider, K.K. Brown, J.L. Pratt, E. Housmann, W. Buchanan

Procter & Gamble Co., Mason, OH, USA; SUNY/Buffalo, New York, USA

775

ABSTRACT

Endotoxin from gram-negative bacteria increases with more severe periodontal disease and stimulates host inflammatory responses associated with tissue destruction. A method to measure endotoxin levels in gingival crevicular fluid (GCF) has been reported (*J Dent Res* 75: 1544, 1996). To refine models of periodontitis progression, a twelve month study followed changes in clinical measurements, digital subtraction radiography and GCF endotoxin levels, in 39 sites among 5 older beagle dogs with natural periodontitis. Five dogs with the highest mean GCF endotoxin levels (x=683 EU/µL) and multiple radiographic bone loss sites at screening exam were followed. Changes in GCF endotoxin, gingival index, inflammation index, pocket depth, attachment loss and digital subtraction radiography were measured at 3 month intervals for 12 months. Differences in measurements at sites over time were compared using the Paired t-test. Six weeks following the screening exam, at baseline, the mean GCF endotoxin levels were significantly lower (x=171 EU/µL, p=0.004). **No subgingival cleaning was performed; yet significant reductions in endotoxin were noted following probing measures and sampling.** By 12 months the mean GCF endotoxin values were significantly elevated over baseline (x=427 EU/µL, p=0.015) but were lower than screening levels. GCF endotoxin levels at 12 months were not significantly different from screening levels (p=0.066). Four of the 5 dogs lost attachment over time; the mean attachment loss from baseline to 12 months approached significance (0.44mm, p=0.068). Mean gingival (0.16, p=0.328) and inflammation (0.01, p=0.970) indices showed no significant changes. Pocket depth changes were also not significant (0.23mm, p=0.139). Analyses of subtraction radiographs indicated bone loss exceeded 1mm^2 at 6 of 39 sites (15%). **Selecting animals with higher endotoxin levels and multiple sites with bone loss identified animals with significant attachment loss and continuing bone loss. Repeated measuring and sampling of periodontal pockets can significantly reduce subgingival endotoxin.**

INTRODUCTION

New more sensitive measures of periodontitis progression could identify patients at high risk and should reduce size and duration of clinical studies. The progression of periodontal disease is associated with increasing numbers of gram-negative bacteria in dental plaque(2, 3). The majority of perio- pathogens are gram-negative bacteria, and as sites progress from health to disease, the proportion of plaque organisms which are gram-negative increases (4, 5). Bacterial endotoxin or lipopolysaccharide (LPS) is a component of cell surface membranes of gram-negative bacteria which is released when the organisms are lysed. LPS stimulates humoral and cellular host inflammation producing pyrogenic responses, complement activation, tissue necrosis and acts as an immunoadjuvant(6). Periodontal disease is the result of the interaction of plaque bacteria and the host inflammatory response.

Measures including attachment loss, gingival index, inflammation index, and pocket depth are currently used to measure progression of periodontitis (7). Dr. Ernest Hausmann has recently developed a dental X-ray positioning system which permits taking a series of dental radiographs with angular deviations between the tooth and X-ray reduced to less than 1 degree. This accuracy is needed to perform digital subtraction analyses of changes in alveolar bone. The EGAD system is an electronically guided, force-sensitive sensor-based alignment device which permits the most accurate measure of progression of periodontitis by digital subtraction radiography(8). To refine models of periodontitis progression, a twelve month study followed changes in clinical measurements, digital subtraction radiography and GCF endotoxin levels, in 39 sites in 5 older beagle dogs with natural periodontitis.

MATERIALS AND METHODS

Animals in study were older (>2 years old), male and spayed female beagle dogs. Fifteen dogs were screened to identify 5 dogs with untreated pre-existing periodontitis which were selected based on attachment loss, pocket depth and inflammation scores (GI, II). Overall status of periodontal bone loss at baseline was observed using lateral jaw X-rays. To qualify for the study dogs must have had at least 2 interproximal sites with untreated periodontitis and radiographic bone loss.

The 5 animals with the highest GCF endotoxin levels at disease sites were enrolled in this study. Following preliminary examination, dental impressions were made and used to fabricate radiographic stents for the study.

The study started with replicate baseline radiographic measures which were analyzed to confirm that films could be subtracted to show no bone change. Clinical measures and duplicate radiographs were repeated at study sites at 3 month intervals during the 12 month study. At baseline and 3 month intervals, samples of gingival crevicular fluid samples and a blood plasma sample were collected for analysis of endotoxin levels.

RESULTS

Figure 1: Shows changes in mean clinical measurements for all dogs from baseline to 12 months. Four of 5 dogs lost attachment and the mean attachment loss (0.44 mm) over 12 months approached significance (p=0.068). Pocket Depth measurements increased in 3 animals, but mean change (0.23 mm) was not significant (p=0.139). Gingival Index increased in 2 of 5 dogs, but mean change of 0.16 was not significant (p=0.328). Inflammation Index increased in only 1 dog and the mean change was 0.01 which was not significant (p=0.970).

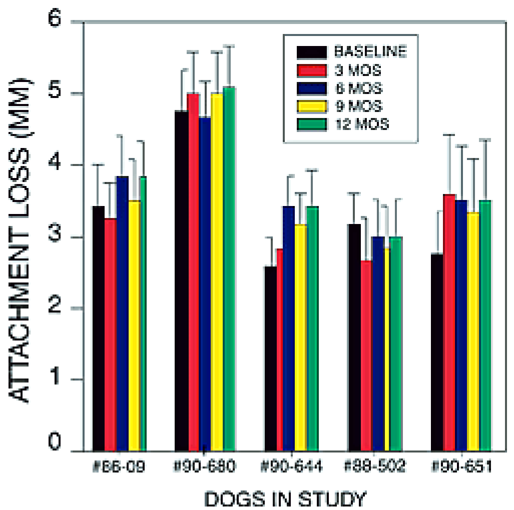
Research presented at the 75th General Session of the IADR; Orlando, Florida March 19-23, 1997

P&G

Endotoxin Changes during Natural Periodontitis Progression in Dogs

R. Donovan-Brand*, A.G. Snider, K.K. Brown, J.L. Pratt, E. Housmann, W. Buchanan
Procter & Gamble Co., Mason, OH, USA; SUNY/Buffalo, New York, USA

MEAN (± SEM) ATTACHMENT LOSS



MEAN (±SEM)INFLAMMATION INDEX

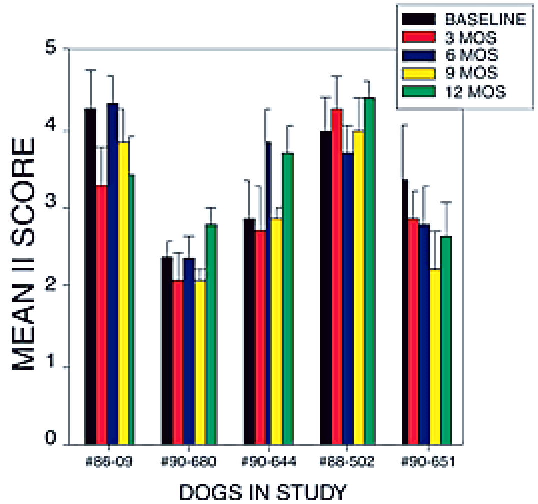
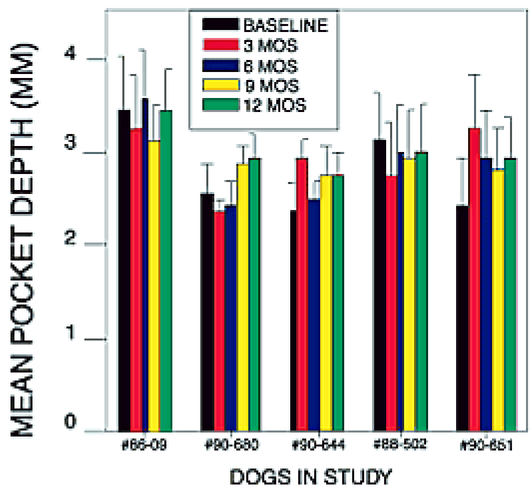


Figure 2: Shows the mean endotoxin levels for all dogs at all timepoints. At screening the 5 dogs with the highest mean GCF endotoxin levels (x=683 EU/μL) were selected. Six weeks later at baseline the mean endotoxin levels (x=171 EU/μL) were significantly (p=0.004) lower. By 12 months the mean endotoxin values (x=427 EU/μL) were significantly (p=0.015) elevated over baseline but did not return to those levels measured at screening. The difference between endotoxin screening values and 12 month values was not significant (p=0.066). Table 1 shows that changes in serum endotoxin levels paralleled changes in GCF endotoxin levels. Radiographs showed bone loss in 6 sites of 39 (15%) exceeding $1mm^2$; four sites in #86-09 lost bone.

MEAN (± SEM) POCKET DEPTHS



MEAN (± SEM) GINGIVAL INDEX SCORES

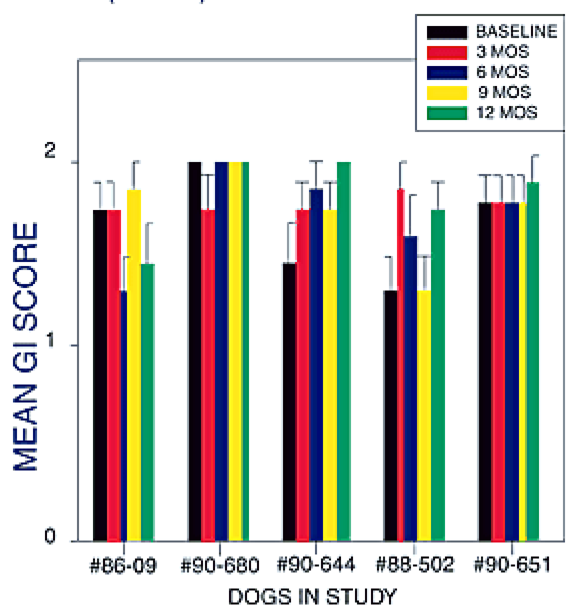
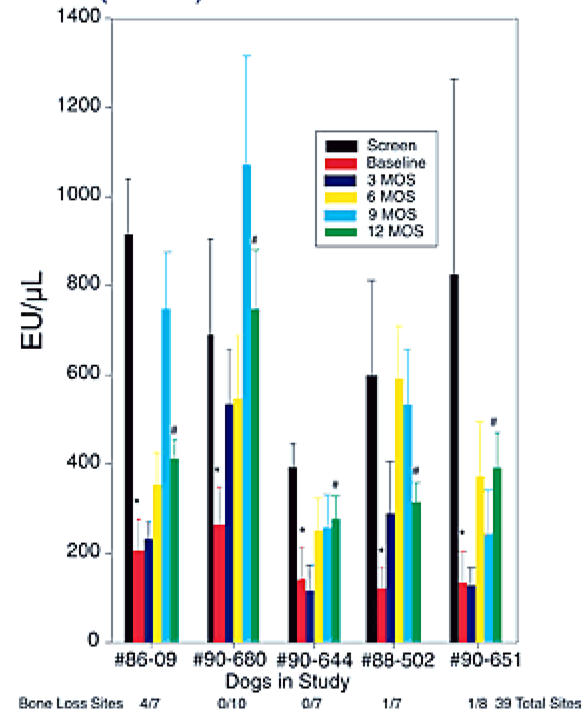


Figure 2.

MEAN (± SEM) GCF ENDOTOXIN LEVELS



P&G

Endotoxin Changes during Natural Periodontitis Progression in Dogs

R. Donovan-Brand*, A.G. Snider, K.K. Brown, J.L. Pratt, E. Housmann, W. Buchanan
Procter & Gamble Co., Mason, OH, USA; SUNY/Buffalo, New York, USA

Significantly below screen $p<0.05$
Significantly above baseline $p<0.05$

TABLE 1: SERUM ENDOTOXIN LEVELS EU/mL

	90-651	90-680	88-502	86-09	90-644	MEAN
BASELINE	0.892	0.908	0.693	0.979	0.849	0.86
3 MONTH	0.29	0.611	0.388	0.38	0.361	0.41
6 MONTH	0.502	0.350	0.383	0.229	0.315	0.36
9 MONTH	0.677	0.421	0.322	0.306	0.514	0.45
12 MONTH	0.879	0.777	0.711	0.857	0.681	0.78

CONCLUSION

-Selecting animals with higher endotoxin levels and multiple sites with bone loss identified dogs with continuing attachment and bone loss.

-No subgingival cleaning was performed; yet significant reductions of endotoxin were noted following probing measures and sampling.

-By 12 months the mean endotoxin value (x=427 EU/μL) was significantly (p=0.015) elevated over baseline but was lower than levels measured at screening. **Changes in serum endotoxin levels paralleled changes in GCF endotoxin.**

-Repeated measuring and sampling significantly reduced subgingival endotoxin. A published study shows that repeated clinical measurements significantly reduce attachment loss(9). **Results from this study suggest that this reduced attachment loss could be due to the disruption of the sensitive, anaerobic gram negative bacteria.**

REFERENCES

1. Donovan-Brand RJ, et al, *J Dent Res.* 1996; 75:Abst.#1544.
2. Loesche WJ, et al, *J Periodontol.* 1985; 56:447-456.
3. Slots J. *J Clin Periodontol.* 1979; 6:351-382.
4. Tanner ACR, et al, *J Clin Periodontol.*1979; 6:278-307.
5. Dzink JL, et al, *J Clin Periodontol.* 1988; 15:316-323.
6. Morrison DC. *Annu. Rev. Med.* 1987; 38:417-421.
7. Williams DM, et al, *Pathology of Periodontal Disease*,1992 Oxford University Press, New York, NY. pp.12-15.
8. Hausmann E, et al, *J of Periodontal Res.*1995, 30:294-297.
9. Lindhe J, et al, *J. Clin. Periodontol.* 1983, 10:433-442.

Research presented at the 75th General Session of the IADR; Orlando, Florida March 19-23, 1997

P&G