J Clin Periodontol 2005; 32: 784–788 doi: 10.1111/j.1600-051X.2005.00743.x

Journal of Clinical Periodontology

Reducing variability and choosing ideal subjects for experimental gingivitis studies

Barbara Shearer[1], Peter Hall[2], Peter Clarke[2], Graeme Marshall[1] and Denis F. Kinane[3]

[1]University of Glasgow Dental Hospital and School, Glasgow; [2]Unilever Research, Port Sunlight, Wirral, UK; [3]School of Dentistry, University of Louisville, Louisville, KY, USA

Shearer B, Hall P, Clarke P, Marshall G, Kinane DF. Reducing variability and choosing ideal subjects for experimental gingivitis studies. J Clin Peridontol 2005; 32: 784–788. doi: 10.1111/j.1600-051X.2005.00743.x. © Blackwell Munksgaard, 2005.

Abstract

Introduction: This study was undertaken to test the hypothesis that male, non-smokers, aged 25–50 years, with a proven track record of developing gingivitis would provide suitable subjects for experimental gingivitis studies because the inter-subject variation would be greatly reduced.

Materials and Methods: Subjects were required to be men aged between 25 and 50 years, in good general health with at least four sound teeth in one posterior quadrant of the mandible. Subjects who were smokers, taking antibiotics, anti-inflammatory drugs or had any other medical, surgical or social condition making participation in the study inadvisable, were excluded. Subjects had previously participated in a 21-day experimental gingivitis study. Subjects with the most gingivitis at the end of the previous study were invited to participate first, moving down the list until the required number of subjects had been achieved. Subjects were randomly assigned to one of three toothpastes: Crest Gum Care, Mentadent P or a placebo (UK Signal). Subjects were asked to brush their teeth twice daily for 1 min., with the tooth-shield in place and containing 1 ml of their assigned toothpaste.

Results: Thirty-five subjects completed the study using the placebo, 36 used Mentadent P and 39 completed the study using Crest Gum Care. Both toothpastes containing active gum health ingredients (Triclosan and Zinc Citrate in Mentadent P and Stannous Fluoride in Crest Gum Care) resulted in statistically significant reductions in gingival bleeding and inflammation. Although a directional reduction, there was no statistically significant reduction in plaque scores. No significant differences between the two active groups were established.

Conclusion: The factors that were controlled or measured and analyzed in these studies were: gender, smoking status, age and chronic gingivitis level. The results add support to the hypothesis that male subjects show less variation, however there is limited evidence linking the female menstrual cycles to variations in gingivitis status. Further research in this area is required before definitive conclusions can be made. Selecting a subject population in order to decrease variation must be undertaken with caution. A subject population selected for particular attributes can be extremely valuable when assessing the anti-gingivitis potential of new agents, but this population would be as biased if used for large efficacy clinical trials. The data obtained will not necessarily be able to be generalized, that is, applicable to the whole population. However there is sufficient evidence to justify balancing for (or excluding) smokers from subject populations in experimental gingivitis studies.

Keywords: experimental gingivitis; gender; gingivitis levels; plaque; smoking; subject variability

Accepted for publication 17 November 2004

The 21-day experimental gingivitis model has been used to test potential anti-gingivitis agents for efficacy over the last 15 years (Saxton & van der Ouderaa 1989). The value of such clinical models is dependent on their ability to be sensitive and reproducible. In the past the inclusion criteria for subjects participating in the 21-day experimental gingivitis model have tended to be inclusive rather than exclusive, however it is recognized that subject-related fac-

tors can influence experimental gingivitis and thus the variability and reproducibility of the model.

There is a wealth of evidence in the dental literature describing the detrimental effects of smoking on periodontal health (Albandar et al. 2000, Haffajee & Socransky 2001, Kinane 2001). Tomar and Asma (2000) analyzed data from NHANES III to determine relationships between tobacco use and periodontal health. Their results suggest that smoking may be responsible for more than half of the periodontitis cases amongst adults in the United States. Evidence suggests that smoking may mask the clinical signs of periodontal disease by reducing blood flow in the microvasculature of the gingivae and altering neutrophil function (Machuca et al. 2000, Obeid & Bercy 2000, Kinane 2001). In experimental gingivitis studies, the suppressed inflammatory response to plaque accumulation has been documented. In experimentally induced gingivitis of 14 days duration, while plaque scores remained similar between smokers and non-smokers, the bleeding scores were half that in the smokers compared with non-smokers even though no major differences in the oral microbiota could be determined (Lie et al. 1998b). This difference in gingival bleeding was further reported by Lie et al. (1998a) and shown to be independent of the bleeding index used. Newbrun (1996) and Lie et al. (1998a) both recommend that smoking should be controlled or smokers identified as a separate group in experimental gingivitis studies.

Hormonal fluctuations in females during pregnancy can lead to an exaggerated response to dental plaque (Raber-Durlacher et al. 1994, Machuca et al. 1999). It is possible that more subtle responses may also be present during the hormonal fluctuations of the female menstrual cycle, and that these effects could introduce confounding background noise into the 21-day experimental gingivitis model. Kovar et al. (1985) showed that decreased levels of estradiol-17β during menstruation were associated with a statistically significant increase in gingival index.

The experimental gingivitis model protocol requires subjects to reach clinical health before beginning the 21-day non-brushing period, however the tendency of the subject to develop gingivitis may also have an impact on gingivitis outcome variables. McClanahan and Bartizek (2002) reported that subjects with higher baseline gingival bleeding scores were more likely to show reductions in gingival bleeding and gingival severity index using triclosan/copolymer in a 3-month clinical trial.

This study was undertaken to test the hypothesis that male, non-smokers, aged 25–50 years, with a proven track record of developing gingivitis would provide suitable subjects for experimental gingivitis studies.

Methods

The Local Ethics Committee granted approval prior to the start of this double-blind parallel clinical study. The study was undertaken according to the methodology described by Saxton and van der Ouderaa (1989). Volunteers who wished to participate gave their informed consent. Subjects were required to be men aged between 25 and 50 years, in good general health with at least four sound teeth in one posterior quadrant of the mandible. Subjects who were smokers, taking antibiotics, anti-inflammatory drugs or had any other medical, surgical or social condition making participation in the study inadvisable, were excluded. Subjects had previously participated in a 21-day experimental gingivitis study. Subjects with the most gingivitis at the end of the previous study were invited to participate first, moving down the list until the required number of subjects had been achieved.

Those subjects accepted onto the study entered the pre-trial phase. The aim of this pre-trial period, of 2–5 weeks duration, was to achieve clinically healthy gingivae. They received scaling and polishing of their teeth and oral hygiene instruction. An impression of the lower jaw was also taken and used to construct a tooth-shield for the experimental period.

At the end of the pre-trial period, the visual symptoms of inflammation and bleeding of the gingivae, as well as plaque, around the subject's five experimental teeth were recorded by the clinical assessor. The visual signs of gingival inflammation were recorded using the modified gingival index (MGI) (Lobene et al. 1986). The MGI is a non-invasive index where the papillary and marginal gingivae of each tooth are assessed on a numerical scale. A score of 0 represents gingival health (absence of inflammation) and a score of 3 indicates a redness and swelling of the entire marginal or papillary unit. Gingival bleeding was assessed using the Gingival Bleeding Index described by Saxton and van der Ouderaa (1989). A CPITN periodontal probe is inserted into the gingival crevice, moved around the tooth at an angle of 60° gently stretching the sulcular epithelium. Gingival bleeding is assessed 30 s after probing; a score of 0 represents an absence of bleeding, a score of 1 is given if bleeding is observed within 30 s after probing and a score of 2 is given if bleeding is observed immediately on probing. Plaque was scored according to Loe (1967) which is weighted in favour of plaque at the gingival margin of the tooth. The index has a four point (0–3) ordinal scale in which the higher value denotes a quantitative increase in plaque.

Any subject with $>25\%$ of sites bleeding (using the Gingival Bleeding index) was withdrawn from the study. That is for a subject with five experimental teeth, there would be a total of 20 sites assessed. If >5 of those sites bled, the subject would be withdrawn. For a subject with only four experimental teeth and a total of 16 sites available for assessment, the subject would be withdrawn if >4 sites bled.

Subjects were randomly assigned to one of three toothpastes: Crest Gum Care, Mentadent P or a placebo (UK Signal). Subjects were asked to brush their teeth twice daily for 1 min., with the tooth-shield in place and containing 1 ml of their assigned toothpaste. The use of other toothpastes, floss or mouthwashes was not permitted. The toothpastes were packed in identical white laminate tubes and labelled with product codes to ensure blindness of the subjects, assessor and study staff.

At the end of the experimental period (21 days), the visual symptoms of inflammation and bleeding of the gingivae as well as plaque levels around the subject's five experimental teeth were again recorded by the clinical assessor. Subjects returned to their normal oral hygiene practices and no further observations were made.

Results

The study population comprised 121 male subjects and was undertaken within the Glasgow Dental Hospital and School. One hundred and ten subjects

completed all phases. Withdrawals were for reasons unrelated to the study. Three adverse events were recorded during the pre-experimental phase, which were unrelated to the test products. Thirty-five subjects completed the study using the placebo, 36 used Mentadent P and 39 completed the study using Crest Gum Care.

The primary outcome was gingivitis measured using two clinical indices (the MGI and Bleeding Index; Lobene et al. 1986, Saxton & van der Ouderaa 1989) as recommended by the American Dental Association (Council on Dental Therapeutics 1986). Analysis of covariance with the baseline data as the covariate was used to assess statistical significance of any difference between the test and placebo product groups. The data was processed using the GINSYS procedure created using Statistics modules of the SAS software system. Only data from those subjects completing all assessments was used in the analyses. Plaque was the secondary outcome variable. The mean scores were compared between groups at the final assessment.

Both toothpastes containing active gum health ingredients (Triclosan and Zinc Citrate in Mentadent P and Stannous Fluoride in Crest Gum Care) resulted in statistically significant reductions in gingival bleeding and inflammation. Although a directional reduction, there was no statistically significant reduction in plaque scores. No significant differences between the two active groups were established (Tables 1 and 2).

Analysis of Subject Variability

Further analyses were undertaken to examine the consistency of plaque, bleeding and gingivitis scores within individuals, between the three indices and over the course of this study and a previous experimental gingivitis study in which they had participated. It is possible that those subjects recruited with high levels of gingivitis have an oral environment pre-disposed to gingivitis and go on to develop high levels of acute gingivitis under the tooth-shield (if they used the placebo product). To test this hypothesis the plaque, bleeding and gingivitis scores measured at enrolment (exam 1) were compared with the scores measured after 21 days of no oral hygiene (exam 3). For each index of interest, each subject was placed in a quartile depending on how his score compared with the rest. Agreement between quartile scores was tabulated and measured with the κ statistic (Table 3).

Overall the level of agreement was only slightly greater than would be expected by chance for plaque and MGI indices, and virtually the same as would be expected by chance for Gingival Bleeding.

The effect of age on the development of gingivitis was examined using the study population. Table 4 below shows the gradient of the best fit of gingival bleeding and MGI score *versus* age at enrolment (exam 1). There is little evidence for an effect of age in this case. Although MGI scores at exam 1 suggest some link, only 2% of the variance in MGI can be explained by age.

The extent to which subjects maintain a consistently high increment in index (change from baseline to final examination) from one study to the next was examined. Thirty-four subjects had participated in two studies and used the same treatment (UK Signal). Similar analyses of the consistency of increments can be performed as above by placing each subject in quartiles and tracking the extent to which quartile score is preserved.

Table 5 shows the κ values for the incremental scores (Exam 3–Exam 1) for this study compared with the incremental scores recorded on a previous study using the same index. The results indicate low consistency. In all three cases, the 95% confidence intervals for κ encompass zero, therefore it is likely that there is no real agreement between the quartile scores.

Table 1. Difference (%) in incremental gingivitis and plaque scores between the two gum health toothpastes (Crest Gum Care and Mentadent P) and the placebo (UK Signal)

Paste	Mean change in MGI from placebo	Mean change in plaque from placebo	Mean change in Bleeding from placebo
Placebo (UK Signal)	–	–	–
Mentadent P (%)	24	4	41
Crest Gum Care (%)	36	8	36

MGI, Modified gingival index.

Table 3. Weighted κ statistics for comparison between exam 1 and exam 3 plaque, gingival bleeding and MGI scores

Index	Weighted κ
Plaque	0.204 ± 0.059
Gingival bleeding	0.090 ± 0.053
MGI	0.254 ± 0.054

MGI, Modified gingival index.

Table 2. Statistical assessment of the differences between the three product groups following analysis of covariance of the 21-day assessment for the gingival indices with baseline as the covariate and analysis of variance for the plaque

	Product Group (no. of subjects)	21-day assessment		Statistical probability	
		observed mean (SD)	adjusted mean (SD)	overall	product
MGI	Placebo (35)	1.425 (0.0749)	1.414 (0.0792)		
	Mentadent P (36)	1.070 (0.0871)	1.0790 (0.0779)	$p < 0.0001$	Placebo > Mentadent P $p < 0.01$
	Crest Gum Care (39)	0.905 (0.0695)	0.907 (0.0746)		Placebo > Crest Gum Care $p < 0.0003$
Plaque	Placebo (35)	1.127 (0.0399)	1.127 (0.0294)		
	Mentadent P (36)	1.077 (0.0270)	1.077 (0.0289)	$p < 0.0903$	
	Crest Gum Care (39)	1.038 (0.0166)	1.038 (0.0278)		
Bleeding	Placebo (35)	0.511 (0.0276)	0.508 (0.0323)		
	Mentadent P (36)	0.295 (0.0313)	0.298 (0.0318)	$p < 0.0001$	Placebo > Mentadent P $p < 0.0003$
	Crest Gum Care (39)	0.326 (0.0358)	0.326 (0.0306)		Placebo > Crest Gum Care $p < 0.0003$

MGI, Modified gingival index.

Table 4. Best fit gradients for bleeding and MGI scores *versus* age

	Gradient	p (gradient = 0)
Exam 1		
Plaque	0.003 ± 0.003	0.6
Bleeding	-0.003 ± 0.02	0.2
MGI	0.011 ± 0.006	0.06

MGI, Modified gingival index.

Table 5. Weighted κ statistics for comparison between plaque, gingival bleeding and MGI incremental scores between two studies

Index	Weighted κ
Plaque	0.103 ± 0.132 (95% confidence limits = -0.157 to 0.362)
Gingival bleeding	0.053 ± 0.122 (95% confidence limits = -0.186 to 0.292)
MGI	0.102 ± 0.124 (95% confidence limits = -0.142 to 0.345)

MGI, Modified gingival index.

Discussion

The results of this study provide further evidence that the experimental gingivitis model originally described by Loe et al. (1965), and modified by Saxton and van der Ouderaa (1989), has the ability to discriminate between toothpastes containing active anti-gingivitis agents (stannous in Crest Gum Care and zinc citrate–Triclosan in Mentadent P).

The results lend support to the hypothesis that decreasing the variation between subjects in terms of gender and smoking status reduces standard errors and results in increased statistical confidence in the results obtained. The reductions in gingival bleeding and gingivitis obtained for Crest Gum Care and Mentadent P are within the range of values seen in previous studies, however the standard errors and p-values are low.

The factors that were controlled or measured and analyzed in these studies were: gender, smoking status, age and chronic gingivitis level. The results add support to the hypothesis that choosing a single gender for study, such as recruiting only male subjects, reduces variation, however there is limited evidence linking the female menstrual cycles to variations in gingivitis status (Kovar et al. 1985). Further research in this area is required before definitive conclusions can be made.

Analyses showed that the links between chronic gingivitis and the gingivitis that developed acutely under the tooth-shield, gingivitis development in previous experimental gingivitis studies and age, could only account for a small amount of the variance.

A similar conclusion was reached by McClanahan and Bartizek (2002) in a 3-month gingivitis clinical study. They examined the relationship between the baseline gingival bleeding sites and the 3-month gingival bleeding sites for the study population. Subjects with at least 40 gingival bleeding sites at baseline demonstrated 4.2% gingival index and 15% gingivitis severity index reduction when using triclosan/copolymer compared with placebo at 3 months. Although the authors conclude that subjects with greater numbers of gingival bleeding sites at baseline may have the required sensitivity to demonstrate treatment benefits for triclosan/copolymer, they consider that additional experimental parameters remain to be fully articulated in order to replicate previously successful trials.

Lie et al. (1995) examined the composition of the oral microbiota and clinical indices (plaque, bleeding, pocket depth and loss of attachment) in subjects who had previously demonstrated either a weak or a strong response to experimental gingivitis. Samples were analyzed for the presence of nine different periodontal species and no differences between the two groups were found either clinically or microbiologically. Understanding the factors involved and their relative importance in decreasing the variation between subjects is critical to the future design of short- and long-term gingivitis clinical trials.

Selecting a subject population in order to decrease variation must be undertaken with caution. A subject population selected for particular attributes can be extremely valuable when assessing the anti-gingivitis potential of new agents, but this population would be as biased if used for large efficacy clinical trials. The data obtained from a selected population is by definition not generalizable, that is, applicable to the whole population. However there is sufficient evidence to justify balancing for (or excluding) smokers from subject populations in experimental gingivitis studies.

References

Albandar, J. M., Streckfus, C. F., Adesanya, M. R. & Winn, D. M. (2000) Cigar, pipe, and cigarette smoking as risk factors for periodontal disease and tooth loss. *Journal of Periodontology* **71**, 1874–1881.

Haffajee, A. D. & Socransky, S. S. (2001) Relationship of cigarette smoking to attachment level profiles. *Journal of Clinical Periodontology* **28**, 283–295.

Kinane, D. F. (2001) Causation and pathogenesis of periodontal disease. *Periodontology 2000* **25**, 8–20.

Kovar, M., Jany, Z. & Erdelsky, I. (1985) Influence of the menstrual cycle on the gingival microcirculation. *Czechoslovak Medicine* **8**, 98–103.

Lie, M. A., Danser, M. M., van der Weijden, G. A., Timmerman, M. F., de Graaff, J. & van der Velden, U. (1995) Oral microbiota in subjects with a weak or strong response in experimental gingivitis. *Journal of Clinical Periodontology* **22**, 642–647.

Lie, M. A., Timmerman, M. F., van der Velden, U. & van der Weijden, G. A. (1998a) Evaluation of 2 methods to assess gingival bleeding in smokers and non-smokers in natural and experimental gingivitis. *Journal of Clinical Periodontology* **25**, 695–700.

Lie, M. A., van der Weijden, G. A., Timmerman, M. F., Loos, B. G., van Steenbergen, T. J. & van der Velden, U. (1998b) Oral microbiota in smokers and non-smokers in natural and experimentally-induced gingivitis. *Journal of Clinical Periodontology* **25**, 677–686.

Lobene, R. R., Weatherford, T., Ross, N. M., Lamm, R. A. & Menaker, L. (1986) A modified gingival index for use in clinical trials. *Clinical and Preventive Dentistry* **8**, 3–6.

Loe, H. (1967) The Gingival Index, the Plaque Index and the Retention Index Systems. *Journal of Periodontology* **38** (Suppl), 610–616.

Loe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 177–187.

Machuca, G., Khoshfeiz, O., Lacalle, J. R., Machuca, C. & Bullon, P. (1999) The influence of general health and socio-cultural variables on the periodontal condition of pregnant women. *Journal of Periodontology* **70**, 779–785.

Machuca, G., Rosales, I., Lacalle, J. R., Machuca, C. & Bullon, P. (2000) Effect of cigarette smoking on periodontal status of healthy young adults. *Journal of Periodontology* **71**, 73–78.

McClanahan, S. F. & Bartizek, R. D. (2002) Effects of triclosan/copolymer dentifrice on

dental plaque and gingivitis in a 3-month randomized controlled clinical trial: influence of baseline gingivitis on observed efficacy. *Journal of Clinical Dentistry* **13**, 167–178.

Newbrun, E. (1996) Indices to measure gingival bleeding. *Journal of Periodontology* **67**, 555–561.

Obeid, P. & Bercy, P. (2000) Effects of smoking on periodontal health: a review. *Advances in Therapy* **17**, 230–237.

Raber-Durlacher, J. E., van Steenbergen, T. J., Van der Velden, U., de Graaff, J. & Abraham-Inpijn, L. (1994) Experimental gingivitis during pregnancy and post-partum: clinical, endocrinological, and microbiological aspects. *Journal of Clinical Periodontology* **21**, 549–558.

Saxton, C. A. & van der Ouderaa, F. J. (1989) The effect of a dentifrice containing zinc citrate and Triclosan on developing gingivitis. *Journal of Periodontal Research* **24**, 75–80.

Tomar, S. L. & Asma, S. (2000) Smoking-attributable periodontitis in the United States: findings from NHANES III. National Health and Nutrition Examination Survey. *Journal of Periodontology* **71**, 743–751.

Address:
Denis F. Kinane
School of Dentistry
University of Louisville
Louisville, KY 40202, USA
E-mail: dfkina01@louisville.edu