



Periodontal conditions in relation to
low-dose aspirin therapy
in ex- and non-smokers

by

Arthur Drouganis BDS, Grad Cert Dent

A thesis submitted for the degree of
Master of Dental Surgery
(Periodontics)

The University of Adelaide
Dental School
November 1999

Dedication

This thesis is dedicated to my loving wife Helen, and my children Vicky, Lambros and Margaret whose support, enthusiasm and tolerance enabled me to complete the work.

TABLE OF CONTENTS

Acknowledgments	viii
Glossary of terms	x
Summary	xi
Chapter 1 Introduction	1
Chapter 2 Review of the literature	5
2.0 SUMMARY OF THE PRESENT UNDERSTANDING OF THE INFLAMMATORY RESPONSE. .	5
2.1 ENDOGENOUS MEDIATORS OF INFLAMMATION	8
2.1.1 Histamine:.....	8
2.1.2 Bradykinin:.....	9
2.1.3 Plasmin:.....	9
2.1.4 Complement:	10
2.1.5 Platelets:.....	11
2.2 EICOSANOIDS	12
2.2.1 General properties of eicosanoids.....	13
2.3 ROLE OF EICOSANOIDS IN PERIODONTAL TISSUES	15
2.3.1 Biosynthesis of eicosanoids.	16
2.3.2 Arachidonic acid pathways: eicosanoid production.....	17
2.3.3 Catabolism of the eicosanoids.....	24
2.4 THE ROLE OF CYTOKINES IN PERIODONTAL TISSUES	25
2.5 CELLULAR EVENTS IN INFLAMMATION	31
2.5.1 Macrophage phenotypes.....	32
2.5.2 Alveolar bone resorption.....	33
2.6 NONSTEROIDAL ANTI-INFLAMMATORY DRUGS IN PERIODONTAL DISEASES	34
2.6.1 History of salicylates.....	34
2.6.2 Physio-chemical properties of aspirin and other salicylates	35
2.6.3 Periodontal studies of the effects of NSAIDs over the last 20 years.....	39
2.7 PERIODONTAL STUDIES WITH ASPIRIN	52
2.7.1 The Waite study:.....	52
2.7.2 The Feldman study.....	53
2.7.3 The Flemmig study	54
2.7.4 The Heasman study	55
2.8 SMOKING AND PERIODONTAL DISEASES	56
2.8.1 The periodontal effects of past smoking and smoking dose	57

2.9 PERIODONTAL MEASURES	58
2.9.1 The experimental unit.....	59
2.9.2 Measurement of extent and severity of periodontal attachment loss.....	59
2.10 NULL HYPOTHESES	61
Chapter 3 Materials and methods	62
3.1. SAMPLE SELECTION	62
3.2 QUESTIONNAIRE	64
3.3 ORAL EXAMINATION	66
3.4 CLINICAL MEASUREMENTS	66
3.4.1 Plaque Index.....	66
3.4.2 Calculus.....	67
3.4.3 Bleeding index.....	67
3.4.3 Tooth mobility.....	68
3.4.4 Furcation involvement.....	69
3.5 DETAILS OF THE STUDY	69
3.5.1 Periodontal attachment loss (PAL).....	69
3.5.2 Periodontal Pocket Depths (PPD).....	70
3.5.3 Gingival Recession (GR).....	70
3.5.4 Examiner standardisation:.....	70
3.5.5 Procedure.....	70
3.6 STATISTICAL METHODOLOGY	71
Chapter 4 Results	72
4.1 INTRA-EXAMINER ERROR	72
4.2 PROFILE OF STUDY POPULATION:	72
4.3 DEMOGRAPHICS	73
4.3.1 Age categories of subjects.....	73
4.3.2 Education status of the subjects.....	74
4.3.2 Oral health behaviour.....	75
4.4 TOOTH LOSS	76
4.5 THE PERIODONTAL STATUS OF THE STUDY POPULATION	76
4.6 ASSOCIATIONS OF ASPIRIN AND EX-SMOKING WITH VARIOUS MEASURES OF PAL ...	78
4.6.1 The associations of aspirin and ex-smoking with mean PAL.....	78
4.6.2 The associations of aspirin and ex-smoking on the extent and severity of PAL.....	79
4.6.3 Associations of aspirin and ex-smoking with the most severe site of PAL (MSS-PAL).....	81
4.6.4 Associations of aspirin and ex-smoking with the extreme worst site of PAL (EWS-PAL).....	82

4.7	THE ASSOCIATIONS OF VARIOUS CLINICAL PARAMETERS ON MEAN PAL.	84
4.7.1	Site and tooth variations in recession and pocket depth by mean PAL	85
4.7.2	Socio-economic factors and periodontal attachment	86
Chapter 5	Discussion	115
5.1	PROFILE OF THE STUDY POPULATION	115
5.1.1	Age groupings	117
5.2	QUESTIONNAIRE	119
5.2.1	Socio-economic status	120
5.3	PERIODONTAL ATTACHMENT LOSS	121
5.3.1	Age associations with PAL	121
5.4	MEASURING PAL.....	122
5.4.1	Case definitions	122
5.5	OUTCOMES OF ASPIRIN AND PAST SMOKING ON PAL.....	125
5.5.1	Mean PAL.....	125
5.5.2	MSS-PAL.....	127
5.5.3	EWS-PAL.....	127
5.5.4	Plaque.....	128
5.5.5	Gingival bleeding	129
5.6	COMPARISONS WITH OTHER ASPIRIN STUDIES.....	130
5.7	SMOKING AND PAL	133
5.8	PREVALENCE OF PERIODONTAL ATTACHMENT LOSS.	133
5.9	FUTURE RECOMMENDATIONS.....	134
Conclusions	135
Appendix A	137
Appendix B	138
Appendix C	139
Appendix D	140
References	144

TABLES

Table 2.1	Interactions of plaque bacteria and their products in inflammation and immunity.....	7
Table 2.2	Composition of eicosanoids.....	12
Table 2.3	Cell sources and actions of prostanoids.....	15
Table 2.4	Major tissue destructive mediators in periodontitis.....	26
Table 2.5	Neutrophil components and function	31
Table 2.6	The types of NSAIDs (and their classification) used in periodontal studies	39
Table 2.7	Periodontal effects of NSAIDs in human studies.....	40
Table 3.1	Inclusion and exclusion criteria.....	63
Table 3.2	Aims of questionnaire.....	65
Table 3.3	Plaque index	67
Table 3.4	Modified Sulcus Bleeding Index (mSBI).....	67
Table 3.5	Tooth mobility index	68
Table 3.6	Furcation index.....	69
Table 4.1	Intra examiner reliability test using kappa statistics.....	87
Table 4.2	The number and percentage distribution of subjects participating in the study by group.....	87
Table 4.3	Distribution of age by group.....	87
Table 4.4	A Scheffés analysis of homogeneity between two groups at a time for mean age differences.....	88
Table 4.5	Scheffés ^a analysis for homogeneity between subsets	88
Table 4.6	Demographics on pension status with group specific characteristics.....	89
Table 4.7	Pension status in relation to denture use.....	89
Table 4.8	Demographic data on schooling of all subjects with group specific characteristics.	89
Table 4.9	A self-evaluation of English language skill.....	90
Table 4.10	Socio-economic factors and dental behaviours.	90
Table 4.11	Population and percentage distribution of subjects since their last dental visit. The time range was from less than one year to never visiting the dentist.....	91
Table 4.12	Missing teeth by age and group.....	92
Table 4.13	Missing teeth and smoking history	92
Table 4.14	The mean plaque index per group.	92
Table 4.15a	Profile of aspirin use and subject numbers.....	93
Table 4.15b	The association of age and past smoking on mean plaque scores with tests of significance.....	93

Table 4.16	Distribution of mean percentage of teeth with calculus by age, aspirin and ex-smoking.	94
Table 4.17	The association of age with mean percentage of calculus between groups..	95
Table 4.18	The association of low-dose aspirin and ex-smoking with the mean percentages of mobile teeth.	95
Table 4.19	The correlation of low-dose aspirin and past smoking with mean PAL.	96
Table 4.20	The association of aspirin dosage with mean PAL.....	97
Table 4.21	The association of aspirin duration with mean PAL.	97
Table 4.22a	The association of past smoking dosage and duration with mean PAL.	97
Table 4.22b	The correlation of the number of cigarettes smoked and duration of smoking with mean PAL with t-test of significance.	98
Table 4.23	Univariate analysis of variance in mean PAL at ≥ 2 , ≥ 4 , ≥ 5 and ≥ 7 mm.....	99
Table 4.24	Univariate analysis of variance on mean % PAL at ≥ 2 , ≥ 4 , ≥ 5 & ≥ 7 mm.	100
Table 4.25	The magnitude of the association of aspirin and smoking history with severity and extent of PAL at ≥ 2 , ≥ 4 , ≥ 5 & ≥ 7 mm PAL using the general linear model (2-way ANOVA) of analysis.....	101
Table 4.26	The correlation of aspirin and past smoking history with MSS-PAL.	102
Table 4.27	The age class distribution of males 50+ years in metropolitan Adelaide in 1996 from census statistics and their appropriate frequency distribution. .	103
Table 4.28	The proportional weights given to each group using the percentage frequency of each class interval from census statistics for metropolitan Adelaide.....	103
Table 4.29	Descriptive statistics of EWS-PAL	103
Table 4.30	The association of aspirin and past smoking history with EWS-PAL using weighted data.....	104
Table 4.31	The ratio of aspirin to smoking on various measurements of PAL.....	105
Table 4.32	Associations of plaque and age with mean PAL with tests of significance.	105
Table 4.33	Associations of calculus and age with mean PAL with tests of significance.	105
Table 4.34	Associations of gingival bleeding and age with mean PAL with tests of significance.....	106
Table 4.35	Socio-economic factors, oral hygiene patterns and mean PAL (mm).....	106
Table 4.36	The statistical power values for most ANOVA analyses	107
Table 4.37	Relative percentage of subjects with medical conditions per group	107
Table 4.38	Outcome of age, ex-smoking and aspirin with various indices of PAL	107

Figures

Figure 2.1	Products and pathways of cyclo-oxygenase	14
Figure 2.2	The chemical structures of PGE ₂ and TxB ₂	20
Figure 2.3	Structure of aspirin	36
Figure 2.4	Effects of aspirin on cyclo-oxygenases	38
Figure 3.1	A copy of an advertisement placed in local press media to recruit subjects	62
Figure 4.1	The mean percentage of sites with gingival bleeding (modified bleeding index).....	108
Figure 4.2	The mean percentage of teeth with calculus.....	108
Figure 4.3	Cumulative distribution of MSS-PAL representing the worst score (site) per mouth per subject, averaged over all subjects.	109
Figure 4.4	Diagrammatic representation of PAL according to smoking and aspirin taking history, showing mean PAL, MSS-PAL and EWS-PAL.	110
Figure 4.5	Cumulative distribution of EWS-PAL. Data were weighted using age class statistics for metropolitan Adelaide population.....	111
Figure 4.6	Variations of recession and pocket depths by tooth- and jaw type for the whole study population.....	112
Figure 4.7	Variation of recession and pocket depths by tooth- and jaw type in the AXS group.....	112
Figure 4.8	Variation of recession and pocket depths by tooth- and jaw type in the NAXS group.....	113
Figure 4.9	Variation of recession and pocket depths by tooth- and jaw type in the ANS group.....	113
Figure 4.10	Variation of recession and pocket depths by tooth- and jaw type in the NANS group.....	114

Signed Statement

This research report is submitted in partial fulfillment of the requirements of the Degree of Master of Dental Surgery (Periodontics) in the University of Adelaide.

The thesis contains no material which has been accepted for the award of any other degree or diploma in any University and that, to the best of my knowledge and belief, the thesis contains no other material previously published or written by another person, except where due reference is made in the text of the thesis.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for photocopying and loan if accepted for the award of the degree.

Arthur Drouganis.

November 1999

Acknowledgments

I wish to take this opportunity to thank those people who have assisted me in completing my candidature. I am particularly grateful to many people but utmost to my wife, and family for their patience and understanding throughout this challenging course.

I am truly indebted to two individuals. Robert Hirsch my supervisor, a true researcher, for his kindness, knowledge and in particular his insight and wisdom who lent me unconditional support, tempered at times, by considerable forbearance. To Bryon Kardachi, for his clinical knowledge, expert guidance and for his enthusiasm. The knowledge I have gained from both of them is, and will be invaluable.

My thanks go to the Colgate Australian Clinical Dental Research Centre for the use of its state-of-the-art facilities and I am especially grateful to Kerrie Ryan and Jane Burns who gave excellent support and assistance. To Colgate Australia for their generosity in supplying the Oral Care Kits which were given to each participant in the study. A special thank you to Professor Felix Bochner, Department of Clinical and Experimental Pharmacology, Division of Health Sciences University of Adelaide for his initial guidance.

I am deeply grateful to Knute Carter for his meticulous statistical analyses of the data. To Jane Carter for her enthusiasm and ideas on the study

These people have inspired and encouraged me to ask questions, to learn to reason and think independently. I truly believe I have been educated.

Thank you.

" Do not be rash to make friends; but, when once they are made, do not drop them"

DIOGENES (412-332 B.C.)

A Greek philosopher

I can quite honestly say that I have made life time friends.

Glossary of terms

ANS	Aspirin Never Smoked group
AXS	Aspirin eX-Smoker group
COX	Cyclo-oxygenase, an enzyme that produces the prostanoid and thromboxane mediators of inflammation
Cytokines	Polypeptide mediators released by cells involved in inflammation healing and homeostasis
EWS-PAL	The extreme worst site of PAL per subject, then averaged across each group
Extent	The proportion of tooth sites of an individual with PAL exceeding 1mm and often measured at various threshold values
GCF	Gingival Crevicular Fluid
IgG	Immunoglobulin-G
IL-1	Interleukin-1 an inflammatory cytokine involved in inflammation, immunity, tissue breakdown and homeostasis
IL-6	Interleukin-6 an inflammatory cytokine involved in inflammation, immunity, tissue breakdown and homeostasis
Low-dose aspirin	≤300mg per day
LPS	Lipopolysaccharide
Mean PAL	The average PAL of all sites per subject, then averaged across each group
MSS-PAL	The most severe site of PAL per tooth per person then averaged across each group
NANS	No Aspirin Never smoked group
NAXS	No Aspirin eX-Smoker group
NSAIDs	Non-steroidal anti-inflammatory drugs
PAL	Periodontal attachment loss
PGE ₂	Prostaglandin-E2. A primary cyclo-oxygenase mediator of inflammation
Prevalence	The proportion of group who have PAL (ie cases)
Severity	The degree of PAL averaged per affected tooth sites
TNF- α	A proinflammatory cytokine with synergistic effects with other cytokines

Summary

In the 1970's, Vane proposed that the anti-inflammatory effects of aspirin and aspirin-like drugs (non-steroidal anti-inflammatory drugs, NSAIDs) were due to inhibition of the enzyme cyclo-oxygenase, which stops the production of prostanoids (prostaglandins and thromboxanes). By the early 1980's, high doses of aspirin and other NSAIDs were shown to significantly reduce gingivitis, periodontal attachment loss and alveolar bone loss in humans. However, long-term use of these agents in periodontal therapy was not advocated, due to their side effects and the inconsistent findings between studies. Often test and control groups were not from the same sample population, results were based on concurrent use of other NSAIDs, dosages and duration varied between groups, and there was no control for smoking effects. Research in the 1990's showed that periodontitis is a multifactorial disease, being dependent on genetic and environmental influences, which modify the host response to the microbial challenge. One of the primary environmental risk factors for periodontitis is cigarette smoking. Ex-smokers lie between non-smokers and current smokers with regard to the severity and extent of periodontal attachment loss and alveolar bone loss; people who quit smoking respond to periodontal therapy similarly to non-smokers.

There is no information in the literature about the periodontal effects of low-dose aspirin on the periodontium in either non-smokers or ex-smokers. The aim of this study was to assess the periodontal status of a self-selected sample of men (aged 50 and above), residing in metropolitan Adelaide, South Australia, with respect to aspirin intake and smoking history. Subjects were targeted by advertisements placed in the local press.

Demographic data were collected from information obtained from a self-administered questionnaire and periodontal health was assessed by a periodontal examination carried out by one operator, blind to each subject's aspirin and smoking history. Measurements of pocket depths and gingival recession were made at six sites of all teeth present and were used to

compute periodontal attachment loss (PAL) for all subjects. Other parameters recorded were plaque and calculus accumulation, gingival and bleeding indices and tooth mobility.

Periodontal assessments were carried out in 392 men, aged 50-85 years. Significant age effects were found on PAL but these were of small magnitude in comparison to the significant influences that aspirin and ex-smoking had on PAL. The subjects were divided amongst four sub-groups:

- aspirin never smoked (ANS),
- aspirin ex-smokers (AXS).
- no aspirin never smoked (NANS)
- no aspirin ex-smokers (NAXS).

The extent and severity of PAL was evaluated against a background of age, ethnicity, socio-economic and dentition status. The study population comprised low, middle and higher educational levels and there were no significant distribution differences between the groups. The study population comprised a much higher group of educated subjects when compared to the general population of Adelaide. Higher educated subjects with good English skills brushed more frequently and had a more recent scale and clean than the lower educated groups. A measure of subjects' economic level was their pension status; pensioners representing low income. Approximately 58.9% of subjects were pensioners; there were no significant differences in mean PAL between pensioners and non-pensioners.

In order to correlate the effects of aspirin and smoking habits on advanced PAL, three measures of PAL were used; mean PAL, the most severe site of PAL (MSS-PAL) and the extreme worst site of PAL (EWS-PAL). Mean PAL was the overall mean PAL of all sites per tooth/per subject/per group. MSS-PAL was the most severe site of PAL of the six sites per tooth/subject. This method associated the effects of aspirin and ex smoking on advanced

PAL by reducing the overwhelming effects of sites with low PAL. EWS-PAL was the extreme worst site of PAL/mouth. The results were as follows:

	Mean PAL mm \pm se	MSS-PAL mm \pm se	EWS-PAL mm \pm se
ANS	2.5 \pm 0.01	3.7 \pm 0.13	6.2 \pm 0.22
AXS	2.8 \pm 0.09	4.1 \pm 0.11	7.0 \pm 0.18
NANS	2.7 \pm 0.08	4.0 \pm 0.10	6.8 \pm 0.17
NAXS	3.1 \pm 0.08	4.4 \pm 0.10	7.5 \pm 0.17

Prevalence was measured using different threshold levels of PAL. Significant positive effects of aspirin for the extent of PAL were found for all threshold levels. At thresholds of ≥ 2 mm PAL, the prevalence of PAL was approximately 94%. At a moderate threshold of 4mm PAL, 28.7% of subjects exhibited PAL ≥ 4 mm with a mean severity score of 4.6 ± 0.03 mm (se), indicating that the percentage of subjects with advanced PAL was low particularly at higher thresholds. Controlling for age, ANOVA analysis showed that the prevalence rate of PAL was significantly lower in aspirin takers when compared to non-aspirin takers and these effects were independent of smoking history. In addition, ex-smokers had significantly more PAL compared to non-smokers and this effect was independent of aspirin history. The prevalence of advanced PAL in subjects (using 7mm PAL as a threshold) was found to be 2.6% with a mean PAL of 7.7 ± 0.05 mm (se).

Epidemiological studies (including this one) attribute all PAL to the effects of destructive periodontal diseases. No account is given to other causes of PAL such as continuous tooth eruption, alveolar dehiscence, cervical enamel projections, cracked or split roots and retrograde periodontitis. Taking these factors into account, the true prevalence of advanced PAL due to periodontitis within the community must be lower than the estimated rate of 10-15%.

My findings suggest that men aged 50 and above may benefit from taking low-doses of aspirin daily in order to reduce their risk of PAL. With the reduced severity and extent of PAL in ex-smokers taking aspirin, it is tempting to speculate that subjects with periodontitis may benefit significantly by taking low-dose aspirin to reduce their periodontal and cardiovascular risks, irrespective of their smoking history. Further research should aim to establish whether patients with periodontitis would benefit from taking low-dose aspirin as an adjunct to periodontal therapy and whether low-dose aspirin modulates the effects of periodontitis in females and current smokers.