CLINICAL AND PHARMACOLOGICAL STUDIES
OF OROFACIAL PAIN

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A thesis submitted for the degree of
Doctor of Philosophy
“The pain in my jaw is like a bad, nagging toothache or migraine pain, which can last up to two or three days. Sometimes I feel like taking my life because of the pain.” - a patient diagnosed with atypical odontalgia
ABSTRACT

For pain research, the orofacial region is unique in a number of ways. The region has complex local anatomy, including substantial sensory innervation from neural pathways, and muscles of facial expression that convey important information concerning pain intensity and associated psychological traits. Although chronic orofacial pain conditions appear prevalent, useful documentation on pain intensity ratings using well established instruments is sparse. In particular, two conditions, atypical facial pain and atypical odontalgia, are poorly understood in aetiology so that definitive treatment modalities are severely limited. The region’s local biofluid, saliva, has been used to diagnose various local and systemic disease states, and to quantitate drug concentrations. However, recent studies indicate that saliva also contains some of the same peptides, e.g. bradykinin, that are involved in pain mechanisms. It may be that pharmacological-pharmacokinetic studies of these peptides could shed more information on the significance of their presence in saliva.

This thesis consists of four major sections. Section 1 comprises of three clinical studies investigating orofacial pain. Section 2 deals with clinical laboratory studies of saliva. Section 3 is concerned with the development of chromatographic methods to assay bradykinin and its pharmacokinetics in saliva. Section 4 uses chromatography for the identification of novel salivary peptides. This thesis, then, presents clinical studies of orofacial pain and pharmacological investigations of saliva as the local biofluid.
Section 1

Study 1 analysed 120 consecutive patients with chronic orofacial pain who completed a comprehensive questionnaire that included pain intensity scales (McGill Pain Questionnaire and visual analogue scale). The most frequent condition diagnosed was atypical facial pain (n = 40), followed by temporomandibular disorder (n = 32), atypical odontalgia (n = 29) and pain arising from recognised pathology of the orofacial region (n = 19). Results showed a disproportionate female : male ratio (88 : 32) (P < 0.001) in the study group, and in the subgroup of patients diagnosed with atypical facial pain (34 : 6) (P < 0.001). Temporomandibular disorder was present in 65% subjects as the sole pain complaint (n = 32) or as a secondary condition (n = 43). The Pain Rating Index (Total) of chronic orofacial pain conditions was similar to other chronic pain conditions including back pain, cancer pain and arthritis. Patients diagnosed with multiple orofacial pain complaints reported higher Pain Rating Index (Miscellaneous and Total) scores than those patients with a single diagnosis. A significant positive relationship was found between visual analogue scores and the Number of Words Chosen rating (P = 0.002).

Study 2 examined patients with a diagnosis of atypical facial pain. The current IASP definition interprets this condition as “psychogenic pain” and specifically excludes an organic basis or component. Results of this study revealed that these patients described pain with sensory qualities, which is highly suggestive of underlying, but undetected, pathophysiology. Furthermore, a majority of patients were diagnosed with an associated temporomandibular disorder. It is proposed that patients with atypical facial pain have an
organic component contributing to pain, but psychological factors can magnify the affective component of ‘pain and suffering’ on clinical presentation.

Study 3 evaluated 50 patients diagnosed with atypical odontalgia. Patients underwent pharmacological tests including topical anaesthetic application and phentolamine infusion. Therapeutic trials of topical capsaicin were carried out to assess its efficacy for pain reduction. Results showed that 34 females and 16 males, with an age range of 21 - 82 years, were diagnosed with the condition. Dental treatment triggered the pain in 74% of patients. The pain was generally “constant” (80% of patients) and “medium” to “severe” in intensity (78%). A secondary temporomandibular disorder was present in 35 patients. EMLA topical anaesthetic cream applied to the site of intraoral pain for five minutes caused a significant reduction in pain intensity as measured by the visual analogue scale (P < 0.0001). Patient-blinded saline / phentolamine infusions demonstrated that there was a variable contribution to the pain condition from the sympathetic nervous system. A four week trial of topical capsaicin resulted in 19 / 30 patients reporting a significant pain reduction (P < 0.0001), which was maintained at long term review in the majority of patients. The response to these pharmacological procedures and the high occurrence of dental treatment in the aetiology of atypical odontalgia is highly suggestive that this condition is a neuropathic pain of the oral cavity.

Section 2

Study 4 assessed whether measurements of concentrations of salivary bradykinin might be useful markers in quantifying pain states. This was a screening study based on preliminary
chromatographic ‘fingerprint’ profiles obtained from patients with pain. The preliminary
work assaying saliva showed that chromatographic profiles of patients with different pain
conditions were markedly different compared to patients without pain; further development
may result in a ‘fingerprint’ of different pain states. Study 5 investigated bradykinin as a
possible marker in these profiles. The results assessing salivary bradykinin concentrations
showed that there was wide intersubject variation among healthy controls and several
groups of patients with pain (cancer pain, arthritis and post-operative pain). Generally,
females and surgical post-operative patients were found to have quantifiable levels of
salivary bradykinin.

Section 3

Based on the results of study 5, the pharmacokinetics of salivary bradykinin were
investigated. For this study, an alternative bradykinin assay to immunoassay was developed
using high-performance liquid chromatography. The purpose of using chromatography is
that other peptides potentially involved in pain pathways could be investigated with relative
ease using identical or similar (i.e. minor changes in mobile phase chemistry)
chromatographic conditions. A chromatographic assay for salivary bradykinin was
successfully developed that is rapid and simple in sample preparation and mobile phase
chemistry. Study 6 assessed the degradation and stability of salivary bradykinin. Metabolic
clearance of bradykinin using an ex vivo model showed that its clearance was much slower
than its known plasma pharmacokinetics. The method required stabilisation of salivary
bradykinin that was achieved at low pH; saliva at pH 2 through the addition of
orthophosphoric acid showed excellent stability for five to nine days at 20°C and for 60
days at 4°C. Study 7 determined the salivary bradykinin concentrations in healthy subjects and it showed this peptide to be present in concentrations at several orders of magnitude greater than reported plasma concentrations.

Section 4

Chromatographic assays were optimised to identify a variety of novel salivary peptides. In conjunction with mass spectrometry, novel salivary peptides defensin HNP-1 and HNP-2 have been identified. These peptides have proven antimicrobial, antifungal and antiviral (including anti-HIV) activities. There were high concentrations of these salivary defensin peptides (2-350 µg/mL) in ten healthy subjects; this may have potentially important therapeutic applications such as the prevention and / or treatment of oral candidiasis and other infections.
Publications


Abstracts


**Letters to the Editor**

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I wish to thank my friends and colleagues throughout the Department who constantly volunteered to partake in the studies, and with only minimal persuasion. Finally, I am grateful to the patients participating in the study, who keenly supported the clinical trials and
screening studies, and for whom I hope the results of these studies may provide future benefit.

This thesis is dedicated to my parents, (the late) E. Russell Vickers (Snr) and Barbara M. Vickers, who instilled in my youth the essential quality of perseverance.
Statement by the Author Pertaining to Original Work

The Human Research and Ethics Committee of the Royal North Shore Hospital gave approval for the studies, where appropriate, in this thesis. All patients and subjects assessed in the studies in this thesis were personally consulted and treated by the author. The method development utilising high-performance liquid chromatography and all analyses using saliva as the investigating matrix was the original work of the author and, in addition, the development of the sample preparation prior to mass spectrometry. The concept of using high-performance liquid chromatography-mass spectrometry for salivary peptide analysis was the original idea of the author. The operation of the mass spectrometer and tentative identification of defensin HNP-1 and HNP-2 peptides and other salivary constituents were carried out in collaboration with Miss Catrin Goebel and Dr Lindsey Mackay and is acknowledged in the appropriate sections of this thesis.
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GLOSSARY OF TERMS

ACTH  adrenocorticotrophic hormone
AFP  atypical facial pain
AFP-TMD  atypical facial pain with secondary temporomandibular disorder
ANOVA  analysis of variance
AO  atypical odontalgia
AO-TMD  atypical odontalgia with secondary temporomandibular disorder
BK  bradykinin
CGRP  calcitonin gene-related peptide
CRPS  complex regional pain syndrome
CSF  cerebrospinal fluid
EEG  electroencephalogram
EMLA  eutectic mixture of local anaesthetics
GIT  gastrointestinal tract
GRS  Graphic Rating Scale
HF  Hageman factor
HMW  high molecular weight
HPLC  high-performance liquid chromatography
HPLC-MS  high-performance liquid chromatography-mass spectrometry
IASP  International Association for the Study of Pain
LMW  low molecular weight
MMPI  Minnesota Multiphasic Personality Inventory
MPQ  McGill Pain Questionnaire
MS  mass spectrometer
NWC  Number of Words Chosen
OPA  o-Phthalaldehyde
PG  prostaglandin
PRI(A)  Pain Rating Index (Affective)
PRI(E)  Pain Rating Index (Evaluative)
PRI(M)  Pain Rating Index (Miscellaneous)
PRI(S)  Pain Rating Index (Sensory)
PRI(T)  Pain Rating Index (Total)
S.D.  standard deviation
SMP  sympathetically maintained pain
TMD  temporomandibular disorder
VAS  visual analogue scale
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**Study 3** evaluated 50 patients diagnosed with atypical odontalgia. Patients underwent pharmacological tests including topical anaesthetic application and phentolamine infusion. Therapeutic trials of topical capsaicin were carried out to assess its efficacy for pain reduction. Results showed that 34 females and 16 males, with an age range of 21 - 82 years, were diagnosed with the condition. Dental treatment triggered the pain in 74% of patients. The pain was generally “constant” (80% of patients) and “medium” to “severe” in intensity (78%). A secondary temporomandibular disorder was present in 35 patients. EMLA topical anaesthetic cream applied to the site of intraoral pain for five minutes caused a significant reduction in pain intensity as measured by the visual analogue scale (P < 0.0001). Patient-blinded saline / phentolamine infusions demonstrated that there was a variable contribution to the pain condition from the sympathetic nervous system. A four week trial of topical capsaicin resulted in 19 / 30 patients reporting a significant pain reduction (P < 0.0001), which was maintained at long term review in the majority of patients. The response to these pharmacological procedures and the high occurrence of dental treatment in the aetiology of atypical odontalgia is highly suggestive that this condition is a neuropathic pain of the oral cavity.

**Section 2**

**Study 4** assessed whether measurements of concentrations of salivary bradykinin might be useful markers in quantifying pain states. This was a screening study based on preliminary
chromatographic ‘fingerprint’ profiles obtained from patients with pain. The preliminary work assaying saliva showed that chromatographic profiles of patients with different pain conditions were markedly different compared to patients without pain; further development may result in a ‘fingerprint’ of different pain states. Study 5 investigated bradykinin as a possible marker in these profiles. The results assessing salivary bradykinin concentrations showed that there was wide intersubject variation among healthy controls and several groups of patients with pain (cancer pain, arthritis and post-operative pain). Generally, females and surgical post-operative patients were found to have quantifiable levels of salivary bradykinin.

Section 3

Based on the results of study 5, the pharmacokinetics of salivary bradykinin were investigated. For this study, an alternative bradykinin assay to immunoassay was developed using high-performance liquid chromatography. The purpose of using chromatography is that other peptides potentially involved in pain pathways could be investigated with relative ease using identical or similar (i.e. minor changes in mobile phase chemistry) chromatographic conditions. A chromatographic assay for salivary bradykinin was successfully developed that is rapid and simple in sample preparation and mobile phase chemistry. Study 6 assessed the degradation and stability of salivary bradykinin. Metabolic clearance of bradykinin using an ex vivo model showed that its clearance was much slower than its known plasma pharmacokinetics. The method required stabilisation of salivary bradykinin that was achieved at low pH; saliva at pH 2 through the addition of orthophosphoric acid showed excellent stability for five to nine days at 20°C and for 60
days at 4°C. Study 7 determined the salivary bradykinin concentrations in healthy subjects and it showed this peptide to be present in concentrations at several orders of magnitude greater than reported plasma concentrations.

**Section 4**

Chromatographic assays were optimised to identify a variety of novel salivary peptides. In conjunction with mass spectrometry, novel salivary peptides defensin HNP-1 and HNP-2 have been identified. These peptides have proven antimicrobial, antifungal and antiviral (including anti-HIV) activities. There were high concentrations of these salivary defensin peptides (2-350 µg/mL) in ten healthy subjects; this may have potentially important therapeutic applications such as the prevention and / or treatment of oral candidiasis and other infections.
PAPERS ARISING FROM THIS THESIS

Publications


Abstracts


**Letters to the Editor**

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The necessary funds that enabled this study to be carried out included a research scholarship (Balthasar scholarship), and I sincerely thank family members of this foundation for much needed financial assistance.

I wish to thank my friends and colleagues throughout the Department who constantly volunteered to partake in the studies, and with only minimal persuasion. Finally, I am grateful to the patients participating in the study, who keenly supported the clinical trials and
screening studies, and for whom I hope the results of these studies may provide future benefit.

This thesis is dedicated to my parents, (the late) E. Russell Vickers (Snr) and Barbara M. Vickers, who instilled in my youth the essential quality of perseverance.
Statement by the Author Pertaining to Original Work

The Human Research and Ethics Committee of the Royal North Shore Hospital gave approval for the studies, where appropriate, in this thesis. All patients and subjects assessed in the studies in this thesis were personally consulted and treated by the author. The method development utilising high-performance liquid chromatography and all analyses using saliva as the investigating matrix was the original work of the author and, in addition, the development of the sample preparation prior to mass spectrometry. The concept of using high-performance liquid chromatography-mass spectrometry for salivary peptide analysis was the original idea of the author. The operation of the mass spectrometer and tentative identification of defensin HNP-1 and HNP-2 peptides and other salivary constituents were carried out in collaboration with Miss Catrin Goebel and Dr Lindsey Mackay and is acknowledged in the appropriate sections of this thesis.
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GLOSSARY OF TERMS

ACTH  adrenocorticotrophic hormone
AFP  atypical facial pain
AFP-TMD  atypical facial pain with secondary temporomandibular disorder
ANOVA  analysis of variance
AO  atypical odontalgia
AO-TMD  atypical odontalgia with secondary temporomandibular disorder
BK  bradykinin
CGRP  calcitonin gene-related peptide
CRPS  complex regional pain syndrome
CSF  cerebrospinal fluid
EEG  electroencephalogram
EMLA  eutectic mixture of local anaesthetics
GIT  gastrointestinal tract
GRS  Graphic Rating Scale
HF  Hageman factor
HMW  high molecular weight
HPLC  high-performance liquid chromatography
HPLC-MS  high-performance liquid chromatography-mass spectrometry
IASP  International Association for the Study of Pain
LMW  low molecular weight
MMPI  Minnesota Multiphasic Personality Inventory
MPQ  McGill Pain Questionnaire
MS  mass spectrometer
NWC  Number of Words Chosen
OPA  o-Phthalaldehyde
PG  prostaglandin
PRI(A)  Pain Rating Index (Affective)
PRI(E)  Pain Rating Index (Evaluative)
PRI(M)  Pain Rating Index (Miscellaneous)
PRI(S)  Pain Rating Index (Sensory)
PRI(T)  Pain Rating Index (Total)
S.D.  standard deviation
SMP  sympathetically maintained pain
TMD  temporomandibular disorder
VAS  visual analogue scale