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 20^0C and for 60

days at 4^0C. 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.

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APPENDICES

GLOSSARY OF TERMS

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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)
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S.D.	standard deviation
SMP	sympathetically maintained pain
TMD	temporomandibular disorder
VAS	visual analogue scale

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