

PREVALENCE OF MAST CELLS WITHIN THE PERIODONTAL LIGAMENT OF THE DEVELOPING RAT MOLAR

A research project submitted in partial fulfilment of the requirements for the degree of Doctor of Clinical Dentistry

RICHARD J. SALMON



Orthodontic Unit

School of Dentistry

Faculty of Health Sciences

The University of Adelaide

South Australia

2000

Contents

Contents	i
Glossary of Abbreviations	v
List of Figures	vi
List of Tables	ix
Summary	x
Signed Statement	xi
Acknowledgements	xii
Chapter 1: Introduction	1
Chapter 2: Aims and Hypotheses	3
Chapter 3: Literature Review	4
Anatomy of the Rat and Chronology of Tooth Development	4
Mast Cells within the Oral Cavity	7
Mast Cells within the Periodontal Ligament	8
Development of Mast Cells	8
Regulation of Mast Cell Differentiation and Proliferation	10
Heterogeneity of Mast Cells	11
1. Functional and Morphological Characteristics of Human Mast Cells	11
2. Functional and Morphological Characteristics of Rodent Mast Cells	15
Mast Cell Activation	16
Degranulation and Recovery of the Mast Cell	19

Mast (Cell Mediators		23
1.	Pre-Formed Mediators (i) Histamine (ii) Heparin and Chondroitin Sulphate (iii) Neutral Proteases ~ Tryptase and Chymase		23 23 24 25
2.	Newly Synthesised Mediators		28
3.	Cytokines	- C.D.	31
Mast (Cell Identification		35
1.	Fixation		35
2.	Staining Techniques (i) Demonstration by Metachromasia (ii) Immunohistochemical Staining Techniques		37 37 39
Chapter 4: I	Methods and Materials		45
Summ	nary		45
Ethics	Approval		45
Pilot S	Study		45
1.	Animals		45
2.	Specimen Retrieval and Fixation		46
3.	Decalcification, Processing and Sectioning		46
4.	Staining and Observation		47
Main	Experiment		47
1.	Animal Care and Specimen Retrieval		47
2.	Fixation and Decalcification		48
3.	Tissue Processing and Embedding		48
4.	Sectioning of Specimens		49
5.	Staining and Immunolabelling		49
6.	Zero Levels		50
7.	Counting Procedure and Microscopy		51
8.	Photomicroscopy		53
9.	Data Collection and Analysis		53

Chapter 5: R	esults	57
Pilot St	udy	57
1.	Fixation	57
2.	Decalcification	57
3.	Staining and Immunohistochemistry •	58
Main E	xperiment	60
۵. «	Tissue Preparation (i) Fixation (ii) Decalcification	60 60 60
2.	Section Preparation (i) Embedding, Sectioning & Mounting (i) Staining	60 60 61
4.	Histological Observations (i) Overview of Tooth Development (ii) Mast Cells in the Surrounding Dental Tissues	63 63 63
5.	Mast Cell Association with Crown Development	68
6.	 Mast Cells Associated with the Developing Mesiobuccal Root (i) Mast Cells related to the Age of the Animal (ii) Mast Cell Distribution between Quadrants (iii) Mast Cell Distribution across the Periodontal Ligament (iv) Mast Cells associated with the Developing Mesiobuccal Root Periodontal Ligament (v) Mast Cell Type Determination 	68 68 75 75 79 79
7	Additional Findings	81
ж. С.		
Chapter 6: D	Discussion	84
The Ex	xperimental Animals	84
Tissue	Preparation	84
1.	Fixation	84
2.	Decalcification	86
Sectio	n Preparation and Staining	88
1.	Zero Levels	88
2.	Sectioning of Specimens	89
3.	Staining with Toluidine Blue (pH 0.5)	89
4.	Staining with RMCP-II	90

#2)

Histol	ogical and Other Observations	91
1.	Mast Cells about the Developing Crown	91
2.	Mast Cells about the Developing Root (i) Mast Cells Prevalence within Tooth Quadrants (ii) Mast Cells across the Periodontal Ligament	92 93 93
3.	Mast Cells in Adjacent Tissue	95
4.	Mast Cell Heterogeneity	- 96
5.	Mast Cells and Blood Vessels	98
6.	Root Resorption	99
Sugge	ested Areas of Future Research	100
Chapter 7:	Conclusions	101
Chapter 8: /	Appendices	103
1. Fix	atives	103
2. De	calcification Solutions	104
3. Tis	sue Processing	104
4. Slie	de Subbing	105
5. Sta	ains	105
Bibliograph	NV	109

Glossary of Abbreviations

ABC	Avidin-Biotin Complex
CD34+	Human Mast Cell Precursor
CFU-Mast	Mast Cell Precursor (Rodent)
сох	Cyclooxygenase
СТМС	Connective Tissue Mast Cell (Rodent)
EDTA	Ethylene-diamine-tetraacetic Acid
FceRI	Antigenic Receptor upon Mast cell surface
FITC	Fluorescein thiocyanate
GAG	Glycosaminoglycan
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
HRP	Horse Radish Peroxidase
IFFA	Formaldehyde / Acetic acid tissue fixative
lgE	Immunoglobulin-E
IL	Interleukin
LT	Leukotrienes
MCT	Mucosal Mast Cell (Human)
MC _{TC}	Connective Tissue Mast Cell (Human)
MMC	Mucosal Mast Cell (Rodent)
PAF	Platelet Activating Factor
PG	Prostaglandins
RMCP-I / RMCP-II	Rat Mast Cell Protease I / II
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumour Necrosis Factor-alpha
TRITC	Tetramethylrhodamine

List of Figures

Figure 1: Basal view of an adult rodent skull	5
Figure 2: Sagittal view of an adult rodent skull	5
Figure 3: Human lung mast cells illustrating variation in granule ultrastructural morphology	13
Figure 4: Degranulation in mast cells: Cross-linking of IgE receptors	18
Figure 5: Human lung mast cell degranulation changes after exposure to anti-lgE	20
Figure 6: Anaphylactic degranulation and recovery of human lung mast cells	22
Figure 7: Biological effects of mast cell mediators, proteases and cytokines	24
Figure 8: Generation of arachidonic acid metabolites and their roles in inflammation	29
Figure 9: Mast cell triggering follows the release of mediators via two major pathways	29
Figure 10: The role of the mast cell in natural and acquired immunity	33
Figure 11: Leukocyte recruitment initiated by selectins	34
Figure 12: The Avidin-Biotin-Peroxidase complex method of staining	42
Figure 13: Identification of zero levels	50
Figure 14: Establishment of quadrants about the mesiobuccal root	52
Figure 15: Determination of bone, middle and tooth thirds of the periodontal ligament for quantitative analysis	52
Figure 16: Determination of RMCP-II concentration for immunohistochemistry	59
Figure 17: Rat intestine fixed in 10% neutral buffered formalin stained with toluidine blue at pH 0.5 and RMCP-II	59
Figure 18: Rat tongue fixed in 10% neutral buffered formalin stained with toluidine blue at pH 0.5 and RMCP-II	59
Figure 19: Toluidine blue staining of the periodontal ligament at pH 0.5	62
Figure 20: Immunohistochemical RMCP-II staining of the periodontal ligament	62
Figure 21: Two-day and one-week development of maxillary first molar	64

VI

Figure 22: Two- and three-week specimens demonstrating eruption times	64
Figure 23: Six-week development of the first maxillary molar	64
Figure 24: Furcation (zero level) of first maxillary molar	65
Figure 25: Identification of the mesiobuccal root periodontal ligament	65
Figure 26: Zero level identification during early root formation	65
Figure 27: Apex of mesiobuccal root of first maxillary molar	66
Figure 28: Periodontal ligament after termination of the mesiobuccal root	66
Figure 29: Apex of the developing six-week mesiobuccal root	66
Figure 30: Mast cells within the lamina propria of palatal tissues	67
Figure 31: Transverse and longitudinal distribution of mast cells about the developing crown assessed with Toluidine blue (pH 0.5) and RMCP-II (two-days)	69
Figure 32: Transverse and longitudinal distribution of mast cells about the developing crown assessed with Toluidine blue (pH 0.5) and RMCP-II (one-week)	69
Figure 33: Transverse and longitudinal distribution of mast cells about the developing crown assessed with Toluidine blue (pH 0.5) and RMCP-II (two-weeks)	69
Figure 34: Mast cells within the dental follicle and in adjacent connective tissue	70
Figure 35: Mast cell within the stellate reticulum of the developing dental follicle	70
Figure 36: Mast cell superior to the developing crown	71
Figure 37: Mast cells within the dental follicle of the cervical loop about the developing crown	71
Figure 38: Mast cells within the buccal tissue and at the cervical loop of a developing two-day crown	72
Figure 39: Mast cell association with cervical development of the crown	72
Figure 40: Mast cell distribution along the length of the mesiobuccal root as determined by horizontal section analysis at 150 μ m intervals to the limit of root formation (two-weeks); Toluidine blue (pH 0.5) versus RMCP-II staining.	73
Figure 41: Mast cell distribution along the length of the mesiobuccal root as determined by horizontal section analysis at 150 μm intervals to the limit of root formation (three-weeks); Toluidine blue (pH 0.5) versus RMCP-II staining.	73

VII

ŝ

Figure 42: Mast cell distribution along the length of the mesiobuccal root as determined by horizontal section analysis at 150 μm intervals to the limit of root formation	
(four-weeks); Toluidine blue (pH 0.5) versus RMCP-II staining.	74
Figure 43: Mast cell distribution along the length of the mesiobuccal root as determined by horizontal section analysis at 150 μ m intervals to the limit of root formation (six-weeks); Toluidine blue (pH 0.5) versus RMCP-II staining.	74
 Figure 44: Mast cell distribution around the developing mesiobuccal root (two-weeks) 	76
- Figure 45: Mast cell distribution around the developing mesiobuccal root (three-weeks)	76
Figure 46: Mast cell distribution around the developing mesiobuccal root (four-weeks)	76
Figure 47: Mast cell distribution around the developing mesiobuccal root (six-weeks)	76
Figure 48: Mast cell distribution within the periodontal ligament (two-weeks)	77
Figure 49: Mast cell distribution within the periodontal ligament (three-weeks)	77
Figure 50: Mast cell distribution within the periodontal ligament (four-weeks)	77
Figure 51: Mast cell distribution within the periodontal ligament (six-weeks)	77
Figure 52: Mast cells within the middle one-third of the periodontal ligament of a three- week specimen	78
Figure 53: Mast cells within the bone one-third of the periodontal ligament of a six-week specimen	78
Figure 54: Mean mast cell count per animal within the periodontal ligament of the entire developing mesiobuccal root (transverse section)	80
Figure 55: Mean mast cell count per animal within the periodontal ligament of the entire developing mesiobuccal root (longitudinal section)	80
Figure 56: Mast cell position within the bone and middle one-third of the periodontal ligament of a six-week specimen	82
Figure 57: Demonstration of a mast cell within the lumen of a blood vessel of the periodontal ligament	82
Figure 58: Mast cell associated with root resorption lacuna	83
Figure 59: Influence of eight fixatives and two stains on the mucosal mast cell count in operative biopsies of normal human jejunum	85

x.

ii.

VIII

List of Tables

Table 1: Chronology of tooth development of the rat	6
Table 2: Summary of characteristics of human mast cell phenotypes	14
Table 3: Summary of characteristics of rat mast cell phenotypes •	15
Table 4: Potential activators of mast cells	17
Table 5: Biological activities of tryptase	26
Table 6: Biological activities of chymase	27
Table 7: Summary of mast cell pre-formed and newly synthesised mediators and cytokines	30
Table 8: Protease content of different tissues in rats	43
Table 9: Recording sheet for transverse mast cell distribution within the periodontal ligament of the mesiobuccal root of the first maxillary molar	55
Table 10: Recording sheet for longitudinal mast cell distribution within the periodontal ligament of the mesiobuccal root of the first maxillary molar	56
Table 11: Mean periodontal ligament widths between age of animals	77
Table 12: Mast cells detected with Toluidine Blue (pH 0.5)	92
Table 13: Quantitative distribution of mast cells within the periodontal ligamentcompared with previous research	94
Table 14: Blood vessel distribution within the periodontal ligament	95

IX

Summary

Mast cells are commonly associated with tissue inflammation and can expel an array of cytokines and mediators during activation or injury to the cell. Mast cells demonstrate two sub-types in both humans and rodents and are distinguished by their neutral protease composition. These are classified as either mucosal or connective tissue-type. This study aims to assess the location and prevalence of mast cells about the developing first maxillary molar crown and periodontal ligament (PDL) of the developing mesiobuccal root. A relative incidence of mucosal and connective tissue-type mast cells within the PDL population was also investigated.

Twenty four Sprague-Dawley rats were divided into six groups and assessed at 2 days, 1, 2, 3, 4 and 6 weeks. Maxillae were fixed in 10% neutral buffered formalin and sectioned either longitudinally or horizontally. Sections were stained histochemically with Toluidine blue (pH 0.5) or immunohistochemically with Sheep anti-rat mast cell protease-II. The mesiobuccal root was examined at 150 μ m levels. The PDL was divided horizontally into thirds (bone, middle or tooth) to determine mast cell position within the PDL. Longitudinal sections provided total mast cell counts.

The unerupted developing crown demonstrated few mast cells randomly distributed about the dental follicle. Development of the root saw mast cell numbers increase within the PDL with increasing age, with the exception of a reduction at four-weeks. This decrease coincided with tooth eruption. Mast cells were distributed evenly along the length of the mesiobuccal root at all ages, however, once in functional occlusion appeared more prevalent in the apical one-half of the root. An even distribution of mast cells about the root circumference was also noted. Within the PDL a shift of the population was noted to occur from the tooth one-third of the periodontal ligament at two-weeks, to the bone one-third of the PDL in the six-week group. Greater counts of mast cells (approximately 20-40%) were identified when labelled with Sheep anti-rat mast cell protease-II antibody than with Toluidine blue (pH 0.5). This difference suggests detection of a non-connective tissue-type of mast cell or an immature mast cell unable to be detected by the histochemical techniques.

Signed Statement

This report contains no material that has been accepted for the award of any other degree or diploma in any other university. To the best of my knowledge and belief, it contains no material previously published except where due reference is made in the text.

I give consent of this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Richard J. Salmon

Acknowledgements

The author wishes to thank the following people for their support in the production of this research report:

Professor W.J. Sampson, P.R. Begg Chair in Orthodontics, The University of Adelaide, for providing the enthusiasm, expertise and motivation in both clinical and research aspects of the post-graduate orthodontic course.

Associate Professor D.F. Wilson, Department of Oral Pathology, The University of Adelaide, for guidance in laboratory techniques and interpretation of results as well as revision of the manuscript.

Dr O.W. Wiebkin, Dept of Medicine, R.A.H., Adelaide, for donation of the RMCP-II immunolabel and invaluable input and enthusiasm given to the research project.

Margaret Leppard and Sandy Powell for their expert technical assistance in all facets of laboratory work, specimen preparation and presentation.

Dr Rob Moore and Dr Jim Manavis, Bone Physiology Research Laboratory, Institute of Medical and Veterinary Science, Adelaide, for donating their time and space in the teaching of immunolabelling techniques.

My wife Catherine for her love and support throughout the course and as well as for the tolerance of the hours I worked.

My colleagues in the post-graduate orthodontic programme, University of Adelaide, for the humour and support that made the slow times fun. Special thanks to Petrina and Daniel for help with specimen preparation, immunological techniques and tolerance of my control over the laboratory radio. Cheers.

Coppers brewery for production of liquid sedation utilised by the author in the production of this research report.

IMVS cafeteria whose tireless effort to revolutionise the way flavour is removed from food prompted laboratory techniques otherwise unexplored.

Australian Society of Orthodontists Foundation for Research and Education for their generous support in this research project.