THE EFFECT OF ORTHODONTIC TOOTH MOVEMENT ON THE MAST CELL POPULATION IN THE RAT PDL

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of the requirements for the Degree of
Master of Dental Surgery

by

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LIST OF ABBREVIATIONS

TEXT

ACTH	adrenocorticotrophic hormone
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
Camp	cyclic adenosine monophosphate
cGMP	cyclic guanidosine monophosphate
C3a C4a C5a	components of the complement cascade
CTMC	connective tissue mast cell
DAG	diacylglycerol
FcεRI	high-affinity Fc receptor for IgE.
FITC-avidin	fluorescein isothiocyanate - conjugated avidin
GMCSF	granulocyte-monocyte colony stimulating factor
H_2O_2	hydrogen peroxide
HRP-avidin	horseradish peroxidase-conjugated avidin
IFAA	isotonic formol-acetic acid
IgE	immunoglobulin E
IgG	immunoglobulin G
IL-3	interleukin 3
IL-4	interleukin 4
IL-5	interleukin 5
IL-6	interleukin 6
LTB_4	leukotriene B
LTC_4	leukotriene C
LTD_4	leukotriene D
LTE_4	leukotriene E
MHC	major histocompatibility complex
MMC	mucosal mast cell
PDL	periodontal ligament
PGD_2	prostaglandin D_2
PGE_2	prostaglandin E_2
PTH	parathyroid hormone
SRS-A	slow-reacting substance of anaphylaxis
TNF	tumour necrosis factor
TRITC avidin	tetramethylrhodamine isothiocyanate-conjugated avidin
48/80	compound 48/80 - a degranulating agent. A product of condensation of *p*-methoxyphenethylmethylamine

SIGNED STATEMENT

This report contains no material which has been accepted for the award of any other degree or diploma in any other university or other tertiary institution and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference has been made in the text.

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

Melinda E. Barva

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SUMMARY

Orthodontic tooth movement may initially cause an inflammatory process in the periodontal tissues. Mast cells have an important function in the initiation of inflammatory responses either as a reaction to trauma, immediate type of immune reactions, or in delayed-type hypersensitivity. Their role, if any, in orthodontic tooth movement has not been determined.

The principal aim of this study was to test the hypothesis that there would be a reduction in the number of stainable mast cells subsequent to orthodontic tooth movement.

The present study utilised sixty male Sprague-Dawley rats: forty of these were experimental animals and twenty were controls. The experimental animals were 83 or 84 days of age at the time of orthodontic appliance insertion. The rats were anaesthetized by intraperitoneal injection of Ketapex[1] diluted in sodium chloride, and Rompun[2] to enable cementation of the appliances. The appliances consisted of bonding mesh adapted as a band around the two maxillary incisors soldered to a palatally positioned supporting wire. A finger spring was welded to the right side of the supporting wire and was adjusted to provide a force of approximately five grams in a buccal direction to the maxillary right first molar. These appliances were cemented to the maxillary incisors using chemically cured composite resin. The finger spring was maintained in the correct position with the use of a 0.08" stainless steel ligature. During the period of orthodontic tooth movement the animals were fed pulverized standard rat pellets and the appliances were checked every second day.

Following completion of the experimental period (15 minutes, 1 hour, 4 hours, 24 hours, 1 week, 2 weeks, 4 weeks and 8 weeks) the rats were sacrificed and the portion of the maxillae supporting the six molars was removed. These specimens were matched with untreated control animals of the same age.

All of the specimens were immersion fixed in Carnoy's fixative and rehydrated before being demineralized in 4% EDTA in cacodylate buffer. The end point of decalcification was

[1] Ketapex = 100mg/mL ketamine (Apex Laboratories, NSW, Australia)
[2] Rompun = 20 mg/mL xylazine hydrochloride (Bayer, NSW, Australia)

determined radiographically and the specimens were processed for routine paraffin wax embedding.

As this study focussed on early changes in the population and distribution of mast cells (i.e. over the period of one week) all of the animals during this time period were examined (N=33). Only one each of the experimental and control 2 week, 4 week and 8 week animals (N=6) were examined to identify any later trends, and the remaining specimens were processed and set aside for later study. The 39 specimens of the current study were sectioned serially in 5 micron thick sections and mounted on APT[3]-subbed slides. There were on average 600 sections per tooth.

To identify zero levels, every tenth section from the first section to section 300, was stained with haematoxylin and eosin. The zero level was taken as the first connective tissue attachment on the mesial of the first maxillary molar. From zero to the last section per block, every twentieth section (i.e. every 100th micron) was stained for mast cells using 0.5% toluidine blue in HCl at pH 0.5. This resulted in approximately 20 levels studied per tooth, depending on its root length.

The root and surrounding periodontal ligament and bone were divided into four quadrants (buccal, distal, palatal and mesial) with the use of a template in the light microscope eyepiece. In addition, the ligament was further subdivided into horizontal thirds (bone, mid and tooth) utilizing a millimetre scale in the same eyepiece. Therefore, there were twelve possible locations (4 quadrants x 3 thirds) per level per tooth where the mast cells could be located. Also, the level at which the alveolar crest completely surrounded the mesiobuccal root was noted. Furthermore, a note was made if the mast cells were located in close proximity (within 12 microns) to any blood vessels.

These data were provided to the statistician as an Excel spreadsheet and were read into a statistical package, S-plus. The data were reduced into a more manageable form by consolidating the levels studied (averaging 20) into three areas (coronal, middle and apical). The data were subjected to analysis of variance, mean mast cell counts and split-plot analysis.

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[3]APT = aminopropyltriethoxysilane (Sigma Chemical Co. St. Louis, USA)

Analysis of variance indicated there were large treatment differences between the left (control) and right (experimental) teeth within the experimental animals (Mean Sq = 21.83, P = 0.0022).

The present study demonstrated a change in the mast cell numbers when comparing experimental (orthodontically moved) and control teeth. The mean mast cell counts throughout the PDL indicated that the control teeth generally had higher mast cell counts than the experimental teeth, except this trend was reversed in the one hour time group.

This change in mast cell counts was not universal throughout the ligament and was highly affected by position (ie. vertical distribution, quadrant distribution and horizontal distribution). Analysis of variance indicated that the horizontal distribution (ie. bone, mid and tooth thirds) showed the most significant differences, followed by the vertical distribution (i.e. coronal, middle and apical levels), with the least significant differences noted in the quadrant distribution (buccal, mesial palatal and distal).

The distribution of mast cells noted in this study is consistent with the hypothesis that suggests a mast cell role in bone remodelling in orthodontic tooth movement, as the cells were preferentially located next to the bone rather than root surfaces of the PDL. Furthermore, in the experimental teeth, the bone third demonstrated the greatest reduction in stainable mast cells, indicating more of these degranulated following tooth movement.

The mast cells were also more numerous in the coronal and apical regions where tooth movements would be greatest. In the experimental teeth, the coronal numbers dropped dramatically, supporting this interpretation.

In the control teeth there were twice as many mast cells in the buccal and mesial quadrants. In the experimental teeth the numbers became more evenly distributed across the quadrants. This may be due to buccal as well as mesial tooth movement.

The mast cell biochemistry is tantalizing for its potential in bone remodelling in both bone formation (by the actions of prostaglandin and histamine) and bone resorption (by the action of prostaglandin and heparin). There is no contradiction between the stimulatory effect of

prostaglandin on bone formation and resorption since these processes are carried out by different cells.

In addition, the mast cells demonstrated a predilection for blood vessels - more than half of the mast cells in control teeth were within 12.5μm of blood vessels. This proportion was higher in experimental teeth, especially at 4 and 24 hours after starting orthodontic tooth movement. This may indicate either a blood-borne passage of mast cells during tooth movement, or a migration of mast cells within the PDL towards blood vessels where their mediators may have most effect.

In conclusion, this study has shown that the number of mast cells detectable using routine histological staining techniques has decreased following orthodontic tooth movement. Interestingly, this decrease was not uniform throughout the ligament and was highly affected by position (i.e. horizontal distribution, vertical distribution and quadrant distribution).

This investigation also revealed that the rat provided a reliable model for the study of the effect of orthodontic tooth movement on the periodontal ligament.

HORIZONTAL DISTRIBUTION

- There were statistically significant differences between bone, mid and tooth thirds of the ligament ($p \leq 1 \times 10^{-7}$).

- For control teeth, the greatest number of mast cells were found in the bone third, followed by the mid third with the least found adjacent to the tooth.

- For experimental teeth, there was a drop in mast cell numbers in each third, however, the distribution stayed the same i.e. bone > mid > tooth.

VERTICAL DISTRIBUTION

- There were statistically significant differences between coronal, middle and apical levels of the ligament ($p \leq 1 \times 10^{-7}$).

- For control teeth, the greatest number of mast cells occurred in the coronal and apical levels, with considerably less found in the middle level.

- For experimental teeth, this distribution changed such that the coronal mast cell numbers dropped dramatically with essentially no change of the mast cell numbers in the middle and apical levels.

QUADRANT DISTRIBUTION

- Although there were differences between the quadrants of the ligament, these were not statistically significant ($p = 0.1271842$).

- For control teeth, there were nearly twice as many mast cells in the buccal and mesial quadrants as in the palatal and distal quadrants.

- For experimental teeth, this distribution changed such that the buccal and mesial quadrants reduced markedly (by approximately half) whilst the palatal and distal quadrants remained essentially the same.

MAST CELLS NEAR BLOOD VESSELS

- For control teeth (of both experimental and control animals), more than half (54.8%) of the mast cells were located near blood vessels.

- For experimental teeth, more than three-quarters (75.7%) of the mast cells were located near blood vessels.

- There was a significant increase in the number of mast cells located near blood vessels at 4hrs and 24hrs after starting orthodontic tooth movement.