AN IMMUNOHISTOCHEMICAL STUDY OF MARMOSET PERIODONTAL LIGAMENT MICROVASCULATURE

A CONFOCAL LASER SCANNING MICROSCOPIC STUDY

A research project submitted in partial fulfilment of the requirements for the degree of Master of Dental Surgery

by

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LIST OF ABBREVIATIONS

CLSM	Confocal laser scanning microscope
DMSO	Dimethyl sulphoxide
DNS	Donkey normal serum
DTAF	Dichlorotriazinyl amino fluorescein
ET-1	Endothelin-1
FITC	Fluorescein isothiocyanate
GCF	Gingival crevicular fluid
IHC	Immunohistochemistry
IL-1	Interleukin-1
MVB	Microvascular bed
PBS	Phosphate buffered saline
PDL	Periodontal ligament
TGFβ	Transforming growth factor β
VEGF	Vascular endothelial growth factor
VSMC	Vascular smooth muscle cells
α-SMA	Alpha smooth muscle actin
I1	Central incisor
I2	Lateral incisor
C	Canine
PM1	First premolar
PM2	Second premolar
PM3	Third premolar
M1	First permanent molar
M2	Second permanent molar
TA	Terminal arteriole
AVA	Arteriovenous anastomosis
AC	Arterial capillary
p-VC	Pericytic venous capillary
a-PC	Apericytic venous capillary
p-PCV	Pericytic postcapillary-sized venule
a-PCV	Apericytic postcapillary-sized venule
CV	Collecting venule

SUMMARY

The biological response to the application of an orthodontic force to a tooth involves various cell types of the periodontium assuming altered functional states (Davidovitch, 1995). The vasculature of the periodontium undergoes functional and morphological alterations simultaneously along with other cellular elements. A 21-amino-acid peptide, endothelin-1 (ET-1, Yanagisawa *et al.,* 1988) is a cytokine with potent vasoactive properties that may play a role in the response of the PDL microvascular endothelium to tooth loading.

A custom designed device was constructed for the application of an intrusive occlusal load to the buccal segment teeth of four anaesthetised marmosets for 1.5 hours. The contralateral teeth served as controls. For immunohistochemical investigations, the jaws, and samples of kidney, skeletal thigh muscle and gut, were extirpated and fixed immediately after the animals were euthanased.

Sagittal sections of each mandibular body were prepared using a diamond wafering blade mounted on a low speed sectioning saw. Each section was 150-200μm thick and incorporated bone, periodontium and teeth. Immunohistochemistry (IHC) was performed on the undemineralised mandibular sections using laboratory methodologies established with pilot studies.

The pilot studies comprised systematic chequerboard tests of indirect immunohistochemical labelling for the identification of ET-1, endothelial cells and periendothelial cells on cryostat-cut sections of marmoset kidney, skeletal muscle and gut. The primary antibodies were anti-ET-1, JC-70A and alpha smooth muscle actin (α-SMA). Immunoglobulin G secondary antibodies conjugated with the fluorochromes DTAF, FITC, Cy3™ and Cy5™ were tested for optimum signal-to-noise ratios. Streptavidin-biotin amplification immunolabelled vascular endothelium for ET-1 but did not prove as satisfactory as the indirect IHC technique.

Fluorescence and confocal laser scanning microscopy were used to ascertain the location of immunolabelled vascular ET-1, endothelial and periendothelial cells. Antibody penetration routinely occurred to a depth greater than 85μm from either side of the mandibular sections.

Endothelin-1 demonstrated an heterogeneous distribution within the microvasculature of the PDL and alveolar bone with the anti-ET-1 primary antibody and the IgG/Cy5™ secondary antibody at dilutions of 1:50. Vascular ET-1 was located within the walls of terminal arterioles, postcapillary-sized venules and collecting venules. Immunolabelling for ET-1 occurred within the cytoplasm of endothelial cells and vascular smooth muscle cells. The presence of this potent vasoconstrictor suggests that the microvascular beds of the PDL and alveolar bone might possess contractile properties. The hypothesis that vascular ET-1 expression varies within the PDL in unloaded versus loaded conditions could not be established as insufficient data were generated for quantification. A method for future quantification of antigen expression was examined.

Endothelial cell, pericyte and vascular smooth muscle cell location and morphology was examined with the JC-70A (1:40) and α-SMA (1:100) primary antibodies respectively in combination with IgG/Cy3™ secondary antibody (1:50). The intercellular distribution of endothelial cell immunolabelling enabled illustration of cellular size and shape. Immunolabelling of intricate actin fibrils within pericytes and vascular smooth muscle cells provided further insight into periendothelial cell morphology. The confocal laser scanning microscope (CLSM) and CoMOS software (Bio-Rad, version 7.0a) enabled the construction of detailed three-dimensional cellular images by computer stacking of the optical sections.

The results of immunohistochemical investigations of vasoactive peptides such as ET-1 may eventually lead to pharmacological targeting of the PDL microvasculature to assist orthodontic tooth movement. Endothelin-1 may play a significant role during angiogenesis within the periodontium concomitant with orthodontic tooth movement. The administration of agents that influence the function of PECAM-1, the antigen to JC-70A, and their effect on the rate of tooth movement might also be investigated. Further research utilising the marmoset experimental and control maxillary sections and the laboratory methodology established in the current study are warranted.

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

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