

UNIVERSITI PUTRA MALAYSIA

INDUCTION OF PERIODONTAL DISEASE VIA RETENTIVE LIGATURE, LIPOPOLYSACCHARIDE INJECTION AND THEIR COMBINATION IN A RAT MODEL

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By

HANA HASSAN MUSTAFA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Veterinary Science

January 2018

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DEDICATION

I wish to dedicate this dissertation work to my entire family. I specifically want to appreciate and express my gratitude to my parents, siblings, husband and daughter whose love and prayers kept me on and saw me through this challenging period of my life.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Veterinary Science

INDUCTION OF PERIODONTAL DISEASE VIA RETENTIVE LIGATURE, LIPOPOLYSACCHARIDE INJECTION AND THEIR COMBINATION **IN A RAT MODEL**

By

HANA HASSAN MUSTAFA

January 2018 : Associate Professor Chen Hui Cheng, PhD Chairman Faculty

Veterinary Medicine :

Periodontitis is a highly prevalent, chronic immune-inflammatory disease of the periodontium that results in progressive degradation of the periodontium and alveolar bone loss. This thesis aims to evaluate the induction of periodontal disease via retentive ligature, lipopolysaccharide, and their combination in a rat model. Seventy two Sprague Dawley rats were distributed into four treatment groups: 1) control group with no treatment; 2) application of 4/0 nylon ligature around 2^{nd} maxillary molars; 3) intragingival injection of *Porphyromonas gingivalis* lipopolysaccharide (LPS) to the palatal mucosa of the 2nd maxilla molars; 4) combination of ligature and LPS injection (ligature-LPS). At 7, 14, 30 days after the induction of periodontal disease, 6 rats of each group were sacrificed. Morphological changes in the gingival tissues were evaluated according to Loe & Sillness Gingival Index and Plaque Index. Alveolar bone loss were evaluated histologically and via microcomputed tomography. Parametric data were analysed using two-way ANOVA followed by Tukey test with significance set at 5%. Non-parametric data were analysed using Kruskal-Wallis followed by multiple comparisons with Bonferroni's correction. The morphological, histological and radiological results revealed significant degenerative changes in the periodontal tissues and alveolar bone following both the ligature and ligature-LPS induction techniques. These changes were evident as early as 7 days; maintained until 14 days, and declined with time. There was minimal difference in the changes induced by ligature-LPS when compared to ligature alone. Injection with LPS alone resulted in minimal increase of the Gingival and Plaque Index, and insignificant histological and radiological changes when compared to the controls. In conclusions, the ligature technique was effective to induce acute periodontal disease. The LPS injection technique in this study was not effective to induce alveolar bone loss, and its combination to ligature added insignificant effect.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

INDUKSI PENYAKIT PERIODONTIUM MELALUI LIGATUR, SUNTIKAN LIPOPOLISAKARIDA, DAN KOMBINASI LIGATUR-LIPOPOLISAKARIDA PADA MODEL TIKUS

Oleh

HANA HASSAN MUSTAFA

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Periodontitis adalah penyakit periodontium radang-imun kronik yang lazim, yang mengakibatkan kemerosotan progresif tisu periodontium dan kelesapan tulang alveolus. Tesis ini menilai induksi penyakit periodontium melalui ligatur, suntikan lipopolisakarida, dan kombinasi ligatur-lipopolisakarida pada tikus. Tujuh puluh dua tikus Sprague Dawley dibahagikan kepada empat kumpulan rawatan: 1) kumpulan kawalan tanpa rawatan; 2) pengikatan sutur nylon 4/0 mengelilingi molar maksila kedua; 3) suntikan lipopolisakarida Porphyromonas gingivalis (LPS) ke mukosa gingiva molar maksila kedua; 4) kombinasi ligatur dan suntikan LPS (ligatur-LPS). Pada 7, 14, 30 hari selepas induksi penyakit periodontium, 6 tikus dari setiap kumpulan dikorbankan. Perubahan morfologi tisu gingiya dinilai berdasarkan Indeks Gingiva dan Plak Loe & Sillness. Kelesapan tulang alveolar dinilai secara histologi dan melalui tomografi mikrokomputer. Data parametrik dianalisa menggunakan ANOVA dua hala diikuti ujian Tukey; nilai ditetapkan pada 5%. Data bukan parametrik dianalisa dengan menggunakan Kruskal-Wallis diikuti perbandingan berganda dengan pembetulan Bonferroni. Hasil morfologi, histologi dan radiologi menunjukkan perubahan degeneratif yang signifikan dalam tisu periodontium dan tulang alveolus berikutan teknik induksi ligatur dan ligatur-LPS. Perubahan ini kelihatan pada 7 hari; kekal sehingga 14 hari, dan menyusut dengan masa. Tiada beza dalam perubahan yang disebabkan oleh ligatur-LPS berbanding ligatur sahaja. Suntikan dengan LPS mengakibatkan peningkatan Indeks Gingival dan Plak yang minimum, dan perubahan histologi dan radiologi yang tidak signifikan jika dibandingkan dengan kawalan. Sebagai kesimpulan, teknik ligatur adalah berkesan untuk mengaruh penyakit periodontium akut. Teknik suntikan LPS dalam kajian ini tidak berkesan untuk mendorong kelesapan tulang alveolus, dan gabungannya kepada ligatur menambah kesan yang tidak signifikan.



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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Veterinary Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

	ANOVA	Analysis of variance
	ABC	Alveolar bone crest
	AL	Attachment loss
	BV	Bone volume
	CEJ	Cemento-enamel junction
	СТ	Connective tissue
	GI	Gingival Index
	H&E	Hematoxylin and eosin
	IACUC	Institutional animal care and use committee
	IL-a	Interleukin alpha
	IL-6	Interleukin- six
	IC	Inflammatory cells
	IQR	Interquarter range
	JE	Junctional epithelium
	L	Ligature
	LPS	Lipopolysaccharide
	M1	Maxillary first molar teeth
	M2	Maxillary second molar teeth
	M3	Maxillary third molar teeth
	M-CT	Micro-Computed tomography
	μm	Micro meter
	μL	Micro Liter
	mm	millimeter
	PD	Periodontal disease
	PI	Plaque index
	PL	Periodontal ligament
	Pg-LPS	Porphyromonus gingival lipopolysaccharide.
	ROI	Region of interest
	SE	Standard error

- TNF-a Tumor necrosis factor-alpha
- UPM Universiti Putra Malaysia
- VOI Volume of interest
- 2-D Bi dimensional
- 3-D Three dimensional



CHAPTER 1

INTRODUCTION

1.1 Introduction

Periodontal disease (PD) is a significant oral disorder affecting humans (Poul 2004). It is a multifactorial disease, caused by bacterial plaque in the periodontium. Research has shown that there are many risk factors involved in pathogenesis of periodontitis, such as breed, sex, age, diet, bedding, water, weight, alcohol, zinc deficiency and stress (Duarte et al., 2010). The disease can be graded according to the degree of change in the gums and teeth. It can range from mild plaque and gingivitis, to gingival recession and degradation of the periodontal ligament, to significant inflammation and loss of teeth (How et al., 2016).

Various animal models have been used to investigate mechanism of the periodontal disease and the host bacterial interaction. Non-human primates, dogs, pigs, ferrets, rabbits, rats and mice have been used as the animal models (Polak et al., 2009). Rodents, with rats in particular, are important models for experimental periodontal research because rats are easy to handle and inexpensive (Oz & Puleo et al., 2011).

Methods that have been described to induce periodontitis in rats include inoculation Porphyromonus gingivaliss pathogens such as and Actinobacillus of actinomycentemcomitans, or their pathogenic products such as lipopolysaccharide (LPS) and, placement of ligatures in the dentogingival area, which allows accumulation of subgingival microorganisms. In the mouse, the ligature model could result in alveolar bone loss within a short period (Molon et al., 2014). Intra-gingival injection of LPS derived from Porphyromonus gingivalis could also induce periodontal inflammation as well as 8 bone resorption in experimental animals. However, to induce the same lesion, this method may need a longer time (Taguchi et al., 2015). Combination of the ligature technique with inoculation of the bacteria P. gingivalis has been reported to induce alveolar bone loss in the rat model (Meulman et al., 2011). Whether combination of ligature with a pathogenic product, such as lipopolysaccharide from P. gingivalis would accelerate the process is not well described. This thesis hypothesis that combination of ligature and injection of LPS would induce periodontal disease in a shorter time compare to use of either ligature or LPS alone.

1.2 Objectives

This thesis aims to find the best model (periodontal disease production in the shortest time) by describe and compare the morphological, histological and radiological changes of periodontal disease induced by ligature, injection of LPS and

combination of ligature-LPS injection in the Sprague Dawley rat model. The specific objectives are: evaluate the:

- Morphological evaluation of the three different models
 Histological evaluation of the three different models
 Radiological evaluation of the three different models.



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