UNIVERSITI PUTRA MALAYSIA

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

HANA HASSAN MUSTAFA

FPV 2018 12
INDUCTION OF PERIODONTAL DISEASE VIA RETENTIVE LIGATURE, LIPOPOLYSACCHARIDE INJECTION AND THEIR COMBINATION IN A RAT MODEL

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

Chairman : Associate Professor Chen Hui Cheng, PhD
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 2nd 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

Januari 2018

Pengerusi : Profesor Madya Chen Hui Cheng, PhD
Fakulti : Perubatan Veterinar

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I willh like to conclude by extending my most sincere love and gratitude to my family, my husband Pshdar Abdulla and daughter Lani for their patience and support during this most trying period of our lives. I salute your courage, resilience and tolerance. May ALLAH spare our lives to benefit from the results of these sacrifices.

Finally, I am grateful to my parents and siblings for their prayers, love and encouragement. I am grateful and may ALLAH reward you abundantly.
I certify that a Thesis Examination Committee has met on 12 January 2018 to conduct the final examination of Hana Hassan Mustafa on her thesis entitled "Induction of Periodontal Disease via Retentive Ligature, Lipopolysaccharide Injection and their Combination in a Rat Model" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

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<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ABC</td>
<td>Alveolar bone crest</td>
</tr>
<tr>
<td>AL</td>
<td>Attachment loss</td>
</tr>
<tr>
<td>BV</td>
<td>Bone volume</td>
</tr>
<tr>
<td>CEJ</td>
<td>Cemento-enamel junction</td>
</tr>
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<td>CT</td>
<td>Connective tissue</td>
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<tr>
<td>GI</td>
<td>Gingival Index</td>
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<td>H&amp;E</td>
<td>Hematoxylin and eosin</td>
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<td>IACUC</td>
<td>Institutional animal care and use committee</td>
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<tr>
<td>IL-a</td>
<td>Interleukin alpha</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin- six</td>
</tr>
<tr>
<td>IC</td>
<td>Inflammatory cells</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquarter range</td>
</tr>
<tr>
<td>JE</td>
<td>Junctional epithelium</td>
</tr>
<tr>
<td>L</td>
<td>Ligature</td>
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<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>M1</td>
<td>Maxillary first molar teeth</td>
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<tr>
<td>M2</td>
<td>Maxillary second molar teeth</td>
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<tr>
<td>M-CT</td>
<td>Micro-Computed tomography</td>
</tr>
<tr>
<td>μm</td>
<td>Micro meter</td>
</tr>
<tr>
<td>μL</td>
<td>Micro Liter</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
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<td>PD</td>
<td>Periodontal disease</td>
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<tr>
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<td>Plaque index</td>
</tr>
<tr>
<td>PL</td>
<td>Periodontal ligament</td>
</tr>
<tr>
<td>Pg-LPS</td>
<td>Porphyromonas gingival lipopolysaccharide.</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>SE</td>
<td>Standard error</td>
</tr>
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<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>TNF-a</td>
<td>Tumor necrosis factor-alpha</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
<tr>
<td>VOI</td>
<td>Volume of interest</td>
</tr>
<tr>
<td>2-D</td>
<td>Bi dimensional</td>
</tr>
<tr>
<td>3-D</td>
<td>Three dimensional</td>
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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 of pathogens such as Porphyromonas gingivalis and Actinobacillus actinomycetemcomitans, 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 Porphyromonas gingivalis could also induce periodontal inflammation as well as 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:

1. Morphological evaluation of the three different models
2. Histological evaluation of the three different models
3. Radiological evaluation of the three different models.
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