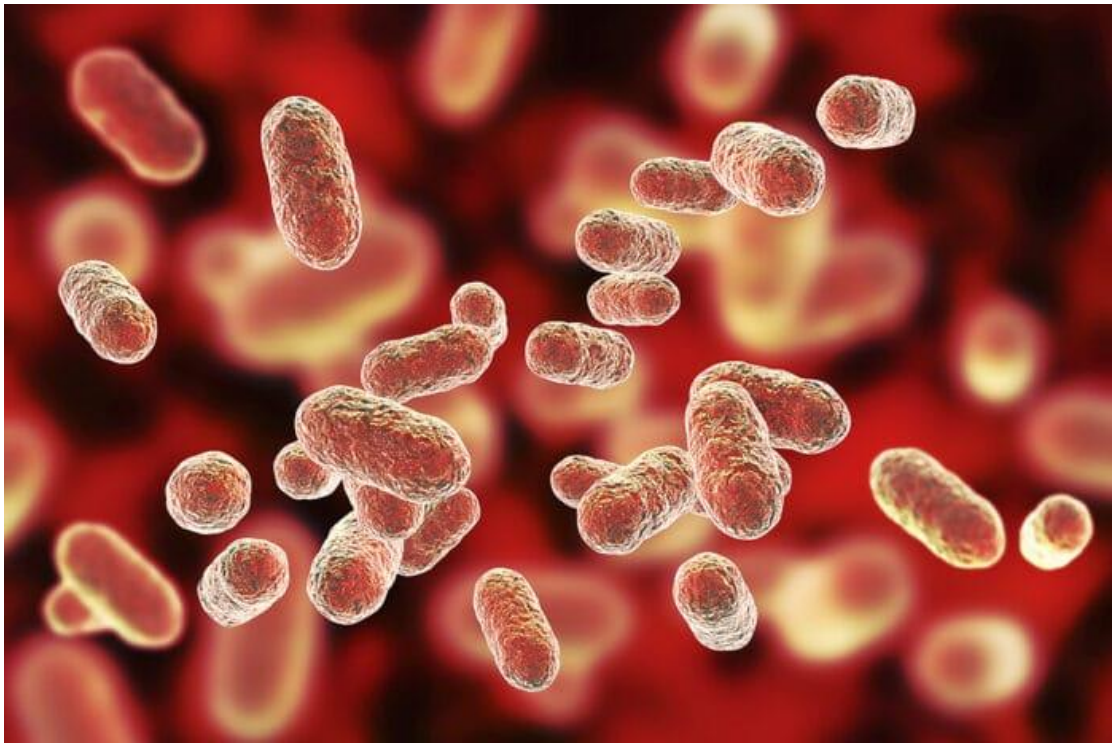
Porphyromonas Gingivalis Linked to Diabetes and Heart Disease



Porphyromonas gingivalis 之內毒素及短鏈脂肪酸對口腔細胞的影響
Effects of Porphyromonas gingivalis LPS and Short-Chain Fatty Acid on Oral Cells

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牙周及牙髓疾病的病原菌能入侵我們的組織，並且影響細胞的表現。微生物和宿主細胞之間的交互作用，在免疫系統中扮演著重要的角色。口腔的病原菌可能本身的構造即具有致病的毒力因子，但也可能會製造出各種致病的代謝產物。目前已知，Porphyromonas gingivalis (P. gingivalis)是造成牙周、牙髓、及根尖疾病的主要致病菌。它是一種革蘭氏陰性厭氧菌，可以產生如丁酸（butyric acid）等短鍊脂肪酸（short chain fatty acid）。丁酸會影響牙周細胞（如：成骨細胞）的活性。P. gingivalis 的內毒素（lipopolysaccharide, LPS）也廣為周知和牙周的嚴重程度相

關。然而，目前的研究對於 P. gingivalis LPS 或是其產物丁酸對於牙周細胞的影響並不是很清楚，而其造成牙髓組織發炎的機制還有待更多的研究以進一步了解。為了瞭解 P. gingivalis 對口腔組織所引起的發炎反應，這個研究分成了三個章節，並以數種生物技術像是：細胞存活率分析（MTT assay）、免疫細胞化學法（immunocytochemistry）、流式細胞術（flow cytometry）、酵素免疫分析法（ELISA）、逆轉錄聚合酶鏈式反應（RT-PCR），以及西方墨點法（Western blot）等進行研究。第一章著重於 P. gingivalis LPS 對牙齦纖維母細胞的影響，針對發炎介質，如：COX-2、Porstanoid、IL-8 等，進行基因及蛋白表現的測試。第二章的研究目的是要評估丁酸在成骨細胞（MG-63 細胞）的細胞毒性、訊息傳導路徑，以及對細胞週期的影響。第三章則聚焦於 P. gingivalis LPS 對於人類牙髓細胞的影響，並瞭解可能的訊息傳導路徑。

經由這幾個部分的研究，我們發現 P. gingivalis LPS 不論對於牙齦纖維母細胞或是牙髓細胞，均呈現低度的細胞毒性，且不會造成細胞型態的改變。低濃度的 P. gingivalis LPS 即可明顯地造成牙齦纖維母細胞表現出發炎介質的基因或蛋白產物。而這些表現和 MEK/ERK 訊息傳導相關，且是牙周發炎致病的關鍵。在人類牙髓細胞，P. gingivalis LPS 可藉由類鐸受器 4 及其銜接蛋白 TIRAP，引發 PI3K/Akt 及 MAPK/ERK 訊息傳導路徑。而 P. gingivalis LPS 所活化的 PI3K/Akt 路徑則可能造成牙髓細胞的細胞凋亡的內在路徑被中止。除此之外，我們發現牙周或根管微生物所分泌的丁酸可能會抑制骨細胞的生長，這可能是藉由引發細胞週期中斷、ROS 的產生，以及抑制膠原蛋白的表現所致。

總結來說，P. gingivalis 的代謝毒性產物及內毒素並不會明顯地影響口腔細胞的細胞活性，但的確會經由幾個訊息傳導路徑啟動細胞的免疫機制，並製造有助於細菌於組織內存活或複製的環境。未來將可開發相關抑制藥物以減少發炎產物的產生，或是阻斷讓此菌得以躲藏寄生之訊息傳導路徑，提供牙周牙髓疾病另一個方向的治療選擇。

Pathogenic microorganisms of periodontal and endodontic diseases can invade our tissue and affect the expression of target cells. The interaction between microorganisms and host cells plays an important role in the immune system. Various toxic products and virulence structures are generated by oral pathogens. Porphyromonas gingivalis (P. gingivalis) is known as a major pathogen of periodontal and pulpal/periapical diseases. It is an anaerobic Gram-negative bacteria and can generated metabolic short chain fatty acid products such as butyrate. Butyrate affects the activity of periodontal cells such as osteoblasts. It is also well known that lipopolysaccharide (LPS) of P. gingivalis relates to the severity of periodontitis. However, the precise effect of P. gingivalis LPS or short chain fatty acids on periodontal cells is not clear, and the inflammatory mechanism of P. gingivalis LPS to pulp tissue await further investigation.

For understanding of the effects of P. gingivalis induced inflammation, this study

was divided into three chapters and using several methods of biotechnology such as: MTT assay, immunocytochemistry (ICC), flow cytometry, reverse transcription-PCR (RT-PCR), Western blot etc. Chapter 1 was dedicated to the effect of P. gingivalis LPS on gingival fibroblasts (GFs). The inflammatory mediators such as COX-2, prostanoids, and IL-8 were exam in gene and protein expression. The specific aim of Chapter 2 was to evaluate the role of butyrate in the cytotoxicity, signaling pathway, and cell cycle of osteoblasts (MG-63 cell line). Chapter 3 focused on the influence of P. gingivalis LPS on human dental pulp cells to elucidate the possible signaling mechanisms.

Through these tests, we found that P. gingivalis LPS showed little cytotoxicity and morphologic changes of gingival fibroblasts and pulp cells. Low concentration of P. gingivalis LPS could obviously stimulated gene expression of differential inflammatory mediators (COX-2 and IL-8) as well as prostanoids and IL-8 production in GFs. These events are associated with MEK/ERK signaling and crucial in the pathogenesis of inflammatory periodontal diseases. On the other hand, we found that the secretion of butyrate by periodontal and root canal microorganisms may inhibit bone cell growth and matrix turnover. This is possibly due to induction of cell cycle arrest and ROS generation and inhibition of collagen expression. In human dental pulp cells, P. gingivalis LPS could in induce PI3K/Akt and MAPK/ERK signaling pathway through TLR4-TIRAP. The P. gingivalis LPS induced activation of PI3K/Akt pathway could arrest the apoptotic process of pulp cells.

In conclusion, the toxic products and virulence structures LPS of P. gingivalis may not have prominent effect on the viability of oral cells, but it indeed induced inflammatory reactions through several signaling pathways for facilitating its intracellular survival. The understanding of immune responses induced by P. gingivalis of pulpal or periodontial cells can offer us a new perspective to develop novel therapeutic strategies against periodontitis and pulpitis.

New evidence suggests that a common bacterium associated with periodontal disease can cause a metabolic syndrome to develop and eventually lead to diabetes and heart disease due to its ability to alter microbiomes in the gut. Metabolic syndrome involves a variety of conditions, including obesity, high blood pressure, altered lipid metabolism, systemic inflammation, and high blood glucose levels that can lead to insulin resistance. The *Federation of American Societies for Experimental Biology* published the study in an article titled, "*Porphyromonas gingivalis* impairs glucose uptake in skeletal muscle associated with altering gut microbiota."

The Dangers of Diabetes and Heart Disease

Diabetes develops when someone has higher than normal blood sugar levels, and their body can't produce enough insulin to convert glucose into energy. The metabolic disorder can eventually cause serious, life-threatening diseases such as chronic kidney disease, cardiovascular heart disease, or a stroke when left untreated. The most common signs and symptoms linked to diabetes include extreme thirst, weight loss, blurred vision, fatigue, and frequent urination.

The Centers for Disease Control and Prevention (CDC) reports there are more than 100 million adults living with diabetes or prediabetes in the United States. Meanwhile, an estimated 9.3 percent of the global adult population were diagnosed with diabetes in 2019, according to Statista. That number is expected to increase to 11 percent by the year 2045, based on their projections.

Heart disease or cardiovascular disease refers to a range of conditions that can interrupt blood flow to the heart or stop it from working properly. Heart disease is the leading cause of death for adults in the United States and around the world. In fact, the CDC reports that a cardiovascular illness causes one out of every four deaths in our nation. The disease also takes an estimated 17.9 million lives each year around the globe, based on data gathered by the World Health Organization.

The Tokyo Medical and Dental University Study

A recent study conducted by researchers from the Tokyo Medical and Dental University (TMDU) revealed oral bacteria *Porphyromonas gingivalis* can alter the composition of the gut's microbiome and cause skeletal muscle metabolic dysfunction. Over time, the dysfunction can alter the skeletal muscle and eventually develop into metabolic syndrome, which may increase someone's risk of being diagnosed with heart disease and diabetes. This is partially because skeletal muscles have a high metabolic capacity due to their essential role in metabolizing glucose.

The researchers enrolled 35 Japanese patients from the Eguchi Hospital and Saga Medical School who were diagnosed with metabolic syndrome in the study. After running tests, they discovered antibodies for *Porphyromonas gingivalis* in their blood – thus showing a correlation between the bacterium, systemic inflammation, and the eventual development of a metabolic syndrome.

Next, the Japanese scientists performed in vivo experiments on lab mice to gather further conclusive evidence. They fed the mice a high-fat diet, so they would develop metabolic syndrome and orally administered *Porphyromonas gingivalis* bacteria twice per week for a duration of six weeks. At the end of the study, they used glucose tolerance and insulin tolerance tests to observe the combined effects of the animal's poor diet and oral bacteria exposure.

The researchers examined the gut microbiome and discovered its microbial network was significantly altered due to *Porphyromonas gingivalis.* The rodent's blood carried antibodies due to the oral bacteria and became systemically resistant to insulin and developed metabolic syndrome over time because of the blood glucose levels. Based on the study's findings, the scientists believe patients who develop periodontal disease due to *Porphyromonas gingivalis* are at high risk for metabolic syndrome and skeletal muscle metabolic dysfunction due to changes in their gut microbiome.