Endotoxin-Secreting Bacteria in Periodontal Disease

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The LPS is recognized by the complex TLR4/MD2, mediated by CD14 and accessory protein LBP, which induces the activation of several transcriptional regulators like factor nuclear kB (NF-kB), activator protein 1 (AP-1) and interferon (IFN) regulatory factors, leading to the expression of genes involved in the host immune response.

endotoxins LPS lipopolysaccharide periodontal disease fluoride therapeutic approach

1. Introduction

The oral cavity is one of the areas of living organisms where the highest rates of microorganisms are located. Among them, bacteria are the most common [1], and Gram-negative bacteria play a key role in oral infections. The virulence factors used by some of the bacteria involved in the evolution of oral infections include the release of lipopolysaccharide, a structural component of the bacterial cell wall that interacts with cells of host oral cavity connective tissue, modulating its immune response and able to cause diseases [2].

One of the oral infections that arouses greater interest due to its epidemiology is periodontal disease and its forms, periodontitis and gingivitis. Periodontitis is characterized by inflammation and destruction of connective and periradicular tissues, resulting from the interaction between microbial factors and the host immune response, which can lead to tooth loss [3]. Literature supports the link between periodontitis and systemic diseases [4], due to a continuous inflammation, bacterial circulation and bacterial products[5].

Among the periodontal pathogens, *P. gingivalis* is one of the most studied, capable of releasing large amounts of external vesicles containing endotoxins [6]. It is a Gram-negative bacterium, present in patients with periodontal disease (PD) that belongs to the group of black-pigmented Bacteroides and is often presented in the form of a coccobacterium. It can produce collagenase, proteases, hemolysins, endotoxins, fatty acids, ammonia, hydrogen sulfide and indole, among other products [7].

2. Endotoxin as a Component of Gram-Negative Bacteria

Lipid A is a phosphorylated glucosamine disaccharide acylated with hydroxyl saturated fatty acids [8][9], responsible for the toxic effects of Gram-negative bacterial infections. Saturated fatty acids further 3-O-acylate the 3-hydroxyl

groups of the fatty acids of lipid A [10][11]. The core oligosaccharide bonds directly to lipid A and contributes to the bacterial viability and stability of the outer membrane. This phosphorylated heterooligosaccharide is also well-preserved in the proximal area to the lipid A.

Even though the biosynthetic pathway and LPS export mechanisms are common to most Gram-negative bacteria, the detailed structure of LPS varies from one bacterium to another and this could affect the virulence. Moreover, some pathogens can modify the basic structure of their LPS during the infection [12][13]. The difference between the LPS of several Gram-negative bacteria is in the length of the fatty acid chains and these seem to be related to the pathogenicity of the bacteria [14][15]. These variations are the basis of altered host immune response [16].

In general, endotoxins are released by secretion, in vesicles formed on the bacterial outer membrane during the bacterium growth phase or are released during cell death, damaging periodontal tissues and triggering inflammation [17]. The vesicles can deliver virulence factors and modulate the host immune system during bacterial pathogenesis. LPS also are released when the cell is chemically treated to remove this glycolipid.

The LPS of *P. gingivalis* provides integrity to the bacterium and offers a mechanism for its interaction with other surfaces, allowing for the formation of biofilms [18]. During its growing phase, pathogenicity factors are released from the outer membrane vesicles (spherical microstructural bodies) [19][20], which are powerful stimulators of the innate immune signal transduction pathways in a tissue/cell-specific manner [21]. The *P. gingivalis* -LPS basic chemical composition is typical of a bacterial endotoxin with a main difference: the Lipid A structure can undergo isomeric acylation in two ways, tetraacylation and pentaacylation, depending on environmental factors such as hemin levels, phosphate availability and incubation temperatures; thus, eliciting differential immunoinflammatory responses [22].

3. Therapeutic Approaches of Oral Diseases and Their Effect on LPS

3.1. Fluoride as Bacterial LPS Inactivator

In general, preventive and therapeutic approach of periodontal and other oral diseases, includes plaque control to reduce microorganism aggregation. This is achieved by sustained and frequent oral hygiene employing mechanical and chemical tools to lower the viral burden. Fluoride has been widely used in dentistry due to its anticaries effect, antimicrobial properties, and desensitizing potential [23][24][25][26][27][28][29].

Madléna et al. (2012) [30] observed a decrease in plaque index, gingival index and bleeding when testing in orthodontic patients using amine fluoride and stannous fluoride (Am/SnF_2) toothpaste in combination or not with a mouth rinse with Am/SnF_2. Under these conditions, the dental plaque shifts towards less acidogenic, thus supporting an antibacterial property of fluoride agents [31][32].

Haught et al. (2016) [33] applied antimicrobial solutions, containing stannous fluoride (SnF_2), to LPS from *E. coli* and *P. gingivalis* to determine, by fluorescence assays and mass spectroscopy, the binding ability of SnF_2. Stannous fluoride interfered with LPS and inhibited the binding to TLR4 in both dying and cellular assays, hence, potentially reducing their effect in the host cells. In another study, stannous fluoride inhibited gene expression response of TLR4 and TLR2 in HEK293 cells, producing a complete inhibition at micromolar concentrations. Moreover, the addition of stannous fluoride suppressed production of TNF_{α}, IFN-g, IL-12p70, IL10, IL-1b, IL-2 and IL-6, and increased secretion of IL-8. Thus, stannous fluoride had the potential to provide benefits in the early signs of periodontal disease, directly decreasing the pathogenicity of plaque biofilms by blocking reactivity of LPS with tissue receptors associated with inflammation [34].

Clinical improvements in gingivitis also have been reported after applying stannous fluoride dentifrice [35]. These authors found significant changes in the number of cultivable Gram-negative organisms in sampled supragingival and subgingival plaque and a considerable reduction in promotion of TLR activation for subgingival plaque samples. Furthermore, Xie et al. (2018) [36], in an expanded analysis of previous studies, observed the hygienic treatment effects of stabilized stannous fluoride tooth paste on chemically measured endotoxins and the activation of TLR based gene expression in TLR2 specific cell line and a THP-1 (multi TLR reporter) cell line. These authors found that SnF_2 dentifrice treatment potentially reduced the endotoxin content and virulence potentiation properties of subgingival dental plaque, therefore concluding that SnF_2 might be beneficial to reduce the pathogenicity of subgingival dental plaque.

3.2. Surgical and Non-Surgical Periodontal Treatment as a LPS Modulator

Authors agree that periodontal treatment can reduce the bacterial burden and therefore the level of LPS, attenuating their potential inflammatory effect in short term. The therapeutic approach, consisting in professional plaque removal together with home-care reinforcement, combined with an antibiotic treatment based in 500 mg amoxicillin and 250 mg metronidazole, three times a day for seven days, was effective in partially modulating LPS responsiveness [37].

Following the periodontal treatment protocol for patients with localized aggressive periodontitis (LAP) described by Shaddox et al. (2013) [38], in which an ultrasonic full-mouth debridement, site-specific scaling and root planning was performed together with the prescription of wide spectrum antibiotics, combined with home-care instructions, a decrease in clinical parameters of the disease was observed probably due to a reduction in the cytokine/chemokine LPS response after treatment [39]. These studies refer to a rebound effect in LPS inflammatory response after six to twelve months, that apparently do not influence the ability of high responsive patients to show reductions in their clinical parameters of disease, likely due to LPS tolerance in these subjects [40][41][42].

3.3. LPS Neutralization by High Density Lipoproteins

Most of lipoproteins have been reported to bind LPS. The high-density lipoproteins (HDLs) seem to be the more efficient for binding and inactivating different types of LPS. According to Meilhac (2012) [43], the plasma phospholipid-transfer protein (PLTP) was then reported to transfer LPS to HDL in conjunction with the LPS-binding

protein (LBP) leading to LPS neutralization. LBP and PLTP can remove LPS from bacterial membranes and transfer it to HDLs [44][45]. Lipid A diglucosamine-phosphate region seems to be responsible for the association of LPS with HDLs and its neutralization relies on LBP, which may form a complex between CD14 and LPS, favoring its binding to HDL particles and subsequent neutralization, revealing a protective and possible contributory potential in periodontal disease treatment [46][47].

References

1. Siqueira, J.F.; Rôças, I.N; Diversity of endodontic microbiota revisited. *J. Dent. Res.* **2009**, *88*, 969–981, 10.1177/0022034509346549.
2. Bertani, B.; Ruiz, N.; Function and biogenesis of lipopolysaccharides. *EcoSal Plus* **2018**, *8*, 10.1128/ecosalplus.ESP-0001-2018, 10.1128/ecosalplus.ESP-0001-2018.
3. Pihlstrom, B.L.; Michalowicz, B.S.; Johnson, N.P.; Periodontal diseases.. *Lancet* **2005**, *366*, 1809–1820., 10.1016/S0140-673667728-8..
4. Bui, F.; Almeida-da-Silva, C.; Huynh, B.; Trinh, A.; Liu, J.; Woodward, J.; Asadi, H.; Ojcius, D.; Association between periodontal pathogens and systemic disease. *Biomed. J* **2019**, *42*, 27–35, doi.org/10.1016/S0140-6736(05)67728-8.
5. Fiorillo, L.; Cervino, G.; Laino, L.; D'Amico, C.; Mauceri, R.; Tozum, T. F.; Gaeta, M.; Cicciù, M.; Porphyromonas gingivalis, periodontal and systemic implications: A systematic review. . *Dent. J.* **2019**, *7*, 114, doi.org/10.3390/dj7040114..
6. Roier, S.; Zingl, F.G.; Cakar, F.; A novel mechanism for the biogenesis of outer membrane vesicles in Gram-negative bacteria.. *Nat. Commun.* **2016**, *7*, 10515, 10.1038/ncomms10515..
7. Fiorillo, L.; Cervino, G.; Laino, L.; D'Amico, C.; Mauceri, R.; Tozum, T. F.; Gaeta, M.; Cicciù, M.; Porphyromonas gingivalis, periodontal and systemic implications: A systematic review.. *Dent. J* **2019**, *7*, 114, 10.3390/dj7040114..
8. Raetz, C.R.; Whitfield, C.; Lipopolysaccharide endotoxins. . *Annu. Rev. Biochem.* **2002**, *71*, 635–700, 10.3390/toxins13080533.
9. Dixon, D.R.; Darveau, R.P.; Lipopolysaccharide heterogeneity: Innate host responses to bacterial modification of lipid a structure.. *J. Dent. Res.* **2005**, *84*, 584–595, 10.1177/154405910508400702..
10. Gao, J.; Guo, Z.; Progress in the synthesis and biological evaluation of lipid A and its derivatives. *Med. Res. Rev.* **2018**, *38*, 556–601, 10.1002/med.21447..
11. Zamyatina, A.; Beilstein, J.; Aminosugar-based immunomodulator lipid A: Synthetic approaches. . *Org. Chem.* **2018**, *14*, 25–53, 10.3762/bjoc.14.3..

12. Wang, X.; Quinn, P.J.; Endotoxins: Lipopolysaccharides of gram-negative bacteria. . *Subcell. Biochem.* **2010**, *53*, 3–25, 10.1007/978-90-481-9078-2_1..
13. Gao, J.; Guo, Z.; Progress in the synthesis and biological evaluation of lipid A and its derivatives. *Med. Res. Rev.* **2018**, *38*, 556–601, 10.1002/med.21447..
14. Varbanets, L.D.; Structure, function and biological activity of lipopolysaccharide lipid A. . *Mikrobiol. Z* **2016**, *78*, 37–49.
15. Steimle, A.; Autenrieth, I.B.; Frick, J.S.; Structure and function: Lipid A modifications in commensals and pathogens.. *Int. J. Med. Microbiol.* **2016**, *306*, 290–301, 10.1016/j.ijmm.2016.03.001..
16. Gronow, S.; Brade, H.; Lipopolysaccharide biosynthesis: Which steps do bacteria need to survive?. *J. Endotoxin Res.* **2001**, *7*, 3-23.
17. Schwechheimer, C.; Kuehn, M.J.; Outer-membrane vesicles from Gram-negative bacteria: Biogenesis and functions.. *Nat. Rev. Microbiol.* **2015**, *13*, 605–619, 10.1038/nrmicro3525..
18. Li, Y.; Shi, Z.; Radauer-Preiml, I.; Andosch, A.; Casals, E.; Luetz-Meindl, U.; Cobaleda, M.; Lin, Z.; Jaberi-Douraki, M.; Italiani, P.; et al.et al. Bacterial endotoxin (lipopolysaccharide) binds to the surface of gold nanoparticles, interferes with biocorona formation and induces human monocyte inflammatory activation.. *Nanotoxicology* **2017**, *11*, 1157–1175., 10.1080/17435390.2017.1401142.
19. Veith, P.D.; Chen, Y.Y.; Gorasia, D.G.; Chen, D.; Glew, M.D.; O'Brien-Simpson, N.M.; Cecil, J.D.; Holden, J.A.; Reynolds, E.C.; Porphyromonas gingivalis outer membrane vesicles exclusively contain outer membrane and periplasmic proteins and carry a cargo enriched with virulence factors.. *J. Proteome Res.* **2014**, *13*, 2420–2432, 10.1021/pr401227e..
20. Xie, H.; Biogenesis and function of Porphyromonas gingivalis outer membrane vesicles.. *Future Microbiol* **2015**, *10*, 1517–1527, 10.2217/fmb.15.63..
21. Jia, L.; Han, N.; Du, J.; Guo, L.; Luo, Z.; Liu, Y.; Pathogenesis of important virulence factors of Porphyromonas gingivalis via toll-like receptors. . *Front. Cell. Infect. Microbiol* **2019**, *9*, 262, 10.3389/fcimb.2019.00262..
22. Cutler, C. W.; Eke, P. I.; Genco, C. A.; Van Dyke, T. E.; Arnold, R. R.; Hemin-induced modifications of the antigenicity and hemin-binding capacity of Porphyromonas gingivalis lipopolysaccha-ride.. *Infect Immun.* **1996**, *64*, 2282–2287, 10.1128/iai.64.6.2282-2287.1996..
23. Marinho, V.C.C.; Higgins, J.P.; Logan, S.; Sheiham, A.; Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents.. *Cochrane Database Syst. Rev.* **2003**, *2003*, CD002782., 10.1002/14651858.CD002782.

24. O'Mullane, D.M.; Baez, R.J.; Jones, S.; Lennon, M.A.; Petersen, P.E.; Rugg-Gunn, A.J.; Whelton, H.; Whitford, G.M.; Fluoride and Oral Health.. *Community Dent. Health* **2016**, *33*, 69–99.

25. Friesen, L.R.; Goyal, C.R.; Qaqish, J.G.; He, T.; Eusebio, R.; Zsiska, M.; Farmer, T.; Schneiderman, E.; Comparative antiplaque effect of two antimicrobial dentifrices: Laboratory and clinical evaluations.. *J. Clin. Dent.* **2017**, *28*, B6–B11..

26. Abdollahi, A.; Jalalian, E.; Effectiveness of Two desensitizer materials, potassium nitrate and fluoride varnish in relieving hypersensitivity after crown preparation. . *J. Contemp. Dent. Pract.* **2019**, *20*, 489–493.

27. Haraszthy, V.I.; Raylae, C.C.; Sreenivasan, P.K.; Antimicrobial effects of a stannous fluoride toothpaste in distinct oral microenvironments.. *J. Am. Dent. Assoc.* **2019**, *150*, S14–S24.

28. Tasios, T.; Papageorgiou, S.N.; Papadopoulos, M.A.; Tsapas, A.; Haidich, A.B.; Prevention of orthodontic enamel demineralization: A systematic review with meta-analyses. *Orthod. Craniofac. Res.* **2019**, *22*, 225–235, 10.1111/ocr.12322.

29. Konradsson, K.; Lingström, P.; Emilson, C.G.; Johannsen, G.; Ramberg, P.; Johannsen, A.; Stabilized stannous fluoride dentifrice in relation to dental caries, dental erosion and dentin hypersensitivity: A systematic review.. *Am. J. Dent.* **2020**, *33*, 95–105.

30. Madléna, M.; Bánóczy, J.; Götz, G.; Márton, S.; Kaán, M.; Nagy, G.; Effects of amine and stannous fluorides on plaque accumulation and gingival health in orthodontic patients treated with fixed appliances: A pilot study. . *Oral Health Dent. Manag* **2012**, *11*, 57–61.

31. Madléna, M.; Bánóczy, J.; Götz, G.; Márton, S.; Kaán, M.; Nagy, G.; Effects of amine and stannous fluorides on plaque accumulation and gingival health in orthodontic patients treated with fixed appliances: A pilot study. . *Oral Health Dent. Manag* **2012**, *11*, 57–61.

32. Koopman, J.E.; van der Kaaij, N.C.; Buijs, M.J.; Elyassi, Y.; van der Veen, M.H.; Crielaard, W.; Ten Cate, J.M.; Zaura, E.; The effect of fixed orthodontic appliances and fluoride mouthwash on the oral microbiome of adolescents—A randomized controlled clinical trial.. *PLoS ONE* **2015**, *10*, e0137318, 10.1371/journal.pone.0137318..

33. Haught, J.C.; Xie, S.; Circello, B.; Tansky, C.S.; Khambe, D.; Sun, Y.; Lin, Y.; Sreekrishna, K.; Klukowska, M.; Huggins, T.; et al.et al. Lipopolysaccharide and lipoteichoic acid binding by antimicrobials used in oral care formulations. *Am. J. Dent* **2016**, *29*, 328–332.

34. Haught, C.; Xie, S.; Circello, B.; Tansky, C.S.; Khambe, D.; Klukowska, M.; Huggins, T.; White, D.J.; Lipopolysaccharide and lipoteichoic acid virulence deactivation by stannous fluoride.. *J. Clin. Dent* **2016**, *27*, 84–89.

35. Klukowska, M.; Haught, J.C.; Xie, S.; Circello, B.; Tansky, C.S.; Khambe, D.; Huggins, T.; White, D.J.; Clinical effects of stabilized stannous fluoride dentifrice in reducing plaque microbial virulence I: Microbiological and re-ceptor cell findings. *J. Clin. Dent.* **2017**, *28*, 16–26.

36. Xie, S.; Haught, J.C.; Tansky, C.S.; Klukowska, M.; Hu, P.; Ramsey, D.L.; Circello, B.; Huggins, T.G.; White, D.J.; Clinical effects of stannous fluoride dentifrice in reducing plaque microbial virulence III: Lipopolysaccharide and TLR2 re-porter cell gene activation. . *Am. J. Dent.* **2018**, *31*, 215–224.

37. Shaddox, L.M.; Gonçalves, P.F.; Vovk, A.; Allin, N.; Huang, H.; Hou, W.; Aukhil, I.; Wallet, S.M.; LPS-induced inflammatory response after therapy of aggressive periodontitis.. *J. Dent. Res.* **2013**, *92*, 702–708, 10.1177/0022034513495242.

38. Shaddox, L.M.; Gonçalves, P.F.; Vovk, A.; Allin, N.; Huang, H.; Hou, W.; Aukhil, I.; Wallet, S.M.; LPS-induced inflammatory response after therapy of aggressive periodontitis. . *J. Dent. Res.* **2013**, *92*, 702–708, 10.1177/0022034513495242.

39. Allin, N.; Cruz-Almeida, Y.; Velsko, I.; Vovk, A.; Hovemcamp, N.; Harrison, P.; Huang, H.; Aukhil, I.; Wallet, S.M.; Shaddox, L.M.; et al. Inflammatory response influences treatment of localized aggressive periodontitis.. *J. Dent. Res.* **2016**, *95*, 635–641, 10.1177/0022034516631973..

40. Shaddox, L.M.; Gonçalves, P.F.; Vovk, A.; Allin, N.; Huang, H.; Hou, W.; Aukhil, I.; Wallet, S.M.; LPS-induced inflammatory response after therapy of aggressive periodontitis. . *J. Dent. Res.* **2013**, *92*, 702–708, 10.1177/0022034513495242.

41. Allin,N.; Cruz-Almeida,Y.; Velsko,I.; Vovk,A.; Hovemcamp,N.; Harrison,P.; Huang,H.; Aukhil,I.; Wallet,S.M.; Shaddox,L.M.; et al. Inflammatory response influences treatment of localized aggressive periodontitis. . *J. Dent. Res.* **2016**, *95*, 635–641, 10.1177/0022034516631973..

42. Nahid, M.A.; Satoh, M.; Chan, E.K.; MicroRNA in TLR signaling and endotoxin tolerance.. *Cell Mol. Immunol* **2011**, *8*, 388–403, 10.1038/cmi.2011.26.

43. Meilhac, O.; Tanaka, S.; Couret, D.; High-density lipoproteins are bug scavengers.. *Biomolecules* **2020**, *10* , 598, 10.3390/biom10040598.

44. Meilhac, O.; Tanaka, S.; Couret, D.; High-density lipoproteins are bug scavengers. *Biomolecules* **2020**, *10*, 598, 10.3390/biom10040598.

45. 159. Vesy, C.J.; Kitchens, R.L.; Wolfbauer, G.; Albers, J.J.; Munford, R.S.; Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from gram-negative bacterial membranes. *Infect. Immun.* **2000**, *68*, 2410–2417, 10.1128/IAI.68.5.2410-2417.2000.

46. Meilhac, O.; Tanaka, S.; Couret, D.; High-density lipoproteins are bug scavengers.. *Biomolecules* **2020**, *10*, 598, 10.3390/biom10040598.

47. Brandenburg, K.; Jürgens, G.; Andrä, J.; Lindner, B.; Koch, M.H.J.; Blume, A.; Garidel, P.; Biophysical characterization of the interaction of high-density lipoprotein (HDL) with endotoxins. *Eur. J. Biochem.* **2002**, *269*, 5972–5981, 10.1046/j.1432-1033.2002.03333.x.