Selective Antimicrobial Therapies for Periodontitis

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Traditional antimicrobial therapies for periodontitis (PD) have long focused on non-selective and direct approaches that give immediate though short lived clinical improvements. Professional cleaning of the subgingival biofilm by instrumentation of dental root surfaces, known as scaling and root planning (SRP), is the mainstay of periodontal therapy and is indisputably effective. Non-physical approaches, used as adjuncts to SRP, such as chemical and biological agents, will be the focus of this review. We will review traditional antibiotic and antiseptic approaches, but will emphasize immunotherapeutic agents under development that indirectly inhibit microbial colonization/growth and/or bone loss by reducing inflammation. Moreover, those agents that have clear molecular targets and defined mechanisms of action will be stressed. Many of these 'host modulation' agents, have only become possible through decades of research on host-pathogen interactions and the immunopathogenesis of PD. More investment in such approaches is warranted and a goal worth pursuing given the tremendous benefits selective approaches might offer to patients with periodontitis.

Keywords: periodontitis ; antimicrobial therapy ; antibiotics ; antiseptics ; NSAIDS ; resolvins ; antimicrobial peptides ; NLRP3 ; inflammasomes ; exosomes ; dysbiosis ; Porphyromonas gingivalis

1. Introduction

Periodontitis (PD) is one of the most prevalent diseases globally, involving aging or inflammaging [1]. Once thought to be transmissible [2], there is no credible evidence for transmission of PD to an otherwise healthy host, though bacterial transmission does occur [3]. Thus, PD is not an infection in the traditional sense, as defined by Koch [4], but is a result of a polymicrobial consortium of key microbial species contained within the oral biofilm [5], called keystone pathogens [6]. These species exert undue influence on the commensal species and induce a dysbiosis, characterized by a disruption of the homeostatic balance needed to respond effectively, resulting in unregulated inflammation and alveolar bone loss. Traditional antimicrobial approaches to PD involve the direct killing of pathobionts, removal or disruption of the biofilm, with little to no selectivity.

2. Direct Antimicrobial Therapies

The advantages and disadvantages of conventional therapies that promote microbial clearance in and around the periodontal pockets are briefly reviewed to provide context for the new unconventional and pioneering therapies.

2.1. Systemically Administered Antibiotics

The use of antibiotics, taken perorally, combined with mechanical debridement, can help control periodontal pathogens in recalcitrant cases of periodontitis (PD). Both broad and narrow-spectrum antibiotics, alone or in combination, have been used as adjunctive therapy to SRP. Broad-spectrum antibiotics can eliminate or inhibit the growth of Gram-negative facultative and obligate anaerobes within the tissues. Typical examples include amoxicillin with or without clavulanic acid, azithromycin, ciprofloxacin, tetracycline, and doxycycline. Antibiotics with a narrower spectrum, such as metronidazole and clindamycin, are generally preferred with selectivity for anaerobic pathogens. Numerous interventional and observational studies highlight the clinical improvements observed in patients receiving systemic antibiotic therapy, particularly in association with mechanical periodontal therapy and oral hygiene instructions [7][8]. However, the question still remains as to whether these short-term gains are worth the cost in terms of antibiotic resistance and its effects on the oral and gut microbiome [9].

Systemic antimicrobials are principally indicated in immune-compromised PD patients or those with metabolic disorders that limit their response to mechanical therapy [10]. The ability of antibiotics to eliminate dysbiotic oral microbes, e.g., *Porphyromonas gingivalis,* from cellular reservoirs may be particularly relevant to efficacy [11][12]. Perhaps the most commonly prescribed antibiotic regimen for periodontal infections is a combination of metronidazole and amoxicillin [13][14][15]. The regimen ablates so-called ‘red complex’ pathogens [16] *Tannerella forsythia*, *Treponema denticola,* and

P.gingivalis, and other potentially beneficial species [5]. Systematic reviews suggest that such a regimen may be useful, in the short term, in treating PD in otherwise healthy patients. Interestingly, this combination also significantly influences inflammation by increasing the stability of regulatory T cells (Treg), reducing their conversion to bone damaging Th17 cells [12]. Various randomized controlled trials have reported clinical benefits of adjunct systemic antibiotics in combination with non-surgical mechanical therapy; however, long-term benefits are inconclusive. Meta-analyses support the clinical benefits for PD, but these benefits decline after 12 months [17]. Antibiotics influence the community composition of the gut [18] and oral microbiomes [19]. Broad-spectrum antibiotics are contraindicated in patients with a history of *Clostridium difficile*-positive ulcerative colitis [20].

2.2. Locally Administered Antibiotics

Administering antibiotics locally can overcome many of the drawbacks of systemic delivery, precluding disturbances of the gut microbiome and patient compliance issues. Sustained release devices, including films, fibers, strips, gels, injectable devices, micro-and nanoparticles, have been used to treat PD since the introduction of tetracycline-infused fibers by Max Goodson in 1979 [21]. Sustained delivery in the periodontal pocket, subjacent to the site of bacterial invasion, makes intuitive sense, with increasing evidence to back it up. Systematic reviews report adjunctive reductions in probing depth, gingival inflammation, plaque scores, and bleeding indices when mechanical debridement was combined with local delivery drugs like minocycline hydrochloride (ARESTIN), chlorhexidine gluconate (PERIOCHIP), 10% doxycycline hyclate (ATRIDOX), and tetracycline hydrochloride (PERIODONTAL PLUS AB) compared to SRP alone [22]. Typical results with sustained local delivery devices in deep sites, following proper mechanical debridement and behavioral risk management, include reducing 0.4 mm in pocket depths and 0.3 mm gain in clinical attachment levels [23].

2.3. Antimicrobial Photodynamic Therapy (aPDT)

Although locally delivery of antibiotics is an effective alternative to systemic approach, the development of antibiotic-resistant strains has been reported [24]. Therefore, another approach for local delivery of antimicrobial agents such as aPDT has been investigated in clinical trials for periodontitis [25]. This technique is based on the use of a light-sensitive dye (photosensitizer) stimulated with visible light of an appropriate wavelength. Upon activation of the dye, free radicals of singlet oxygen are formed, cytotoxic to periodontal pathogens [26]. However, aPDT, when used as an adjunctive treatment, showed similar improvements in PD reduction and CAL gain compared with conventional periodontal therapy in periodontitis patients [25][27]. In addition, available evidence on the adjunctive therapy with aPDT is limited by the short-term follow-up, the low number of randomized controlled studies, and study design inconsistency [28][29].

2.4. Oral Antiseptics

Oral antiseptics have multiple applications in periodontal care, including home rinses, pre-procedural rinses, post-surgical care, and pocket irrigation. Particularly apt are applications for patients with physical or developmental disabilities, oral mucositis from radiation or chemotherapy, recurrent aphthous ulcers, and oral candidiasis. The most widely used classes of oral antiseptics are bisbiguanides (chlorhexidine), essential oils (eucalyptol, menthol, thymol), and quaternary ammonium compounds. These are often solubilized in alcohol and delivered via various vehicles: oral rinses, gels, pastes, chewing gums, lozenges, aerosols, varnishes, and sustained release devices (reviewed in Reference [30]). Mechanisms of action include permeabilization of the plasmatic membrane, precipitation of cytoplasmic proteins, cell wall disruption, inhibition of bacterial enzymes, extraction of endotoxins derived from lipopolysaccharide (LPS) of Gram-negative bacteria, and anti-inflammatory action based on antioxidant activity [31][32][33][34][35][36][37][38][39]. The chief advantage of chlorhexidine is its substantivity, or the ability to bind reversibly to oral tissues. This results in sustained antimicrobial effect (up to 12 h) [40][41][42].

3. Indirect Antimicrobial Therapies

It is becoming increasingly clear that to win the battle and war against PD; novel antimicrobial strategies need to be developed that do not wipe out the resident flora. Instead, approaches targeting microbial mechanisms of colonization, nutrient acquisition, or accelerate their clearance by the immune system are required.

3.1. Probiotics

Probiotics are a well-accepted approach to treating dysbiotic conditions of the gut, such as *Clostridium difficile* infection. Fecal microbiota transplants from healthy patients can restore microbial homeostasis in the gut by replacing pathogens with commensal microbiota [20]. However, the application of probiotics for gingivitis and PD is presently in its infancy, with long-term studies yet to be carried out. Nonetheless, intriguing studies have been reported (reviewed in [43]. For example, *Lactobacillus reuteri* was applied in a randomized, placebo-controlled clinical trial to a small cohort of 59 gingivitis patients

over two weeks. The results indicate significant improvement in plaque index and gingival index in the test group, not observed in the placebo group [44]. Recent studies also showed that an adjunctive consumption of *Lactobacillus* reuteri lozenges improved CAL change at molar sites with ≥5 mm deep pockets [45] without influencing pocket colonization with periodontal pathogens [46]. Another meta-analysis supported the adjunctive use of L. reuteri to SRP in deep pockets [47]. In an 8-week study, *Lactobacillus salivarius* WB21 was applied, in tablet form, to volunteers without severe PD, using a randomized, double-blind, placebo-controlled study design. Significant improvements in plaque index and probing pocket depth was observed in current smokers in the test group [48]. The use of a probiotic yogurt supplemented with *Bifidobacterium animalis* was shown to have a positive effect on reducing plaque accumulation and gingival inflammatory parameters [49]. Another study suggested that the use of *Bifidobacterium lactis* HN019 as an adjunct to SRP could provide additional clinical, microbiological, and immunological improvements in periodontitis patients [50]. Further long-term studies in larger patient cohorts and that measure clinical attachment loss and alveolar bone are required before conclusions can be drawn about clinical efficacy of oral probiotics.

3.2. Host Modulation with NSAIDS, Bisphosphonates, and SDD

Uncontrolled inflammation, triggered by microbial dysbiosis [6], is the principal cause of tissue destruction in PD. Dysbiotic oral pathogens benefit from inflammation and tissue proteolysis products, essential nutrients for their growth [51]. Thus, a vicious cycle occurs, with infection triggering inflammation and inflammation fueling infection. Therefore, a prudent strategy is to break the cycle by targeting the inflammation, infection, or both. This can consist of selectively starving the pathogens, preventing their colonization, enhancing their clearance [52], or resolving inflammation [53]. Host modulation using agents such as non-steroidal anti-inflammatory drugs (NSAIDS), bisphosphonates, and tetracycline have been reported. The sole drug approved by the FDA in this regard is sub-antimicrobial dose doxycycline (SDD). In early studies by Golub, short-term administration of SDD reduced collagenase activity [54]. Subsequent clinical trials increased the dosing regimen to longer periods, showing a reduction of inflammatory biomarkers. Cessation of therapy reversed collagenase activity to pre-treatment levels [55]. The increased duration of SDD therapy to regimens of 3 and 6 months up to 1 and 2 years have been proven with significant clinical efficacy, safety, and substantivity by various types of studies that followed [56][57][58][59][60][61]. These types of therapies inhibit collagenases without applying selective pressure that could drive antibiotic resistance.

3.3. Monoclonal Antibodies/Cytokine Inhibitors

More selective and targeted strategies are needed to modulate collateral tissue damage. Cytokine inhibitors such as anti-TNF-α, anti-IL-17A, and anti-IL-6 have been used in other inflammatory-driven diseases such as rheumatoid arthritis (RA). Compelling evidence shows that circulating levels of TNF-α are associated with the more severe manifestation of PD, especially in the elderly population [62]. The influence of treatment of rheumatoid arthritis with infliximab (anti-TNF-α) on comorbid PD has been examined [63]. Intriguingly anti-TNF therapy unexpectedly aggravated gingival inflammation. However, attachment loss was decreased [63], with the overall conclusions drawn that periodontal tissue destruction and gingival inflammation are distinct events in PD pathogenesis. Comparative studies of patients with autoimmune diseases, including rheumatoid arthritis, demonstrated that these patients have higher periodontal indices (bleeding on probing, pocket depth, clinical attachment loss) and higher TNF-α levels in gingival crevicular fluid than healthy controls, with anti-TNF-α effectively reversing this disparity [64][65]. Another study compared the periodontal condition in patients with RA and PD before and after therapy with an IL-6 receptor inhibitor, tocilizumab. Anti-IL-6 receptor therapy showed beneficial effects in serum inflammatory mediators, decreasing serum levels of TNF-α, total IgG, and serum amyloid A, though serum IL-6 and soluble IL-6R were significantly increased [66]. Neither study analyzed the influence on alveolar bone loss. In diabetic mice subjected to *P. gingivalis* oral infection, anti-TNF-α antibody reduced serum TNF-α, IL-6, and fasting blood glucose levels [67]. This same study showed marked improvement of wound healing in diabetic mice after *P. gingivalis* inoculation [67]. Wistar rat study of ligature-induced PD showed infliximab significantly reduced granulocyte blood counts, gingival IL-1β, TNF- α, and MPO levels, and diminished MMP-1/-8 RANK, and RANK-L in bone. Periodontal histopathological scores were also improved, and alveolar bone loss was inhibited [68].

3.4. Pro-Resolving Mediators

In this context, the ‘keystone pathogen’ hypothesis of PD pathogenesis [6] should be reiterated. Keystone pathogens, such as *P. gingivalis*, are those that assert undue influence on the local microbiome, causing dysbiosis and resulting in chronic inflammation. In parallel, but not at odds with this hypothesis [69], is the notion that unresolved inflammation, especially neutrophil-mediated, is the principal driving force for pathogenesis (reviewed in [70]). This notion has received considerable attention of late, involving novel therapeutic approaches targeting neutrophil emigration and clearance through manipulation of CXCL8 [71][72][73] or applying pre-resolving mediators. The latter include resolvins, protectins, maresins, and lipoxins. Given the importance of leukocyte trafficking in inflammation, endogenous positive and negative

signaling mediators of inflammation have been referred to as local ‘go’ and ‘stop’ signals. Lipoxins (LX) are an important ‘stop’ signal produced in vivo during inflammation [74]. In an experimental PD model in rabbits, application of LXA4 or over-expression of 15-lipoxygenase promoted reduced inflammatory phenotype and were protective against alveolar bone loss [75]. Resolvin E1 (RvE1) has emerged as particularly efficacious. RvE1 is biosynthesized from eicosapentaenoic acid (EPA) and selectively interacts with specific receptors to inhibit leukocyte infiltration, obtund cytokine generation, and promote PMN apoptosis. The latter favors PMN clearance by macrophages and restoration of tissue homeostasis [76]. Nanomolar doses of RvE1 inhibit RANKL-induced osteoclast growth and differentiation, downregulating bone resorption in vitro [77]. A study of human periodontal ligament stem cells showed that in pro-inflammatory milieu, pluripotency, viability, and cell migration were suppressed, whereas maresin-1 (MaR1) and RvE1 restored tissue regenerative capacity [78]. RvE1 application is also effective at promoting bone preservation in the mouse calvaria model [79] and regenerating bone in the ligature-induced periodontitis in rats [80]. Moreover, shifts in the subgingival microbiota, i.e., dysbiosis, induced with ligature were markedly altered by RvE1; namely, *P.gingivalis* was reduced. Simply put, RvE1-mediated regulation of inflammation appears to reverse the dysbiosis coincident with prevention and treatment of inflammatory disease [80].

3.5. Biologics

Peptides derived from the innate immune defense system (anti-microbial peptides), from oral microbes, and specific inhibitors of NLRP3 inflammasome or senescence (senolytic agents) can be a therapy.

4. Exosome-Based Therapies

The discovery in 1987 of exosomes (EXO) [81] was a major breakthrough in understanding how cells communicate with other cells (reviewed in Reference [82]). EXO are nano-sized extracellular vesicles secreted by all cells. Originally considered cellular waste, EXO have emerged as important ‘packets’ of molecular information, reflective of the physiologic and pathologic state of the source cell [83][84][85][86][87]. Their presence in most body fluids and content of a repertoire of proteins [87][88][89][90], mRNAs, and miRNAs [87][91] has raised excitement for potential in disease diagnostics [92]. Saliva exosomes, for example, are under intensive study for early disease biomarkers [93]. Exogenously created EXO are being used for various therapeutic applications [94][95]. These include the use of dendritic cell (DC) derived immunoregulatory EXO for experimental PD [96]. Distinguishing features of EXO include their size (20–150 mm), shape [97], gradient density [98], and mode of biogenesis. The extracellular domain of EXO contains various adhesion molecules, including tetraspanins and integrins that promote binding to and activation of host immune cells [95][96][99] and regulation of antigen-presenting activities [95]. The miRNA content [96] and proteome [99] of dendritic cell-derived exosomes, both natural and engineered to regulate alveolar bone loss, have been extensively characterized by our group. EXO protects their cargo from proteolytic degradation [96] and can transfer it to local acceptor immune cells in the gingiva, for example [96], or to distant sites through the bloodstream, such as the lungs, liver, and secondary lymphoid organs [99]. Several other studies have established the capability of EXO as a natural nano delivery approach for a variety of infectious and immune/inflammatory diseases [100][101][102][103][104][105][106].

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