Review Article

The implication of the PD-1/PD-L1 checkpoint in chronic periodontitis suggests novel therapeutic opportunities with natural products

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SUMMARY

An analysis of the implication of the PD-1/PD-L1 immune checkpoint in periodontitis is provided with the objective to propose a novel therapeutic approach. An exhaustive survey of the literature has been performed to answer two questions: (1) Is there a role for PD-1 and/or PD-L1 in the development of periodontitis? (2) Which natural products interfere with the checkpoint activity and show activity against periodontitis? All online published information was collected and analyzed. The pathogenic bacteria *Porphyromonas gingivalis*, through its membrane-attached peptidoglycans, exploits the PD-1/PD-L1 checkpoint to evade immune response and to amplify the infection. Three anti-inflammatory natural products (and derivatives or plant extracts) active against periodontitis and able to interfere with the checkpoint were identified. Both curcumin and baicalin attenuate periodontitis and induce a down-regulation of PD-L1 in cells. The terpenoid saponin platycodin D inhibits the growth of *P. gingivalis* responsible for periodontitis and shows a rare capacity to induce the extracellular release of a soluble form of PD-L1, thereby restoring T cell activation. A potential PD-L1 shedding mechanism is discussed. The targeting of the PD-1/PD-L1 immune checkpoint could be considered a suitable approach to improve the treatment of chronic periodontitis. The plant natural products curcumin, baicalin and platycodin D should be further evaluated as PD-1/PD-L1 checkpoint modulators active against periodontitis.

1. Introduction

Chronic periodontitis is considered as an inflammatory disease implicating microbial factors that induce a series of host defense responses. The main pathobiont implicated in the disease is the oral Gram-negative bacteria *Porphyromonas gingivalis*, present in the mouth and which can penetrate deep into the tissues. It can invade host cells and dispose of an array of virulence factors, such as lipopolysaccharide (LPS), fimbriae and gingipain proteases, which are used to induce an inflammatory environment and to subvert the immune response to evade bacterial clearance [1]. *P. gingivalis* is one of the main microbes implicated in the biofilm formation of bacterial plaque, responsible for local gingivitis and systemic inflammation. The gum disease is characterized by progressive destruction of gingival connective tissue and in the resorption of alveolar bone, leading ultimately to tooth loss. As a chronic non-communicable disease, periodontitis has a high prevalence affecting 11% of the world's population and considered the sixth most common human disease [2]. In the USA, the periodontitis prevalence has been estimated recently to 42.2% of the population with 7.8% of people experiencing severe periodontitis [3].

Chronic periodontitis refers to a progressed situation, beyond a local and reversible gingivitis into a chronic, destructive, irreversible inflammatory disease state (Fig. 1). The disease can contribute to systemic inflammation. Links have been discussed between periodontal disease and other diseases, such as atherosclerosis, ischemic cardiac disease [4] and obstructive sleep apnea [5]. In fact, many comorbid diseases have been suggested like Alzheimer's disease, macular degeneration, chronic kidney disease, hypothyroidism, nosocomial pneumonia, psoriasis and rheumatoid arthritis [6–11]. Periodontal disease is also considered as a risk factor for hematopoietic and lymphatic cancers [12]. Administration of *P. gingivalis* promotes resistance to the anticancer drug paclitaxel in mice bearing oral squamous cell carcinoma (OSCC) tumors [13]. The risk of developing OSCC seems to increase with periodontal disease [14]. The proposed links rely on the fact that the bacteria-
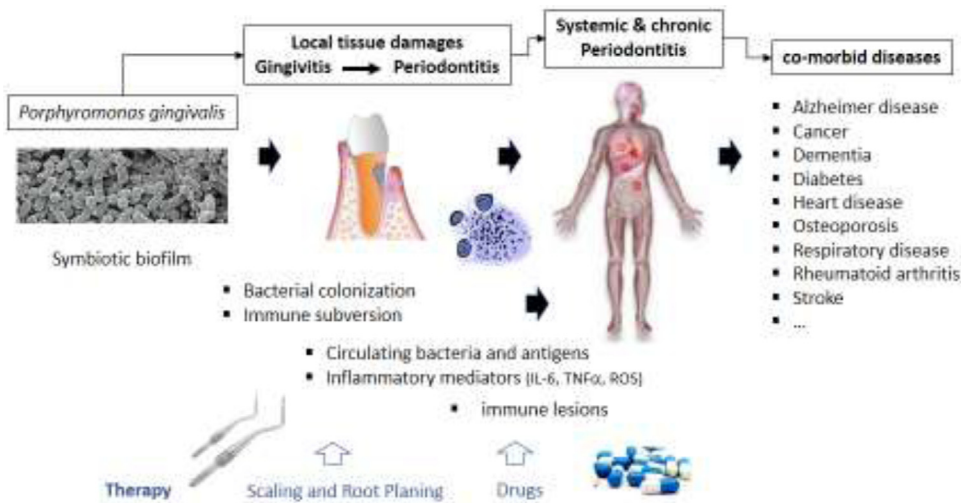
Fig. 1. Illustration of periodontal disease progression, from initial tissue colonization by *P. gingivalis* and other pathogenic bacteria (perturbing the oral microbiota) to tissue damages caused by the spread of the bacteria and/or antigenic components. The inflammatory and immune lesions that accompany disease progression can contribute to the development of comorbid pathologies, as those indicated. The therapeutic approaches need to be adapted to the disease evolution, with mechanical treatment at the early stage of the disease (SRP) and then local and systemic drug treatments.

induced local inflammatory process can intensify inflammation at distant sites, to favor the occurrence of other diseases. A local bacterial colonization and inflammation in the mouth can lead to the introduction of pathogenic microorganisms and/or the distribution of proinflammatory cytokines at distant sites, notably in the central nervous system (Fig. 1). Moreover, these effects can be aggravated by the excessive consumption of alcohol which increases the risk of periodontitis [15]. In this context, it is essential to identify new treatments to combat these hyper-inflammatory immune lesions responsible for tissue damages. The recent characterization of the role of PD-1/PD-L1 checkpoint in the development of chronic periodontitis suggests novel therapeutic opportunities. This topic is discussed here.

2. Inflammation and periodontitis

The pathogen *P. gingivalis* plays a major role in the reduction, if not the suppression, of the host's adaptive immune system (Fig. 2). In particular, the bacterium potently inhibits interleukin 2 (IL-2) synthesis and secretion, thereby reducing the activation of T and B cells and thus the secretion of interferon γ (IFNγ). The pathogenic mechanism is complex, chiefly implicating IL-2 and IFNγ production inhibition but also the down regulation of several immune response-regulated genes and a modulation of the Th17/Treg imbalance. Via these different signals/routes and through other virulence factors, the pathogen diminishes adaptative immunity, thereby contributing to the development of periodontitis and other associated inflammatory pathologies as a result of the dissemination of the pathogen in the circulation [16]. Other cytokines play a role in periodontitis notably IL-1β, IL-6, IL-17 and IL-18 which are produced after inflammasome activation that induces maturation of these cytokines through their cleavage by caspase-1 [17,18]. Notably IL-1β reaches a high level in the serum of patients with chronic periodontitis and it represents an attractive target to combat the disease [19]. *P. gingivalis* induces a high level of IL-1β and IL-6 by peripheral CD4$^+$ T helper cells and these pro-inflammatory cytokines contribute to the pathogenesis of aggressive periodontitis [20]. Consequently, the use of products able to attenuate the expression of proinflammatory cytokines in periodontal cells is considered for the treatment of the disease (see below).

3. The PD-1/PD-L1 pathway in periodontitis

The PD-1 (receptor, CD279)/PD-L1 (ligand, B7-H1, CD274) co-inhibitory pathway plays critical roles in the immune response and autoimmunity via the regulation of T cell activity. Many tumor cells express high levels of PD-L1 which exerts immunosuppressive effects and strongly limit treatment efficacy. The checkpoint is also functional in tumor-associated macrophages [21]. This checkpoint is recognized as a major driver in many types of cancers and anti-PD-L1 + anti-PD-1 drugs (monoclonal antibodies) have revolutionized the therapy of cancer [22–24]. The targeting of this pathway could also be useful to treat non-oncological diseases, such as cardiovascular disease, Alzheimer and other pathologies [25–27].

Recent studies have demonstrated that the PD-1/PD-L1 pathway is involved in periodontitis. PD-L1 is physiologically expressed on the oral masticatory mucosae in the oral cavity, with regulated expression on both prickle cells and basal keratinocytes. PD-L1-expressing keratinocytes importantly contribute to the regulation of CD4$^+$ T cell-mediated local tissue inflammation and thereby they protect against excess tissue damage [28]. PD-L1 mRNA is detected in the saliva from patients with periodontitis and the levels correlate with the severity of the disease, thus reflecting the progression of the disease [29]. A procedure has been designed to detect exosomal PD-L1 in the saliva of patients in both the localized oral disease and the systemic disease [29]. A link between PD-1 or PD-L1 expression and the periodontal condition was also established upon analysis of the expression of the checkpoint on T lymphocytes from patients with periodontitis versus healthy control. The expression of both PD-1 and PD-L1 was found to be significantly higher on CD4$^+$ and CD8$^+$ T lymphocytes from periodontitis patients versus control and, interestingly, the level of expression diminished after initial therapy [30]. Another study indicated that the expression of PD-L1 was significantly higher in the periodontal tissue of the mild chronic periodontitis compared to the severe periodontitis, therefore suggesting a negative regulation by PD-L1 of the inflammatory periodontal tissue damages [31]. More recently, apical periodontitis lesions were found to be more infiltrated by PD1$^+$ and PD-L1$^+$ lymphocytes than control samples, and the cytokine levels were also significantly higher [32]. Finally, it is worth mentioning also a recent study showing that mesenchymal stem/stromal cells isolated from dental pulp, gingival tissue, and
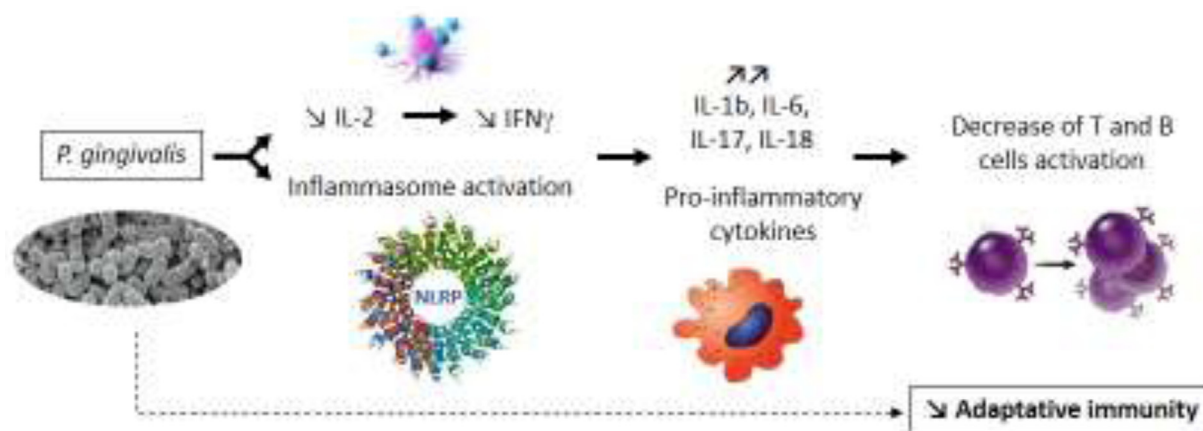
Fig. 2. Schematic illustration of the immunogenic impact of *P. gingivalis* colonization. The pathogen activates inflammasomes, inhibits IL-2 synthesis and secretion, leading to the enhanced production of pro-inflammatory cytokines, and then a deactivation of B and T cells. These effects, together with other inflammatory processes, concur to reduce adaptative immunity.

periodontal ligament all present an upregulation of PD-L1 and show immunoregulatory properties similar to those from bone marrow mesenchymal stem/stromal cells [33].

The up-regulation of PD-L1 in cells can be caused by the pathogenic agent *P. gingivalis*. This was initially shown in human gingival keratinocytes and using the SCC-25 and BHY squamous cell carcinoma cell lines [34]. A prominent increase of the expression of PD-1 and PD-L1 has been observed in CD4+ T cells and CD11b+ cells, respectively, in mice primed with *P. gingivalis*. The upregulation of PD-1 was completely dependent on IL-10 signaling, but not that of PD-L1 [35]. In fact, an up-regulation of PD-L1 frequently occurs under inflammatory conditions, in order to adjust the immune response via a down-regulation of active T cells. An increased expression of PD-L1 can also attenuate the immune system and thus facilitate a chronic bacterial infection. A study of the mechanism by which *P. gingivalis* induces PD-L1 expression in gingival keratinocytes has shown that the up-regulation can be caused by membrane fractions of the bacteria (both inner and outer membrane fractions), and suggested that the peptidoglycans attached to these fractions within the periplasm would be responsible for the PD-L1 inducing activity [36]. By so doing, *P. gingivalis* exploits the PD-1/PD-L1 checkpoint to evade immune response and to amplify the infection. The authors have recently confirmed this hypothesis by showing that the peptidoglycans internalize into oral carcinoma cells via bacterial outer membrane vesicles and then trigger cytosolic receptors to induce PD-L1 expression via a mechanism implicating the protein kinase RIP2 (receptor-interacting serine/threonine-protein kinase 2) [37]. A simplified schematic illustration of the mechanism leading to PD-1/PD-L1 activation and immune escape triggered by *P. gingivalis* is shown in Fig. 3. Drugs capable of blocking PD-1/PD-L1 interaction or reducing their functions could be useful to improve treatment of chronic periodontitis.

4. Therapeutic implications

The standard treatment of local periodontitis is scaling and root planning (SRP) which is a minimally invasive and non-surgical mechanical action to control the disease progression, but it does not eliminate completely pathogenic microorganisms. An adjunct local drug therapy is often recommended. When the disease has reached a chronic phase, the use of systemic drugs is inevitable. Tetracycline antibiotics and nonsteroidal anti-inflammatory drugs (e.g. flurbiprofen), as host-modulation compounds, can be effective against the periodontal disease, in combination with a local therapy.

Minocycline, doxycycline, clindamycin, amoxicillin/metronidazole are frequently used [38]. Therapeutic modalities aimed at blocking or reducing pro-inflammatory cytokines such as IL-1β are also considered, such as drugs targeting the NLRP3 inflammasome (e.g. β-hydroxybutyrate), inhibitors of the P2X7 receptor (e.g. AZ106006120) and direct antagonists of IL-1β or its receptor (e.g. bioproducts such as Rilonacept and Anakinra) [19]. A variety of plant extracts and natural products have shown activities against gingivitis and/or periodontitis, notably epicatechin gallate, the bile acid ursodeoxycholic acid, certain carotenoids, polyphenol components of green tea leaves as well as aged garlic extract [39–42]. Other modalities such as the subgingival administration of a Xanthan-based chlorhexidine gel, and oral prebiotics like Akkermansia muciniphila to restore the local microbiota can be beneficial as well [43,44].

The discovery of the significant implication of the PD-1/PD-L1 checkpoint in the development of chronic periodontitis raises novel opportunities to block disease progression. Given the role of *P. gingivalis*-induced up-regulation of PD-L1 in the chronicity of the periodontal disease, the objective with drugs would be to interfere with PD-L1, either directly with PD-L1 targeting drugs, or indirectly via a decrease of PD-L1 production or its functional activities, or via an increase of PD-L1 degradation. Different options are evoked below, with the specific use of three plant natural products or derivatives with a known activity against periodontitis (Fig. 4).

4.1. Curcumin and derivatives

Curcumin, a well-known naturally occurring nutraceutical compound, displays a range of therapeutic and biological activities such as antioxidant, anti-inflammatory, anti-diabetic, antitumor, and cardioprotective, through its action on the JAK/STAT signaling pathway [45]. Curcumin attenuates the production of IL-1β and TNFα stimulated by LPS in gingival fibroblasts in vitro [46]. Combined with a standard treatment (SRP), the use of a curcumin gel has shown that a local delivery of the drug post-SRP is efficient in restoring gingival health in patients [47]. The tricarbonylmethane curcumin derivative CMC2.24, considered as a Ras inhibitor, is able to reduce alveolar bone resorption and the number of osteoclasts in a lipopolysaccharide-induced model of periodontitis [48]. It functions as a pleiotropic matrix metalloproteinase inhibitor to reduce local and systemic inflammation and to prevent bacteria-induced connective tissue destruction [49]. In vivo, the drug is active orally at a low dose (1 mg/kg) and was found to reduce TNFα and IL-10 production in vitro [50].
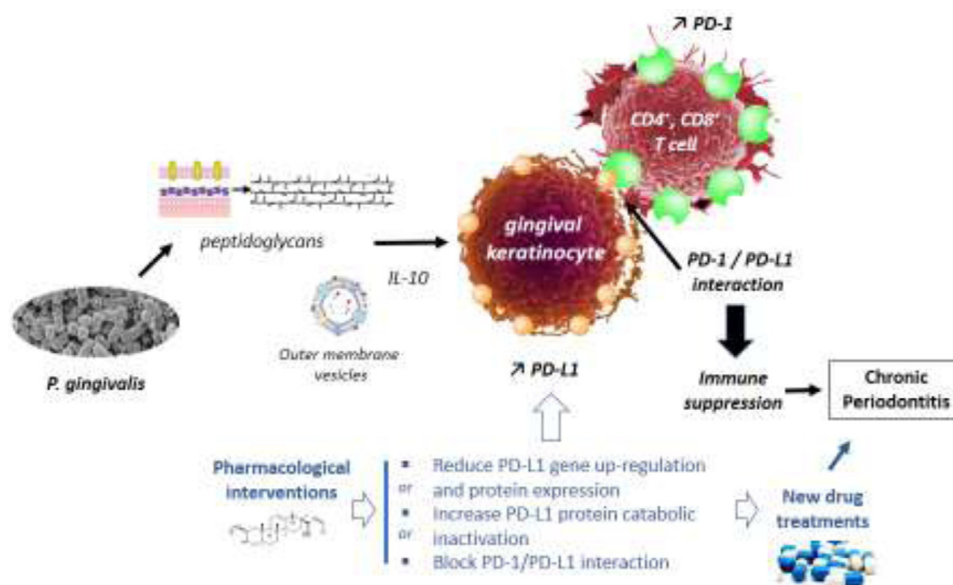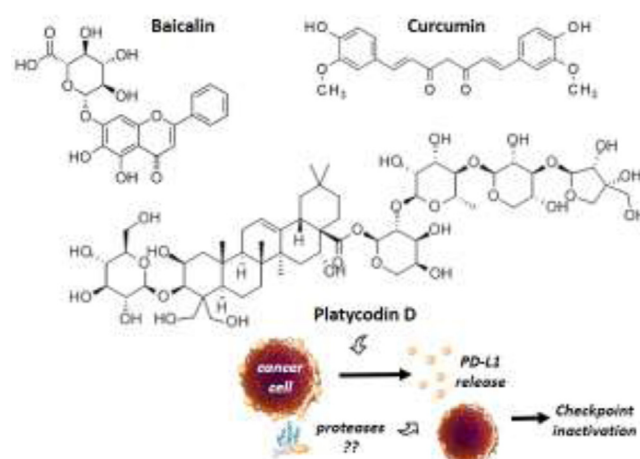
Fig. 3. The PD-1/PD-L1 checkpoint in periodontitis. Peptidoglycans from *P. gingivalis* induce an up-regulation of PD-L1 and PD-1 expressed on gingival keratinocytes and lymphocytes T cells, respectively. The PD-1/PD-L1 interaction causes immune suppression which contributes to the development of periodontitis. Drugs capable of reducing PD-L1 expression or its functions could be useful to reduce immune escape and inflammation associated with periodontitis.

In a dog model of natural periodontitis, capsules of CMC2.24 (10 mg/kg) administered once a day for 3 months were found to significantly decrease the clinical signs of periodontitis, decreasing the production of pro-inflammatory cytokines, metalloproteinases MMP-9 and MMP-2, and cell-signaling molecules TLR-2 and p38 MAPK [51]. There is no data on the potential effect of CMC2.24 on the PD-1/PD-L1 checkpoint but the parent product curcumin was found to inhibit PD-L1 expression in oral cancer cells [52,53] and the closely related product bisdemethoxycurcumin was found to combine well with an anti-PD-L1 antibody, increasing intratumoral CD8+ T cell infiltration and elevating the level of circulating IFN-γ [54]. Curcumin and derivatives provide a first example of drugs that may be used to interfere with the PD-1/PD-L1 checkpoint to improve the treatment of chronic periodontitis.

4.2. Baicalin

The glycosylated flavonoid baicalin, mainly isolated from the root of Chinese medicinal herb *Scutellaria baicalensis*, displays multiple pharmacological activities including anti-inflammatory and antioxidant properties. It is considered as a neuroprotective, cognition-enhancing and anticancer agent [55]. The drug has the capacity to down-regulate IFNγ-induced PD-L1 expression on cancer cells, thereby restoring T cell sensitivity to kill tumor cells. It also suppressed IFN-γ-induced expression and the promoter activity of PD-L1, via inhibition of STAT3 activity in cancer cells [56,57]. In addition, the drug potently prevents the development of periodontitis. Several in vitro studies using human oral keratinocytes, gingival epithelial cells and periodontal ligament cells [58–60] as well as in vivo studies in rat periodontal models [61,62] have revealed the capacity of baicalin to down-regulate the secretion of inflammatory cytokines and to inhibit TLR2/4 expression and the downstream signaling. Baicalin (and its analog baicalein) clearly exhibits regulatory effects on innate immune response, in addition to antibacterial and cytoprotective effects [63,64]. Encapsulated in polymeric micelles due to its poor water solubility, baicalin reduced the destruction of alveolar bone and gingival fiber in a rat model of periodontal disease [65]. A study using minipigs showed that a slow-release chitosan thermosensitive

Fig. 4. Structures of the three drugs mentioned in this study, active against periodontitis and known to interfere with the PD-1/PD-L1 checkpoint. The unusual mechanism of action of platycodin D, inducing the release of soluble PD-L1 in the extracellular milieu [71], is illustrated. The implication of a shedding mechanism, with proteases releasing soluble PD-L1 is only a hypothesis.

hydrogel system containing baicalin can facilitate the regeneration of periodontal defects [66]. And finally, the botanical composition UP446 containing baicalin significantly reduced gingivitis in a dog model [67]. Collectively, this set of data demonstrates the anti-inflammatory activity of baicalin and its beneficial action to limit the development of periodontitis. The contribution of the PD-1/PD-L1 checkpoint to the effects is not entirely defined but it is clear that baicalin has the potential to down-regulate PD-L1.

4.3. Platycodin D

The triterpenoid saponin platycodin D is mainly isolated from the roots of Platycodon grandiflorum (Platycodi Radix or Jiegeng in Chinese), a plant with a long history of use as a traditional herbal medicine [68]. This natural product displays multiple biological and pharmacological properties, including anti-cancer, immunoregula-

tory and anti-inflammatory activities, as well as anti-nociceptive, anti-atherosclerosis, antiviral, anti-obesity and hepatoprotective [69]. The anticancer activity relies in the capacity of platycodin D to block activation of the transcription factor NFκB (nuclear factor-kappa B) and the JAK2/STAT3 signaling pathways (Janus kinase 2/signal transducer and activator of transcription 3) [70]. In addition, the drug was found recently to down-regulate the expression of PD-L1 on NCI-H1975 lung cancer cells, while enhancing the secretion of IL-2 by co-cultured Jurkat T cells. Platycodin D reduces PD-L1 expression on the cancer cells by an atypical process, by triggering its extracellular release into the cell culture medium [71]. It does not reduce PD-L1 mRNA level and does not promote PD-L1 protein degradation, but it rapidly triggers the extracellular release of the membrane protein in the extracellular milieu. Via this release, platycodin D regulates immune cells to restore T cell activation. The release process could be abolished upon treatment of the cells with chlorpromazine, an inhibitor of clathrin-mediated endocytosis. But the silencing of clathrin did not restore PD-L1 release induced by the drug [71]. Therefore, chlorpromazine must be acting by another, yet undefined mechanism.

This mode of action of is very interesting, somewhat reminiscent to the protease-mediated termination of inflammation seen in other situations. For example, a neutrophil-driven proteolysis of inflammatory mediators was found to work as a built-in safeguard for inflammation in Papillon-Lefèvre syndrome which is characterized by nonfunctional neutrophil serine proteases and fulminant periodontal inflammation [72]. Apparently, platycodin D has the capacity to trigger cytokines, chemokines and protease release. It is known that membrane PD-L1 can be proteolytically cleaved by cell surface metalloproteases, such as ADAM10 and ADAM17, to generate a N-terminal protein fragment (about 37 kDa) released to the media. The calcium ionophore ionomycin is an activator of ADAM10 and phorbol 12-myristate 13-acetate is an activator of ADAM17. They can both induce the release of soluble PD-L1 to the media [73]. Other proteases, such as matrix metalloproteinases MMP-7 and MMP-13, frequently upregulated in oral squamous cancer cells, can induce PD-L1 cleavage. Notably, MMP-13 is clearly involved in the shedding of PD-L1 in OSC cells, thereby contributing to the depletion of PD-L1 [74]. The extracellular release of PD-L1 induced by platycodin D could implicate a proteases activation process, implicating ADAMs, MMPs or other proteases known to modulate PD-L1 function (e.g. the ubiquitin-specific protease 22 (USP22)) [75]. Alternatively, it may result from a drug-induced release of extracellular vesicles embarking PD-L1.

The traditional medicine Hainosan (Painongsan), that contains three plant components including the dried roots of *P. grandiflorum*, was found to suppress dose-dependently the growth of *P. gingivalis* bacterial isolates in vitro. The antibacterial activities of extracts of this traditional Japanese and Chinese medicine were attributed to the presence of platycodin D [76]. The activity of the drug against *P. gingivalis* on the one hand, and its capacity to down-regulate PD-L1 on the other hand, should encourage further evaluation of this triterpenoid saponin for the treatment of chronic periodontitis. Moreover, platycodin D is also known to inhibit lipopolysaccharide-induced production of ROS, TNF-α, IL-6, and IL-1β microglia cells [77]. Its mode of action is not limited to PD-L1 down-regulation, but this activity could be beneficial to improve periodontitis treatment.

5. Discussion

The PD-1/PD-L1 immune checkpoint is a major target in oncology. Monoclonal antibodies targeting either PD-1 or PD-L1 have considerably improved the treatment of some cancers, such as melanoma, lung carcinoma and lymphoma. The blockade of PD-1/PD-L1 axis also represents a very promising approach to activate

antitumor immunity in oral malignancies and in particular oral squamous cell carcinoma, one of the most common malignancies in humans [78]. Given the more and more evident implication of the PD-1/PD-L1 immune pathway in periodontitis, the use of drugs impacting this pathway should be really considered, notably for the treatment of chronic periodontitis. Drugs interfering with the PD-1/PD-L1 pathway could also be of interest to treat odontogenic keratocysts which are PD-L1 expressing jaw cystic lesions characterized by local invasion and high recurrence rate [79].

Periodontitis is characterized by a deleterious inflammation, with a significant perturbation of the gingiva-resident memory T cell population. Chronic periodontitis patients have an increased level of activated cytotoxic T cells as a result of inflammation and these T cells could cause severe tissue damages, leading to a rapid and severe loss of periodontal tissues [80]. It seems important and promising to develop further immunotherapeutic approaches to improve periodontitis treatment. Immunomodulatory drugs targeting TNFα or interleukins are already considered for the treatment of apical periodontitis [81] but there is little consideration of anti-PD-1/PD-L1 drugs. At present, this checkpoint can only be targeted with monoclonal antibodies (e.g. anti-PD-1 pembrolizumab and anti-PD-L1 durvalumab) but orally active small molecules targeting PD-L1 are actively searched [24]. Alternatively, one can also target the pathway indirectly via the use of drugs affecting PD-L1 expression or its deactivation. This is the point raised here, though the mention of specific natural products known to have an impact on the PD-1/PD-L1 checkpoint and with a proven anti-periodontitis activity. Curcumin and baicalin (and derivatives) have this dual property to down-regulate PD-L1 and to attenuate periodontitis. With no doubt, they warrant further investigation to establish a functional relationship between these two activities. The case of platycodin D is even more interesting because the drug reduces PD-L1 expression via an atypical mechanism, inducing the extracellular release of a soluble form of PD-L1 and restoring T cell activation. The drug, as well as plant extracts containing platycodin D, deserves further studies in the field of periodontitis.

We mainly focused on natural products active against periodontitis and with a known action of PD-L1 but different synthetic molecules could be considered as well. This is the case of the thienotriazolodiazepine derivative JQ1, a potent inhibitor of the BET family of bromodomain proteins (BRD2, BRD3, BRD4, BRDT) which is a potent suppressor of PD-L1 in cancer cells, including oral squamous cell carcinoma [82,83] and has also shown a marked anti-inflammatory activity in gingival fibroblasts and gingival epithelial cells from periodontitis patients [84]. In a murine periodontitis model, the systemic administration of JQ1 significantly inhibited inflammatory cytokine expression in diseased gingival tissues [85]. Other compounds could be cited, such as the serine protease inhibitor nafamostat mesylate which suppresses IFNγ-induced PD-L1 up-regulation in cancers [86] and was found to attenuate gingival granulocyte infiltration in a rat model of periodontitis [87]. Collectively, the literature analysis reported here supports the idea that targeting the PD-1/PD-L1 immune checkpoint should be further considered to improve the treatment of chronic periodontitis.

Funding

Conflicts of Interest

The author declares no conflict of interest.

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