Porphyromonas gingivalis bacterium as a risk factor for Alzheimer's disease!

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Abstract

Alzheimer's disease remains a significant socio-economic problem in modern medicine. This disease is a neurodegenerative one that is characterized by the plaque formation and neurofibrillary tangles, due to the accumulation of β-amyloid peptide in the medial regions of the temporal lobe and the neocortical regions of the brain. It is impossible to name the sole cause of Alzheimer's disease; however, the features of pathogenesis of this disease are known, including cholinergic deficiency; beta-amyloid toxicity, hyperphosphorylation of a microtubule-associated protein Tau, synaptic dysfunction, oxidative stress, and neuro-inflammation. Unfortunately, Alzheimer's disease is still incurable; however, data are increasingly appearing on the participation of the bacterium *Porphyromonas gingivalis* that colonizes the periodontal pockets, in the pathogenesis of this disease. This article aimed to describe the possible involvement of *Porphyromonas gingivalis* in the pathogenesis of Alzheimer's disease. Meanwhile, in patients with Alzheimer's disease; several structures of the *Porphyromonas gingivalis* including the nucleic acids were found in the brain tissues and the cerebrospinal fluid. It can be assumed that part of the effect of *Porphyromonas gingivalis* on the brain cells is mediated by the transport of active metabolites of this bacterium into the outer membrane vesicles. These outer membrane vesicles contain the main *Porphyromonas gingivalis* virulence factors; gingipains, and iron-binding proteins. Indirect penetration of the *Porphyromonas gingivalis* pathogenicity factors into the brain tissue through the outer membrane vesicles and/ or as part of the bacterial cell structures leads to neuro-inflammation and accumulation of the amyloid plaques. It is concluded that focusing on this bacterium as a risk factor for Alzheimer's disease development will help to develop an effective therapy and/ or a set of preventive measures.

Keywords: Alzheimer's disease, Neuro-inflammation, *Porphyromonas gingivalis*, Gingipains

1. Introduction

Over the past decade, information on the possible relationship between periodontal tissue diseases and systemic diseases has emerged. The influence of the microorganisms that infect the oral tissues on the development of diseases of the cardiovascular system; the respiratory tract, and even on the course of pregnancy, has been established (Offenbacher and Beck, 2014; How *et al*., 2016; Xu *et al*., 2020). In this regard, involvement of the oral microbiota in the progression of the neurodegenerative diseases cannot be excluded.

Alzheimer's disease (AD) is a multifactorial disease and remains the most common neurodegenerative pathology. In rare cases, the development of AD is associated with mutations in three main genes; mainly those encoding for the amyloid-beta precursor protein (APP); presenilin 1, and presenilin 2; resulting in clinical symptoms of the disease that appear before the age of 65 years. Sporadic forms of AD are much more common and are clinically manifested after the age of 65 years (Scheltens *et al*., 2021; Ju and Tam, 2022; Bellenguez *et al*., 2022). In the etiopathogenesis of AD, structural changes in the brain tissues are of paramount importance and are manifested by the formation of amyloid plaques outside the cells and neurofibrillary tangles (NFTs) inside the neurons, which are the cause of several neurodegenerative changes (De Strooper and Karran, 2016). The formation of amyloid plaques is associated with an abnormal accumulation of the amyloid-β peptide (Aβ) in the brain extracellular space.

Aβ is produced from APP, which is sequentially exposed to the action of several proteolytic enzymes; mainly β-; α-, and γ-secretase (Long and Holtzman, 2019). The accumulation of Aβ provokes the release of cytokines, leading to the development of neuro-inflammation and oxidative stress, which is accompanied by a violation of the energy exchange processes and functioning of the cell ion channels that ultimately leads to death of the neurons (Rather *et al*., 2021).

Abnormal accumulation of Aβ initiates the activation of kinases that provide hyper-phosphorylation of the Tau proteins (Tiwari *et al*., 2019). There are previous data on the antimicrobial activity of Aß; showing that the expression of Aß protects against the fungal and bacterial infections (Kumar *et al*., 2016). Tau proteins are part of the microtubules that form the cytoskeleton, while Tau hyper-phosphorylation leads to the formation of insoluble NFTs in the neurons (Ju and Tam, 2022). This may cause disintegration of the microtubules; disruption of the intracellular transport, and signal transduction between the cells, which leads to neuronal apoptosis.

To date, there is no clear reason for the accumulation of amyloid plaques and progression of AD; however, signs of neuro-inflammation, such as activation of the complement system and synthesis of the various cytokines, may indicate the importance of an infectious agent (Kim *et al*., 2021; Liu *et al*., 2023). There is also evidence that the pathogenesis of AD is affected by *Porphyromonas gingivalis* (PG) infection. A previous study reported by Dominy *et al*., (2019) that various PG structures, including DNA and lipopolysaccharides, have been isolated from the brain tissues and cerebrospinal fluid of the AD patients. Thus, considering PG as the cause of neurodegenerative changes in the brain may lead to the emergence of new methods for AD treatment and prevention.

The objective of this review was to analyze the currently available data on the involvement of *Porphyromonas gingivalis* in the pathogenesis of Alzheimer's disease.

2. Search methodology

Searches were conducted in the Medline database (PubMed) and articles published from 1998 to 2023 were analysed. Researches devoted to the microbiological; biochemical, and pathogenesis aspects of Alzheimer's disease were studied. The authors mainly selected articles from the past 10 years; however, they also referenced important older reports and seminar descriptions. Articles in English were selected and original articles; reviews, systematic reviews, and case reports were included.

3. *Porphyromonas gingivalis*

Porphyromonas gingivalis is a Gram-negative anaerobic bacterium that is capable of producing black pigment (Dominy *et al*., 2019; Lunar Silva and Cascales, 2021; Chen *et al*., 2023). PG mainly colonizes the gingival tissues of the periodontal pockets, and along with other representatives of the "red complex"; it is the causative agent of the destructive processes of the periodontal tissues. Even in small amounts, PG can cause destructive periodontal diseases by altering the quantitative and qualitative composition of the commensal microbiota, which leads to dysbiotic conditions of the oral cavity (Mei *et al*., 2020; Zhang *et al*., 2021; Liu *et al*., 2023). It is known that this bacterial pathogen is isolated from the majority of patients with periodontitis; however, in about 25 % of the cases, PG existed in healthy people that do not suffer from oral diseases (Dominy *et al*., 2019).

Motility and adhesion of the PG cells is provided by long and short pili (Mysak *et al*., 2014). PG fimbriae not only provide invasion into the host cells, but also they are full-fledged antigenic structures. PG fimbriae are known to provide adhesive properties to the bacteria when interacting with both of the macro-organism cells and the other microorganisms, which lead to a biofilm formation. In addition, fimbriae can induce pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukins (IL): IL-1β; IL-6, IL-8 (Mysak *et al*., 2014; Chen *et al*., 2023).

According to Kim *et al*., (2021), the other important factor in the pathogenicity of PG is the lipopolysaccharides (LPS) of the cell wall, which cause inflammatory reactions in the gingival tissues, thus creating favorable environment for development of the periodontal tissue diseases.

Porphyromonas gingivalis has the ability to produce enzymes with high proteolytic activities, which cause destructive lesions of the periodontal tissues (Benedyk *et al*., 2019; Lunar Silva and Cascales, 2021). The main part of the proteolytic activity of PG is provided by the gingipain enzymes (Bi *et al*., 2021). A study conducted by Guo *et al*., (2010) revealed that PG gingipains belong to the family of cysteine proteases and are represented by two categories; mainly arginine-specific gingipains (RgpA and RgpB) and lysine-specific gingipains (Kgp). Recently, Chen *et al*., (2023); Liu *et al*., (2023) highlighted that bacterial gingipains are considered to be the main virulence factor of PG; since they can destroy entire groups of various proteins, including collagens and fibronectin; in addition, they can change the permeability of the blood vessel walls and provoke the secretion of pro-inflammatory cytokines.

Furthermore, gingipains hydrolyse fibrinogen and fibrin, which aggravates the destruction of the gingival tissues; inhibits the mechanism of hemostasis, and increases the blood bleeding. This contributes to the intake of large amounts of heme-containing and iron-containing proteins, which are also degraded by the gingipains; thus providing the PG with a large amount of iron required for their growth and the formation of pathogenicity factors (Guo *et al*., 2010; Aleksijević *et al*., 2022).

Of particular interest is the ability of PG to form outer membrane vesicles (OMV), as revealed by Gui *et al*., (2016). The OMV are represented by two-layers of spherical structures with a diameter of approximately 50 - 250 nm, which are continuously released from the host cell surface during the process of PG growth without losing integrity of the membrane (Zhang *et al*., 2021). The OMV have a wide range of

bacterial virulence factors and play an important role in microbial reproduction; inter-species bacterial communication, biofilm formation, invasion, and activation of the macro-organisms protection factors (Yilmaz *et al*., 2008; Liu *et al*., 2023). Meanwhile, PG actively produces OMV during its vital activity in the form of pathogenic polymicrobial biofilms, which can act as additional growth factors for the other pathogens (Gui *et al*., 2016). OMV contain gingipains; iron-binding proteins, and micro-RNAs, which can inhibit the expression of certain cytokines in T-cells and induce apoptosis (Zhang *et al*., 2021; Fan *et al*., 2023). Moreover, OMV inhibit proliferation of the fibroblasts and the endothelial cells and suppress angiogenesis; resulting in slower healing of the periodontal tissues (Bartruff *et al*., 2005).

Thus, PG has a wide range of pathogenicity factors that contribute in the inflammation and destruction of the periodontal tissues. This raises the question: is it possible to translocate the PG or its pathogenicity factors outside the oral cavity without the development of inflammatory and destructive processes in the other organs and tissues?

4. Alzheimer's disease and *Porphyromonas gingivalis*

Daily oral hygiene and dental procedures promote the PG entry into the bloodstream and its spread to the other loci; especially in those patients with chronic periodontitis (Dominy *et al*., 2019; Ryder, 2020). Microorganisms, including PG can cross the blood-brain barrier (BBB) (Yoshida *et al*., 2022; Liu *et al*., 2023; Lei *et al*., 2023). In patients with chronic inflammation of the periodontal tissues, the level of pro-inflammatory cytokines; chemokines, nitric oxide, and reactive oxygen species (ROS) is increased, which may lead to the destruction of the structural proteins of the BBB and facilitates penetration of the microorganisms into the brain (Singhrao and Harding, 2020). In addition, there are data on the perineural spread of the bacteria and the OMVs into the central nervous system (CNS) via the trigeminal nerve (Kanagasingam *et al*., 2020; Ma *et al*., 2023).

As already noted, in patients with Alzheimer's disease; the PG structures such as nucleic acids, have been detected in the brain tissues and the cerebrospinal fluid (Jungbauer *et al*., 2022; Dominy *et al*., 2019). Meanwhile, despite the signs of existence of a microorganism in the CNS; however, it is not yet been possible to isolate and cultivate the viable bacteria from the brain of a sick person and/ or laboratory animals. In this regard, it can be assumed that part of the effect of PG on the brain cells is mediated by the transport of active metabolites in the composition of OMV (Liu *et al*., 2023). It is well known that OMVs are about two thousand times smaller than a microbial cell and are not affected by the host proteases, which allow them to penetrate into the host deep tissues and the bloodstream (Cecil *et al*., 2016; Aguayo *et al*., 2018). OMVs contain the main PG virulence factors; gingipains, and iron-binding proteins (Zhang *et al*., 2021).

The recent study conducted by Yoshida *et al*., (2022) showed that PG OMVs were detected in the brains of laboratory mice after intraperitoneal injection. These results confirm the importance of OMV in the transfer of the various biologically active substances of the microorganisms into the CNS from the other loci.

Indirect penetration of the PG pathogenicity factors into the brain tissue through OMV or as part of the bacterial cell structures leads to neuro-inflammation (Kim *et al*., 2021; Liu *et al*., 2023). The action of the bacterial fimbriae and LPS on the cells activates the secretion of pro-inflammatory cytokines, which in turn lead to the formation of APP in the intercellular space and its subsequent transformation into the Aβ (Kim *et al*., 2021). PG gingipains degrade the normal Tau proteins into fragments; while kinases that are activated by Aβ and inflammation provide phosphorylation to these fragments; eventually resulting in the formation of insoluble NFTs in the neurons (Tiwari *et al*., 2019; Dominy *et al*., 2019; Kim *et al*., 2021). Also of interest is the ability of gingipains to degrade the iron-binding proteins such as ferritin.

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The release of free iron from the intracellular store and iron-containing proteins causes oxidative stress in the cells and generally exacerbates neuro-inflammation (Liu *et al*., 2023). Accumulation of the amyloid plaques and formation of the insoluble NFTs is accompanied by a disruption in functions of the neurotransmitters and the metabolic processes in the cell, which leads to destruction of the synaptic connections and loss of the neurons.

Conclusion

The number of Alzheimer's patients is increasing every year worldwide. Although there is no clear cause of AD to date; however, there are increasing reports of the role of *Porphyromonas gingivalis* in the progression of this neurodegenerative disease. It is possible that focusing on the microorganisms as risk factors for AD development will help to develop an effective therapy or a set of preventive measures. Treatment with broad-spectrum antibiotics has been shown to reduce the severity of AD; but rarely results in PG eradication, and may lead to bacterial resistance and dysbiotic disorders. In the previous conducted studies, the synthesized low-molecular-weight inhibitors targeting gingipains were tested, and this led to a decrease in the bacterial load. In addition, low-molecular-weight inhibitors blocked the production of Aβ1-42; reduced neuro-inflammation, and repaired the neurons in the hippocampus. It is possible that the use of gingipain inhibitors will give a good result in the complex treatment of periodontitis and the other infectious and inflammatory diseases of the oral cavity, which can serve as AD prevention. Thus, we have evidence for the significance of *Porphyromonas gingivalis* in Alzheimer's disease, which is of interest in studying the roles of infectious agents in the various systemic diseases.

Conflict of interest

The authors declare that there is no competing interests exist.

Ethical approval

Not applicable in this study.

Authors' Contributions

Conceptualization, A.V.K. and A.A.N.; Data curation, A.A.N. and A.V.Y.; Investigation, A.A.E., and A.V.L.; Supervision, A.V.K. and A.V.L.; Validation, A.V.K.; Roles/Writing - A.V.K. and A.A.N.; Writing – review and editing, A.A.E., A.V.Y. All authors have read and agreed to the published version of the manuscript.

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