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Research progress on the correlation between oral diseases and Alzheimer's disease

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Abstract. Increasing elderly population are suffering from dementia in China. Researches have shown that sleep disorders, oral diseases caused by oral microbiota, and genetic factors are associated with the development of Alzheimer's disease (AD) based on relevant factor research. *Porphyromonas gingivalis* (Pg) and its protease in patients' brain tissue anatomy with gum bleeding have been identified as primary pathogenic agents for periodontitis. As a result, more and more people believe that oral diseases are critical inducing factors for AD. Reducing the risk of developing AD through daily common oral problems is particularly important. Given that, this review summarized researches from periodontitis associated with AD and oral microbial community and the mechanisms induced by oral diseases during the development of AD.

Keywords: oral microbiota, Alzheimer's disease, *Porphyromonas gingivalis*, gingipain, periodontitis.

1. Introduction

Alzheimer's disease (AD) is a chronic neurological disorder as well as the most common cause of dementia. Clinically it is characterized by progressive cognitive impairment (memory loss, learning difficulties, attention deficits, spatial cognition dysfunction, impaired problem-solving abilities) leading to societal maladaptation. And it is widely believed that toxicity due to Aβ42 aggregation may be the major pathogenic factor promoting AD development [1].

As one of the human body's four major bacterial reservoirs, the oral cavity is the site for eating. An adult's mouth has many commensal bacteria, and every milliliter of unstimulated saliva contains approximately 1.5×10^8 bacteria. Under normal conditions, these microorganisms exist in a relatively balanced relationship among symbiosis, competition, and antagonism without posing problems to oral health. However, if not correctly managed or disturbed by factors such as elevated concentrations of certain microbes resulting from other environmental stimuli, the balance might be disrupted, leading to the onset of oral diseases.

Periodontitis is one of the most typical oral diseases. Many factors contribute to periodontitis, such as smoking, plaque, and psychological factors. Among these factors, *Porphyromonas gingivalis* (Pg) is a primary causative agent. Related research papers have shown that mice were given continuous oral *Pg*

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eventually showed physiological indicators associated with a confirmed diagnosis of AD [2], including neuroinflammation, neurodegeneration, production of amyloid β1-42 (Aβ42), and phosphorylated tau protein at Ser396 [3-5]. Similarly, it has also been shown that disorders of oral flora can be correlated with the induction of AD, such as the possible effects of Leptospira mitis and Tannerella congenita on the host immune response.

This article primarily focuses on the potential mechanisms linking AD with oral diseases, such as how Pg may disrupt communication channels between neurons and erode tight junction proteins in endothelium via its production of gingipains. This disruption can trigger a cascade of events, including activation of GSK-3-mediated immune reactions affecting tau phosphorylation in brain cells leading to inflammation.

2. Alzheimer's disease-related periodontitis and oral microbiota

2.1. Porphyromonas gingivalis is a critical pathogen in periodontitis

Periodontitis is inflammation involving tissues surrounding teeth, usually resulting from chronic infections from periodontal pathogens, with some cases being non-bacterial inflammations [6, 7]. It often presents itself as gingivitis in its early stages, characterized by swelling and pain of gums that may lead to bleeding and bad breath or bleeding of gums when brushing; in severe cases, it progresses to forms of periodontitis where the main features include the formation of periodontal pockets and inflammation of pocket walls, along with gum recession due to bone resorption resulting in tooth movement and even loss, severely affecting patient's oral chewing function [8]. Periodontitis is primarily initiated by bacteria inside dental plaque accumulated on the gum surface near teeth cervix edge, secreting toxins causing inflammation towards host tissue mainly via bacterial lipopolysaccharide[9]. The critical pathogen causing chronic periodontal disease is Pg, which changes the whole biofilm state inside the mouth by destructing host immune system reaction while infiltrating human epithelial cells endothelial cells and enhancing diverse microbiomes' virulence [10]. Pg and its toxic products including fibrinase, gingival protease, and outer membrane LPS, are able to get into bloodstream and promote protein expression such as cytokines, prostaglandins, and growth factors. Dental plaque persists within pockets, creating an anaerobic environment enabling deep hiding spots for Pg regeneration, inducing continual boost into other adjunctive non-oral diseases through OMVs creation.

2.2. Discovering Porphyromonas gingivalis and its product gingipain in the brain structures of AD patients

Research has indicated that Pg can reach the brain through various means from an infected periodontium. The presence of Pg and Treponema denticola (Td) has been identified in the brains of AD patients by PCR techniques and immunoassay of species-specific dense spirochete antigens. Similarly, through the testing of Pg genomic DNA and fluorescent in situ hybridization (FISH) analysis after oral ingestion of pathogens by ApoE-/- mice, it was found that periodontal pathogens/products were present in their brains. Pg is an intracellular pathogen that induces AD via mouse dental infection experiments. A model for periodontitis produced the hallmark changes seen in AD within mouse brains. The model demonstrated that after oxidative stress caused by Pg infection occurred, Aβ42 protein could fissure into multiple oligomer sizes from precursor proteins, stimulating tissue protease B inside the incision body/lysosomes.

Furthermore, Pg is also known to produce gingipains, opening avenues for further research. Gingipains may erode tight junction protein within endothelial cells while having a significant relationship with cognitive impairment mechanisms exacerbated by age-related blood-brain barrier defects related to older adults, potentially leading to another contributing factor to clinical-like features of small rodent models of AD.

In addition, soluble β amyloid had been suggested as capable of disturbing synapses through complement activation, which leads to cognitive dysfunction.

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2.3. Types of oral bacterial population and their correlation with Alzheimer's disease

Bacteria in the oral cavity mainly belong to the phylum of firmicutes, bacteroidetes, actinobacteria, and proteobacteria, while Candida albicans dominate fungi [11]. Compared to healthy individuals, AD patients have a higher proportion of treponema denticola and tannerella forsythia in their dental plaque based on detection tests. In addition, there was a significant increase in bacteria in the AD brain compared to the non-AD brain. Td is a G- anaerobic spirochete that contributes significantly to periodontal diseases and Pg, also commonly found in oral flora. Pg can promote its growth by metabolizing succinate produced from Td's metabolism, and isobutyric acid secreted by it can stimulate growth. Tannerella forsythia is a G- anaerobic bacterium belonging to bacteroidetes related to periodontitis [12]. Studies show that T. denticola and T. forsythia may trigger AD by altering the immune response in the host via changes within microbiota which affects normal prevalence. Nonetheless, one study indicates that microbial diversity of mouth tends to be lower among AD patients than normal healthy ones despite an elevated level of dental plaque observed, suggesting some specific types of oral microbiomes could correlate notably with AD [13].

3. Mechanisms related to Alzheimer's disease-related periodontitis and oral microbiota

3.1. Identification indicators for Alzheimer's disease patients

Early signs and symptoms of AD include memory impairments, trouble in concentrating, planning, or problem solving, and disorientation towards geographical location and time awareness; visual or spatial disturbances, such as inability to judge driving distance, getting lost or putting things in the wrong place; language issues, like unintelligible expressions or decreased vocabulary in speaking and writing; non-participation of work activities or society; mood changing, such as depression or other behavior and character changes. Laboratory tests may also be needed to exclude other conditions that can lead to similar symptoms, for example, the thyroid disease or vitamin B-12 deficiency.

3.2. Research of mouse oral Pg

First, patients with AD have pathological features of TNFα, IL1β, and IL6 expression and microgliosis and astrocyte proliferation compared to normal subjects and have significant neurodegeneration and a high number of degenerated neurons. Therefore, it is possible to determine whether experimental mice are induced with AD by detecting the presence of the above pathological features [2].

Orthogonal analysis on the brain images of oral Pg/gingival protease induced chronic periodontitis mice has confirmed the nuclear localization of Pg and neuropathological signs were detected in the hippocampal region. Besides, microglia hyperplasia and astrocyte proliferation were also demonstrated. Pg was found localized at intranuclear and perinuclear sites in microglia, astrocytes, and neurons, and additionally is also evident extracellularly. Extracellular Aβ42 and phosphorylated Tau (Ser396) protein were detected in the parenchyma. These findings proved the neurodegeneration and extracellular Aβ42 development after repeated oral administration of Pg. [2].

The study revealed noteworthy findings regarding the impact of Pg infection on gene expression levels and accumulation of inflammatory cytokines and proteins in mice. The gingival tissues of both App KI and WT mice showed increased gene expression of TLR2 and cytokines including IL-1b, IL-6, and TNF-α post-infection [14]. Additionally, the gene expression of C3, a crucial component of the complement system, was significantly elevated in the gingival tissues of both mouse groups. Interestingly, the accumulation of soluble hAβ42 and amyloid plaque deposition in hippocampal cells of App KI mice was also elevated post-infection with Pg. The study also indicated that Pg 16S rRNA expression was notably higher in the brains of both mouse groups post-infection. However, Pg 16S rRNA was not found in the brains of uninfected App KI mice, indicating that the access of Pg to the brain only after infection. And therefore, it helps develop neuroinflammatory environment that fosters the development of AD through complement system activation [14]. These findings provide valuable insights into the potential role of periodontal infection in promoting AD and its underlying mechanisms.

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3.3. Mechanisms of periodontitis-induced Alzheimer's disease

Dominy et al. demonstrated that Pg and/or gingipains, which are proteases found in the gums, play a core role in developing AD, and further evidence showed that they exist as antigens within the AD brain. As a consequence, it is necessary to know how Pg plays an inducing role in AD. The potential mechanisms of periodontitis-induced AD of the related findings can be summarized as follows (Figure 1) [5].

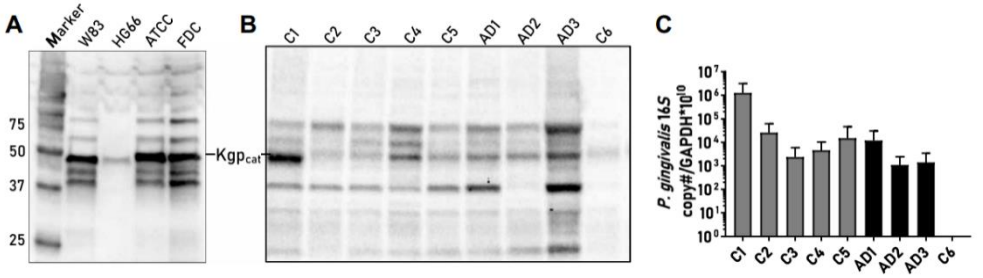

Figure 1. Identification of Pg–specific protein and DNA in cortex.

3.4. Porphyromonas gingivalis disrupts neuronal communication channels and interferes with synapses through complement activation

Pg may activate complement through crosstalk with TLR [5, 15]. Activated complement leads to significant synaptic pruning activity in microglia, resulting in synaptic deficits and reduced memory. Several proteins such as IL-6, IL-1β and TNF-α (conditioners) release and act on neurons during complement activation. Depending on the site where the conditioner binds to the neuron, for example, at the synaptic cleft, the conditioner could disturb the neuron communication and cause cognitive impairment [2]. Furthermore, continuing this cyclic cascade reaction would produce more cytokines such as IL-1β and lead to more severe cognitive deficits. Structural changes in IMR32 neurons are one of the reasons for communication failure between adjacent cells in vitro.

Researches have shown that direct administration of LPS from G- bacteria to the peritoneum or brain induces neuroinflammation through glial cell activation. Learning and memory deficits in individuals often accompany the induced inflammatory response. This is one of the results of IL-1β secretion in the organism after peripheral injury by LPS because soluble IL-1β protein can also interfere with synapses via complement activation. Systemic administration of LPS to mice with Pseudomonas gingivalis leads to impaired recognition of the IL-1β receptor-dependent pathway on their neurons following Aβ release, thus resulting in the inability of this receptor to undergo a rational recognition response and IL-1β recognition of the cytokine is associated with synaptic deficits, which are units of memory. Therefore, it can be concluded that this cytokine has a role in causing cognitive deterioration.

3.5. Porphyromonas gingivalis induces the production of the pathogenic protein gingival protease, which erodes endothelial tight junction proteins through gingival protease

Pg can manipulate the abundance of the microbial community beneath the gingiva and the host immune system so that after Pg triggers oxidative stress in the host, Aβ in the host is likely to be cleaved by its precursor protein into various oligomeric-sized molecules, which in turn activates histone protease B within the endosomes/lysosomes in the host cells [16], and the two types of cysteine proteases (gingival proteases), lysine-specific Kgp and arginine-specific RgpA gingival proteases produced by Pg [17], have the same ability as histone B to hydrolyze the biochemical structure of tau proteins and therefore erode endothelial tight junction proteins. In addition, it has been proposed that oligomeric Aβ in the solubilized state can disrupt synapses and lead to cognitive impairment through the activation of complement system [18].

Pg is known to secrete outer membrane vesicles (OMVs) that carry LPS and related proteases. In this study, it was found that Pg OMVs decrease the expression of Claudin-5, ZO-1, and occluding genes, which encode proteins that are essential for tight intercellular junctions, leading to altered blood-brain

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barrier (BBB) permeability. Furthermore, Pg OMV was observed to increase the average level of phosphorylated tau at Thr231 in hippocampus. Additionally, Pg OMV increased astrocyte number and IL-1β (+) cells, altering their neuronal protein flow. Pg OMV was also found to activate NLRP3 inflammatory vesicles in the mouse hippocampus, as indicated by the significant increase in NLRP3, ASC, and caspase-1 protein levels [19]. Subsequent experiments revealed that incubation of Pg OMV-treated microglia-conditioned media (MCM) with N2a neurons caused a significant increase in the average extent of phosphorylated tau at Thr231, while pre-treatment with an additional NLRP3 inhibitor diminish the level of phosphorylated tau at Thr231 [19]. These results suggest that Pg OMV-induced neuronal tau phosphorylation is indirectly mediated by microglia activation.

In summary, these findings suggest that Pg OMVs can enhance BBB permeability and promote the degradation of tight junction proteins, leading to neuroinflammation and phosphorylation of tau protein mediated by microglia. Pg OMV can also induce the production of IL-1β by macrophages and monocytes through the activation of NLRP3 inflammatory vesicles in the host, further promoting the inflammatory response.

Thus, Pg contributes to cognitive deficits by hydrolyzing the biochemical structure of tau proteins, eroding endothelial tight junction proteins, and by complement activation.

3.6. GSK may affect brain tau protein phosphate through Porphyromonas gingivalis-mediated immune responses

The serine/threonine kinase glycogen synthase kinase 3 (GSK3) is critical regulator in producing pro- and anti-inflammatory cytokines in mammals. GSK3 exists in two main isoforms, GSK-3α and GSK-3β, which are encoded by distinct genes. These isoforms share a high degree of sequence homology, and both are involved in cell proliferation, differentiation, apoptosis, and inflammation responses. GSK-3β can mediate the body's production of pro-inflammatory cytokines such as IFN-β in vivo during bacterial infection, as inhibition of GSK-3β leads to a significant decrease in the body's responsiveness to oral pathogens. GSK3 inhibitors can suppress the host inflammatory response and protect from inflammation-mediated pathological responses. Experimental induction of arthritis in mice using type II collagen and full Freund's adjuvant has been widely employed to investigate the pathogenesis of rheumatoid arthritis. In this context, pharmacological inhibition of GSK3 has emerged as a promising therapeutic strategy. Administration of GSK3 inhibitors to mice subjected to arthritis induction resulted in significantly lessened paw edema, decreased weight reduction, diminished generation of inflammatory mediators, and inhibited bone erosion in comparison to the control. These findings suggest that GSK3 inhibition may effectively ameliorate the severity of arthritis in mice by modulating inflammatory responses and preventing tissue damage. In vitro, LPS-treated macrophages showed that GSK-3β stimulates the production of interferon IFN-β through the c-Jun pathway, activating an ATF-2-dependent mechanism. GSK-3β also down-regulates the production of IL1R, an endogenous IL-1β antagonist, by modulating the capacity of MAPK and ERK1/2 in LPS-stimulated cells, decreasing the effect on IL-1β inhibition. IL-1β has a role in causing cognitive deterioration, ultimately leading to cognitive impairment.

Thus, Pg may contribute to cognitive deficits by reducing the inhibition of the cytokine IL-1β in vivo during its infestation of the host by promoting the body's production of GSK-3β.

4. Conclusion

As discussed above, there is a strong correlation between oral disease and AD, which indicates that the oral disease is AD related inducer. The pathogen of periodontitis, Pg, can enter the brain and hippocampal tissue to disrupt neuronal communication channels and interfere with synapses through inflammatory responses or complement activation, leading to the development of cognitive impairment. However, although various experiments have shown that periodontitis and certain oral microorganisms do have a strong correlation with AD and can induce physio pathological symptoms through the corresponding pathways, more researches are needed to reveal whether they are the main contributors to AD and how to explain the processes that induce the corresponding inflammatory responses further.

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Therefore, it is crucial to investigate and study the oral disease and flora in more AD patients in the future to reveal the relationship between them more accurately. This review concludes that these findings have significant implications for reducing the risk of developing AD by addressing oral health issues in the future and providing direction for further research specifically focusing on Pg and related factors.

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