ORIGINAL RESEARCH

Analysis and Research on the Relationship between Oral Microorganisms and Alzheimer's Disease

Chuanying Dong, BD; Fei Xue, BD; Xinying Ji, MD; Di Zhang, BD

ABSTRACT

Background • The common neurodegenerative disease among the elderly is Alzheimer's disease, which in severe cases can affect the quality of life of patients and their families. It has been reported that oral microorganisms are involved in the progression of Alzheimer's disease.

Objective • To analyze the relationship between oral microorganisms and Alzheimer's disease.

Methods • The oral microbial population, a comprehensive analysis of relevant literature was conducted. Immunofluorescence was adopted to assess albumin deposition in the cerebral cortex of mice. Western blot was used to detect expression level of CYP46 in mouse brain.

Results • It can be concluded that the population of oral microorganisms includes bacteria, viruses, fungi, and spirochetes, which can cause various oral diseases. They can enter the human brain through the blood and surrounding nerves, leading to permeability increase of the blood-brain barrier and neuroimmune related inflammation. They will participate in and worsen the pathological process of Alzheimer's disease, leading to damage to neurons and cerebral blood vessels. The intervention methods for oral microbiota population include vaccination and phage therapy. Vaccines provide suitable treatment methods for periodontal disease, and phage therapy is a new method for controlling oral infections. At the same time, postoperative patients with oral diseases can use gel containing ethanol extract of Brazilian green propolis to ensure oral hygiene. In the rat blood-brain barrier model, porphyromonas gingivalis bacteremia enhanced barrier permeability, and immunofluorescence showed an increase in albumin deposition in the rat cerebral cortex. The expression of cytochrome P450 46A1 (CYP46A1) enzyme in the brain of Alzheimer's disease mice aged 24-56 weeks after long-term administration of SLAB51 increased.

Conclusion • The elderly population should develop good living habits, maintain a clean mouth, and adjust the oral environment through methods such as oral and Alzheimer's disease promotion, combined with medication treatment. (*Altern Ther Health Med.* [E-pub ahead of print.])

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INTRODUCTION

With the global aging population, the probability of dementia among the elderly is gradually increasing. According to 2018 statistics, 50 million people worldwide have dementia, and it is expected to increase to 152 million by 2050.[1] The total medical cost of dementia worldwide is $1 trillion, and it is expected to reach $2 trillion by 2030. In some developed countries, early prevention can to some extent reduce the incidence of dementia. Alzheimer's disease is a progressive neurodegenerative disease that initially manifests as memory impairment, aphasia, apraxia, agnosia, impairment of visuospatial skills, executive dysfunction, and personality and behavioral changes.[2] Its pathological feature is the reduction of neuronal synapses caused by abnormalities in Amyloid β-protein (Aβ) and microtubule associated protein tau, which accounts for 50% -70% of dementia types and is the most common one. Alzheimer's disease can generally be diagnosed through consultation, electroencephalography, magnetic resonance imaging, and other examinations. Consultation usually requires cognitive function testing, neuropsychological testing, etc. The results of electroencephalography usually indicate abnormal brain electrical activity, while magnetic resonance imaging generally indicates a decrease in hippocampal volume or atrophy of the medial temporal lobe.[3] Related studies have found that the recovery rate of the brain during the stage of cognitive impairment is 8%, while the recovery rate of normal cognition in healthy individuals is 25%. The cognitive

level of Alzheimer's disease patients can be improved through non pharmacological treatment methods, including cognitive therapy, physical exercise, and music therapy. There are many microbial populations in the oral cavity, and human oral microbiome data shows that there are 775 types of microorganisms in the oral cavity, of which 57% have been officially named, 13% have been identified but not named, and 30% have not been identified.[4] Imbalance of periodontal microbial system can lead to local inflammation, disrupt the blood-brain barrier, spread to the brain, and exacerbate Aβ Sedimentation.[5] The oral health of Alzheimer's disease patients has attracted widespread attention, and research on oral microbiota and Alzheimer's disease is reviewed in order to provide systematic data for the oral health of Alzheimer's disease patients.

METHODS

Animals

Eight-month-old Male APPswe/PS1dE9 (APP/PS1) heterozygous mice and wild type C57BL6/L mice were purchased from Shanghai Laboratory Animal Centre (SLAC). The APP/PS1 mice expressed human APP with Swedish mutations (K595N/M596L) and human PS1 gene with deletion of exon 9. All animals were raised under a 12 h light/dark cycle with food and water ad libitum.

Immunofluorescence staining

The brain sections were washed in PBS, infiltrated with PBS containing 0.5% Triton X-100 oscillating at room temperature for 15 min, rinsed in PBS, and then sealed with 5% bovine serum albumin (AR1006, BOSTER, Wuhan, China) in PBS at room temperature for 1 hour. The slices were then incubated with the primary antibody overnight at 4°C and then placed in a wet box containing a small amount of water. After rinsing, the slices were incubated with a suitable fluorescently coupled secondary antibody at room temperature for 1 hour.

Western blot

Mice were perfused through the hearts with 30 ml saline solution and rapidly decapitated, and then the brains were immediately removed. The hippocampus tissue was dissected carefully on ice, immediately frozen in liquid nitrogen, and then stored in a freezer at -80°C. The tissue was homogenized in a cold radioimmune precipitation assay (RIPA) buffer, followed by centrifugation of the homogenation to collect the supernatant. The total protein concentration of the samples was determined with the bicinchoninic acid (BCA) protein assay kit (Solarbio, Beijing, China). The total protein of each sample was separated by 12% or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. After 2 hours of blocking with 5% BSA at room temperature, incubate at 4°C overnight with the required primary antibody, and then incubate at room temperature with the enzyme-labeled secondary antibody for 2 hours. After repeated washing, protein bands were developed with an electrochemiluminescence (ECL) detection kit (Beyotime, Shanghai, China).

Statistics

Data were exhibited as mean ± standard deviation (SD). SPSS version 24 (IBM, USA) was adopted for data analysis. Two-tailed unpaired *t* test and analysis of variance (ANOVA) analyses with post hoc Tukey's multiple comparison test were applied to the group comparison. The difference was considered statistically significant only when the $P < .05$

RESULTS AND DISCUSSION

Oral microbiota associated with Alzheimer's disease

Oral microbiota population: In healthy conditions, the oral microbiota population maintains a steady-state relationship with the host. The aggregation of microorganisms colonized in the human oral cavity is known as the oral microbiota population, which contains over 700 microorganisms and can be divided into bacteria, viruses, fungi, and spirochetes. The hiding places of oral microorganisms are relatively complex, including saliva, gums, gingival sulcus, and other locations.[6] Under the regulation of specific local factors, the oral microbial population undergoes symbiosis in the host environment. In a healthy human state, the number of oral microbial populations maintains a stable relationship with the host immune system. A healthy oral microbiota population contributes to the overall oral health of the human body, helps shape and drive a healthy gut microbiota, regulates saliva buffering capacity, reduces the number of pathogenic acid bacteria, prevents gum disease, and reduces gingivitis.[7] Oral microbiota population imbalance refers to significant changes in the types and quantities of various microorganisms in a normal population due to certain reasons. Common causes of dysbiosis include antibiotic abuse and chronic consumptive diseases. According to the degree of dysbiosis, the dysbiosis of the bacterial community can be divided into three degrees.[8] The treatment of oral microbiota imbalance requires a clear understanding of the patient's current infection status and targeted treatment. Microbial dysbiosis is also known as dysbiosis or alternation of microbiota, and the oral and gastrointestinal areas are prone to dysbiosis. The main reasons for dysbiosis of the microbiota are abuse of antibiotics, chronic inflammatory diseases, etc. In clinical practice, many patients may experience dysbiosis of the microbiota after extensive use of certain vitamins.[9]

Related oral microbiota population: The oral microbiota population can enter the bloodstream due to infection, trauma, or iatrogenic factors and directly enter the brain, producing toxic substances that can cause inflammation and further cause Aβ deposition, Tau protein hyperphosphorylation, and ultimately irreversible damage to neurons, even leading to neuronal apoptosis.[10] In the bacterial microbial population, periodontal pathogens and Helicobacter pylori are associated with Alzheimer's disease.

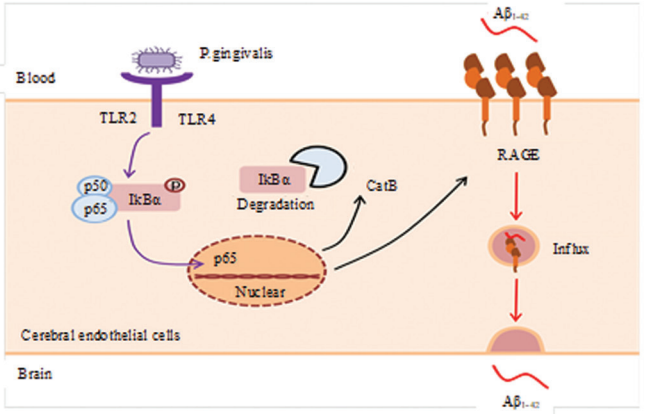
Most of the microorganisms infected in periodontitis are Gram negative anaerobic bacteria, and Porphyromonas gingivalis is an important periodontal pathogen associated with early Alzheimer's disease.[11] The oral cavity is an important storage area for *Helicobacter pylori*, and the infection rate of Helicobacter pylori in the oral cavity is higher than that in the stomach, making it a risk factor for Alzheimer's disease. Among viral microorganisms, herpes simplex virus type 1 is a neurophilic virus that invades the human body through the oral cavity and squats around the human nervous system, entering the human brain at appropriate times. Its properties and behavior are similar to that of human herpesvirus type 6. Herpesviruses also contain cytomegalovirus, which can cause lifelong asymptomatic infections in the host body. Alzheimer's disease has higher antibody levels than the normal population.[12] Similarly, Epstein-Barr virus can reproduce within the epithelial cells of the human oropharyngeal region and lurk in lymphatic tissue, which is activated when the human immune system decreases, leading to infection. Candida albicans, Candida tropicalis, and Candida smooth are fungi related to Alzheimer's disease, mainly distributed in periodontal pockets, root canals, mucosa, and denture tissue surfaces. They have a higher pathogenicity in vulnerable elderly populations. Spirochetes are flagellated spiral Gram-negative bacteria with strong activity and affinity for nerves. They can cause persistent chronic infections, invade brain nerves, cause neuronal damage, and are also associated with Alzheimer's disease.[13]

Oral microbiota population and brain entry pathways related to Alzheimer's disease

Blood pathway: Oral infections caused by microbial populations can create a local to systemic inflammatory environment, and the bacterial toxins and inflammatory factors produced can disrupt the blood-brain barrier through blood circulation to enter human brain, participating in the development of Alzheimer's disease.[14] Oral microorganisms should break through the blood-brain barrier in order to ultimately enter brain tissue. This barrier is located between peripheral and central nervous systems.[15] The blood-brain barrier's tight cellular connections prevent the entry of potentially destructive substances into the brain tissue. This barrier's permeability is influenced by various complex factors. In elderly individuals with Alzheimer's disease, the increased permeability of the blood-brain barrier facilitates the entry of oral microorganisms into brain tissue, contributing to the development of Alzheimer's disease.[16] Furthermore, certain oral microorganisms can disrupt barrier integrity to cause damage to the central nervous system.[17] While neurons in the brain are Aβ's primary source, peripheral cells also contribute to its production.[18] Figure 1 illustrates the significant RAGE role in brain endothelial cells following gingival infection in rat experiments.

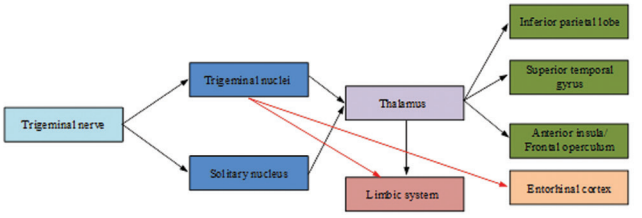
In Figure 1, gingival infection bacteria activate the nuclear factor-κB (NF-κB) pathway, thereby triggering receptor for advanced glycation end products (RAGE) and

Figure 1. Schematic diagram of the key RAGE role in brain endothelial cells following gingival infection.



Abbreviations: TLR2, toll-like receptor 2; TLR4, toll-like receptor 4; CatB, catalase-encoding gene; RAGE, receptor for advanced glycation end products.

Figure 2. Anatomical connection of trigeminal nerve

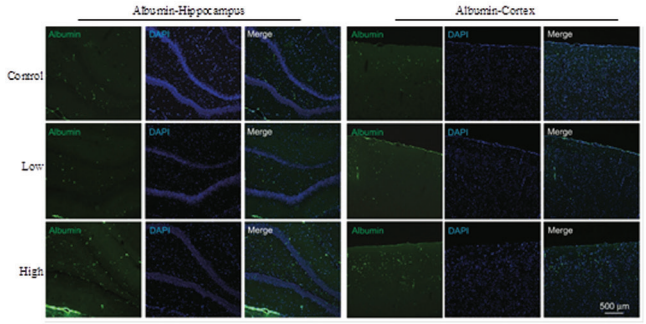


catalase-encoding gene (CatB) transcription. CatB, through regulating activation of NF-κB, is involved in the upregulation of RAGE. Ultimately, the elevated RAGE leads to Aβ influx from bloodstream into brain. In comparison to the control group, gingival infection results in a decreased integrity of the blood-brain barrier, with a quantity reduction of tight junction-associated proteins and an influx increase of peripheral Aβ into the brain.[19] These studies suggest that oral microbiota damages the blood-brain barrier either directly or through their toxic factors for entering brain.

Neural pathway: Some oral microorganisms can break through nerves connected to brain, including the olfactory and trigeminal nerves. Neuroanatomical evidence suggests a close proximity between trigeminal nucleus and locus coeruleus. The anatomical connection of the trigeminal nerve is shown in Figure 2.[20]

The black arrow in Figure 2 represents the confirmed path, while the red arrow represents the assumed path. Spirochetes belong to red complex microbiota with synergistic effects, exhibiting obvious neurotropism. Treponema serrata is a larger bacterium that can easily enter the central nervous system. Additionally, blood-brain barrier cannot affect trigeminal nerve pathway, indicating that it is easy for the neural pathway to enter the brain and may bypass initial immune recognition, continuously hiding within the ganglia.[21] Spirochetes and other organisms can invade adjacent neural anatomical regions of the brain, affecting the release of neurotransmitters and causing depressive symptoms. These bacteria eventually spread to other areas of

Figure 3. Immunofluorescence shows an increase in albumin deposition in the cerebral cortex of mice.

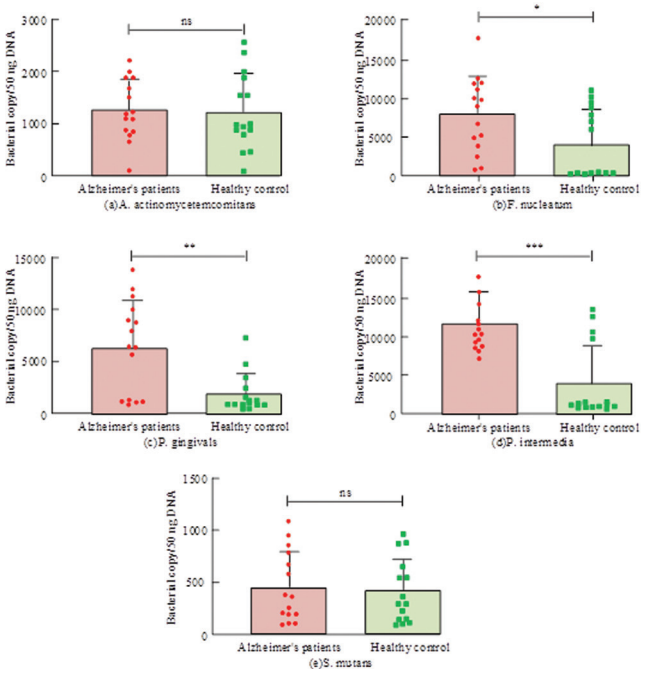


Abbreviation: DAPI, 4'-6-diamidino-2-phenylindole.

Table 1. Probes and primers used for gene sequence detection

Target bacteria	Primer/ probe	Oligonucleotide sequence (50e30)	Product size (bp)
Porphyromonas gingivalis	Primer F	TCGTCGAAACGATCGAAACC	162
	Primer R	GCAGAGCGGTGTAATACGTC	
	Probe	TTCGCGGTATCTTGCCGGCC	
Aggregatibacter actinomycetemcomitans	Primer F	CCACGCCGTTAATGTTCCAT	120
	Primer R	GCCCGTAAGCCTTGCTATTC	
	Probe	AAACGCCTGTGTGCCGCGCC	
Fusobacterium nucleatum	Primer F	AGCTACAAGAGAAGAAAATGAAAATGG	105
	Primer R	CCAACTCCTACAAATCCAGTAACC	
	Probe	TTACTTCATACCATACACGAGGATCTACTT	
Prevotella intermedia	Primer F	AAGACCGTGTTCAACCAACG	102
	Primer R	TGTCATCACTTCCTGCTCGT	
	Probe	CTGGCGCAGGCTTACTCGCA	
Streptococcus mutans	Primer F	TGGAACAATCTCACCAGCCA	112
	Primer R	TCGTCAGTTCTTCACCACGA	
	Probe	TGCTGCTTCCAAGGCTTGTTCCAGC	

Figure 4. Number of bacterial copies of two groups of personnel. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$.



the brain affected by Alzheimer's disease, leading to neuroinflammation, promoting the formation of landmark lesions, and affecting the permeability of the blood-brain barrier, allowing more oral bacteria to enter the central nervous system through the bloodstream.[22] Therefore, slow-growing bacteria can hide in the ganglia and evade the host's immune response, observing prolonged lag in disease formation and final diagnosis.

The mechanism of oral microbial population-connected Alzheimer's disease

Blood-brain barrier disruption: The blood-brain barrier can prevent harmful substances from entering the brain through the blood, achieving the effect of brain tissue protection. Aging can lead to changes in the function and structure of the blood-brain barrier, and its increased permeability can lead to pathogens in oral microorganisms easily entering the brain. Researchers have linked pathogens associated with periodontitis to neuroinflammation in vivo and in vitro, as well as Alzheimer's pathology, and found that the presence of periodontitis related bacteria is associated with Alzheimer's disease, as DNA and toxin factors have been confirmed in brain samples of human Alzheimer's disease subjects. However, the mechanism by which bacteria penetrate the brain and potentially affect neuropathology is still unclear.[23] Some oral microorganisms may also alter blood-brain barrier permeability to damage central nervous system. Therefore, oral microorganisms may disrupt the barrier and enter the brain through their own or their toxic factors.[24] In the rat blood-brain barrier model, porphyromonas gingivalis bacteremia enhanced barrier permeability, and immunofluorescence showed an increase in albumin deposition in the rat cerebral cortex, as shown in Figure 3.

In Figure 3, the immunofluorescence staining results demonstrated a significant increase in levels of albumin in the hippocampus and cortical tissues in the high-intensity group,[25] which was consistent with previous study.[26] Mice infected with gingival porphyromonas monocytes exhibit decreased integrity of the blood-brain barrier, manifested primarily by a reduction in the quantity of tight junction-associated proteins.

Neuroinflammation: Neuroinflammation is the main cause of neuronal necrosis and a triggering mechanism for Alzheimer's disease. Porphyromonas gingivalis, Pseudomonas forsythiae, and Treponema serrata among oral bacteria have immunity and can evade immune monitoring for long-term survival and growth, causing severe maintenance and toxic environments.[27] In a comparative study of oral microbiota between 15 Alzheimer's disease patients and 15 healthy individuals, quantitative real-time polymerase chain reaction was used to detect the composition of oral microbiota, and enzyme-linked immunosorbent assay was used to evaluate systemic inflammatory cytokines levels of individuals. The detection probes and primers are shown in table 1.

The target detection bacteria include Porphyromonas gingivalis, Actinobacillus actinomycetes, Clostridium nucleatum, etc. Quenching dye BHQ and dye molecule FAM labeled the 3 and 5 ends of all TaqMan probes. The 3 end of each probe was phosphorylated to prevent probe extension. The bacterial copy numbers of the two groups of personnel are shown in Figure 4.[28]

In Figure 4, the quantitative analysis of bacterial genera shows that Porphyromonas gingivalis is more common in Alzheimer's disease patients. The number of Actinobacteria in the oral microbiota of the Alzheimer's disease group was higher than that of the control group, and there was a significant difference in the abundance of Clostridium nucleatum between the Alzheimer's disease group and healthy individuals. Alzheimer's disease group also exhibited higher abundance of intermediate Prevotella oral microbiota bacteria. In addition, although Alzheimer's patients showed higher actinomycete population, there is no significant difference compared to healthy individuals, and the difference in Streptococcus mutans is similar. Related studies have found that dysbiosis of oral microbiota can disrupt the gut brain axis, leading to intestinal inflammation and systemic inflammatory response in the host.[29] Consistently, it has been reported that Porphyromonas gingivalis, the keystone pathogen in chronic periodontitis, is identified in the brain of Alzheimer's disease patients.[30]

Increased pathological progression: Neuroinflammatory plaques and neurofibrillary tangles are neuropathological features of Alzheimer's disease, primarily composed of Aβ and P-tau. Aβ deposition leads to synaptic and neuronal damage that progressively worsens the patient's condition, ultimately resulting in neuronal deformation and death. Periodontal disease occurs as various diseases develop such as atherosclerosis, etc.[31] In patients with periodontitis, peripheral blood levels of inflammatory cytokines including interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNF-α) are elevated. These inflammatory mediators may exacerbate brain inflammation in Alzheimer's disease patients, thereby aggravating the pathological process. The relationship between periodontal disease and cognitive decline is illustrated in Figure 5.[32]

Periodontal disease, characterized by bacterial infections and chronic inflammation, can compromise the integrity of the blood-brain barrier and increase the susceptibility to cerebrovascular disease. This, in turn, can trigger inflammation in the brain. It may indirectly lead to the pathological deterioration of Alzheimer's disease through the deterioration of diabetes. The loss of teeth and other conditions can lead to cognitive decline, which can directly or indirectly exacerbate the condition of dementia.[33]

Neuronal damage: Various oral microorganisms have a direct damage effect on neurons, and Pg, dental treponema, Forsythian and their components are present in the brains of Alzheimer's disease patients. In the Alzheimer's disease mouse model, Pg is present in the nuclei and perinuclei of neurons, microglia, and astrocytes in the hippocampus, and has cytotoxicity.[34] Arnsten et al. hypothesized that tau pathology can trigger sporadic Alzheimer's disease, and the transported P-tau may infect neurons in the intracellular environment. The adjacent TRE/ER samples of tau pathology in fragile cortical circuits are seeded as shown in Figure 6.

Implanting abnormal tau proteins at the earliest time points in these cortical regions is the most effective. In the early stages of aging, the production of a large amount of P-tau in the endoplasmic reticulum may be caused by early calcium imbalance in the circuit.[35] In short, various microbial populations in the oral cavity can break through the blood-brain barrier, trigger immune cascade reactions, and directly cause damage to the nervous system; On the other hand, it can secrete pro-inflammatory factors and act on neurons, causing indirect damage to them.[36]

Cerebrovascular damage: In addition to causing systemic inflammatory responses that affect the nervous system, oral bacteria may also contribute to cerebrovascular damage.[37] Multiple studies have indicated that cerebrovascular pathological changes may be a contributing factor to Alzheimer's disease, with approximately 86% of Alzheimer's patients exhibiting multiple cerebral vascular lesions.[38] In the study of pathogenic bacteria in dental caries, it has been suggested that infection by Streptococcus mutans, upon entering the central nervous system, can damage cerebral blood vessels by activating metalloproteinases. Experimental evidence supports the significant role of oral bacteria in cerebrovascular damage, and the impaired cerebrovascular function is closely associated with the onset and progression of Alzheimer's disease.[39] In periodontitis research, bacterial products can induce inflammatory cells to release free radicals and various enzymes, damaging the vascular endothelium, activating the coagulation system, and leading to thrombotic occlusion of cerebral blood vessels, ultimately resulting in indirect neuronal damage. Platelet aggregation also promotes the deposition of Aβ plaques and phosphorylation of tau protein, triggering or exacerbating Alzheimer's disease.[40]

Figure 5. The relationship between periodontal disease and cognitive decline

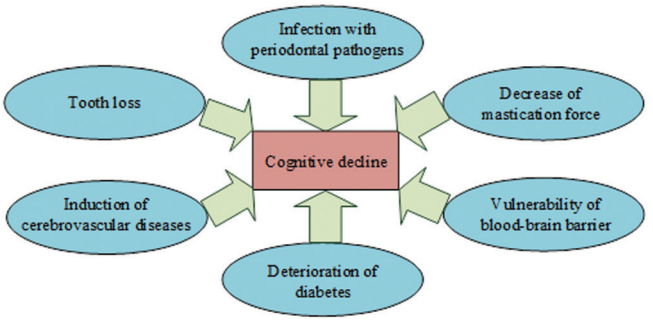


Figure 6. Seeding from adjacent TRE/ER samples.

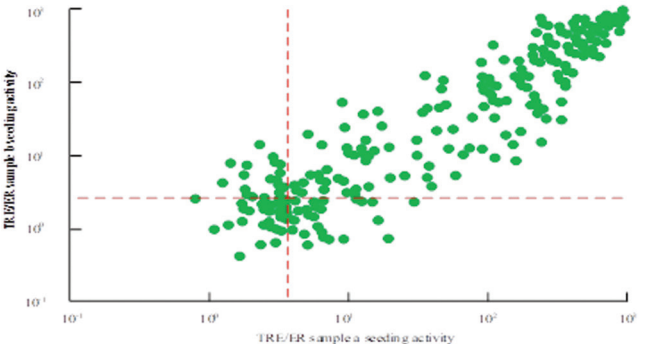


Figure 7. Comparative results of SER-109 drug efficacy

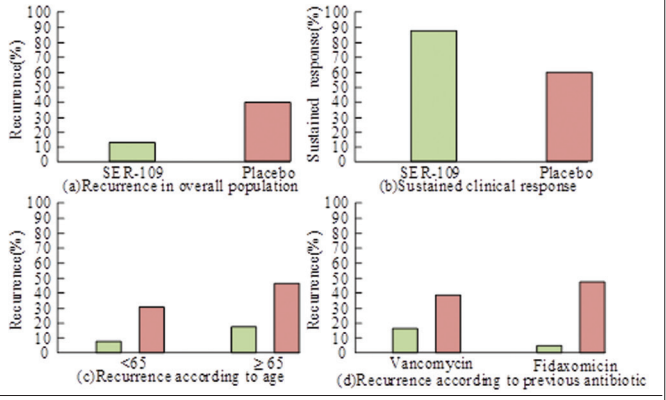
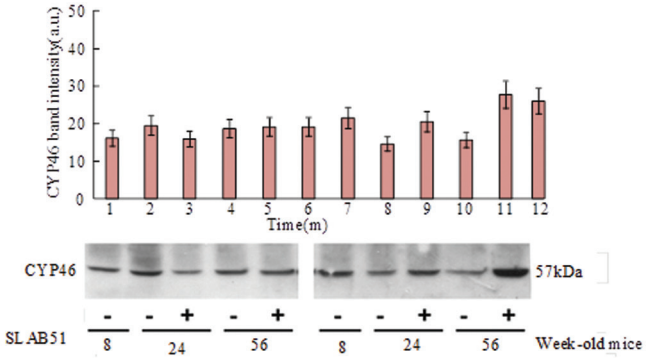


Figure 8. Expression level of CYP46 in mouse brain. CYP46: Cholesterol 24S-hydroxylase; SLAB51: multi-strain probiotic formulation.



Other mechanisms: Oral microbiota can also participate in the regulation of Alzheimer's disease risk genes, while microglia play a crucial role in neuroinflammation and their associated genes are implicated in the onset of Alzheimer's disease.[41] The protein encoded by the triggering receptor expressed on myeloid cells-2 (TREM-2) gene has immunomodulatory and neuroprotective properties, but infection by Porphyromonas gingivalis significantly inhibits the expression of TREM-2, potentially leading to aggravated neuroinflammation and subsequent neuronal apoptosis.[42] Additionally, oral bacteria may also influence the expression of genes such as complement receptor 1 (CR1) and CD33, resulting in imbalanced metabolism of Aβ and affecting the occurrence and progression of Alzheimer's disease.[43]

Targeted oral microbiota population comprehensive intervention

Habits and customs: Biological habits are closely related to the health status of the human body. Alzheimer's disease patients can adjust and cultivate their oral microbiota population through lifestyle adjustments.[44] Oral health knowledge and methods are promoted for maintaining oral health among Alzheimer's disease patients and their related populations, and good lifestyle habits should be cultivated. Firstly, diet can affect the composition of oral microbial populations, and specific dietary patterns help maintain microbial homeostasis, achieving the effect of preventing Alzheimer's disease.[18] Foods with antibacterial active substances, such as garlic and olive oil, have a certain effect on the prevention of Alzheimer's disease. The important way to maintain oral cleanliness every day is to brush teeth. The protease added to toothpaste can cause changes in the oral microbial population, reduce periodontal disease related bacteria, and increase saliva defense.[45]

Medication: The conventional treatment for oral microbiota is drug therapy, while antibiotics are directly targeted therapy.[46] SER-109 is an oral microbiome therapy in development, consisting of highly purified Firmicutes spore, for the prevention of recurrent C.difficile infection. SER-109 is designed to reduce the recurrence of Clostridium difficile infection by regulating the damaged gut microbiome to a state that is resistant to the colonization and growth of Clostridium difficile.[47] In the clinical study of the drug SER-109 for the recurrence of Clostridium difficile infection, patients taking placebo were used as controls. The efficacy comparison results of the SER-109 drug are shown in Figure 7.

As shown in Figure 7, SER-109 achieved its main therapeutic goal and reduced Clostridium difficile infection recurrence. The recurrent percentage in SER-109 was significantly lower than in the other. In analyzing alternative indicators, it was found that 88% of SER-109 subjects had sustained clinical response, while 60% of placebo subjects had sustained clinical response. In age stratified analysis, SER-109 resulted in a lower recurrence rate of Clostridium difficile infection compared to placebo.[47] The ability of simvastatin to inhibit the growth of Porphyromonas gingivalis can be more than 1300 times higher than normal, making it a candidate drug for adjuvant treatment of chronic periodontitis. The chitosan delivery system has strong biological adhesion and permeability, and atorvastatin administration significantly increases anti-inflammatory effects while promoting bone and tissue healing.[48] In addition, lactoferrin, distributed in the mucus cells of the parotid and submandibular glands, can enhance the inhibitory effect of antibodies on microorganisms and kill streptococcus.[49] Unlike antibiotic therapy, antibiotic therapy is ineffective against biofilm bacteria, adherent bacteria, and bacteria with intracellular lifestyles. Local use of lactoferrin has been shown to have simultaneous activity in microbial proliferation, biofilm, adhesion, invasion, and inflammation, and has been shown to effectively treat related oral diseases without any adverse reactions.[50]

Supplement probiotics and transplant oral microbiota: Probiotics are bacteria that can generate beneficial microorganisms and help maintain the balance of the microbiota in the human body, preventing the proliferation of harmful bacteria. In oral health, probiotics play a specific role in the formation of dental plaque and biofilm. Long-term use of the dietary supplement multi-strain probiotic formulation (SLAB51) probiotic formulation can modulate the gut microbiota in Alzheimer's disease mice, improving glucose metabolism, inflammation, and oxidative status, while partially restoring damaged neuronal protein hydrolysis. This ultimately reduces Aβ and tau aggregation, improves cognitive function, and delays the progression of Alzheimer's disease. Cholesterol

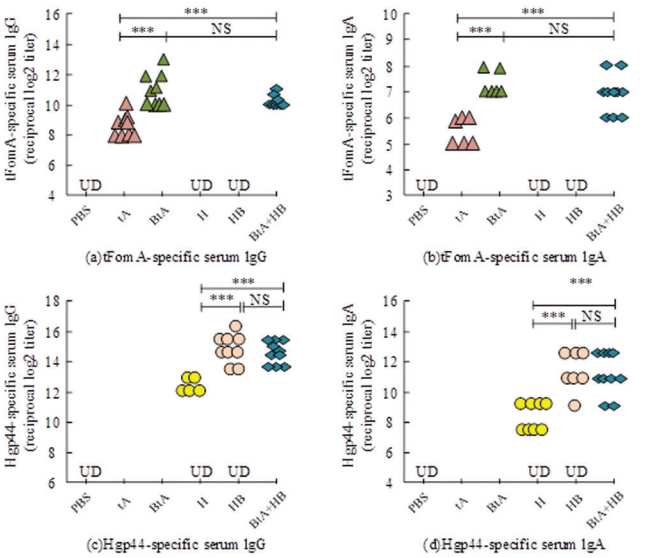
24S-hydroxylase (CYP46) mediates conversion of cholesterol to 24S-hydroxycholesterol, which is a mechanism of the elimination of excess cholesterol and maintenance of cholesterol homeostasis in the brain.[51] It has been shown that in patients with Alzheimer's disease the level of 24S-hydroxycholesterol in serum and cerebrospinal fluid (CSF) is increased in the early stage of the disease.[52] The expression levels of brain CYP46 in mice orally administered SLAB51 probiotics for 1 month and 12 months were shown in Figure 8.

In Figure 8, statistically significant differences in CYP46 expression levels were observed when comparing mice of the same genotype with the control group. The expression of cytochrome P450 46A1 (CYP46A1) enzyme in the brain of Alzheimer's disease mice aged 24-56 weeks after long-term administration of SLAB51 increased, indicating that probiotics induce brain cholesterol update,[53] which was in line with previous study.[54] Related studies have shown that the therapeutic effect and disease relief ability of probiotics are achieved by inducing immunity. In addition, probiotic administration has been shown to regulate the composition of gut bacteria and act along the gut brain axis, indicating the presence of immune regulatory activity after probiotic use in healthy human subjects.[55] The safety of supplementing probiotics and transplanting oral microbiota is high, and it does not cause other diseases and has high genetic stability. Research has found that strains such as Lactobacillus curlicus do not cause dental caries in the artificial oral system, have significant antibacterial activity against periodontal bacteria in rat models, and reduce the risk of infectious endocarditis.[56]

Other interventions: Intervention methods for oral microbiota populations include vaccination and phage therapy. Vaccines provide suitable treatment methods for periodontal disease, and phage therapy is a new method for controlling oral infections.[57,58] In the development of bivalent mucosal vaccines, FlaB-tFomA and Hgp44-FlaB fusion proteins were mixed to target the virulence factors of the inflammatory bacteria Clostridium nucleatum and Porphyromonas gingivalis. The antigen-specific antibody response after bivalent mucosal vaccination is shown in Figure 9.

As shown in Figure 9, monovalent and divalent vaccines exhibit corresponding tFomA and Hgp44 specific antibody titers that are comparable. The results indicate that hybrid vaccines do not interfere with each other's immune responses. Compared with monovalent vaccines, bivalent vaccines can effectively induce the same level of tFomA and Hgp44 specific responses in both systemic and mucosal compartments.[59] The gel containing the ethanol extract of Brazilian green propolis can reduce pathogenic bacteria and opportunistic microorganisms, so as to maintain the oral mucosal wound hygiene of patients with oral diseases after surgery, and will not affect the number of normal physiological flora and bacteria.

Figure 9. Antigen specific antibody response after mucosal vaccination of bivalent vaccinesn. $^{***}P < .001$.



Abbreviations: PBS, phosphate buffer saline; NS, No significance; IgG, immunoglobulin G; IgA, immunoglobulin A; HB, Hgp44-FlaB, BtA, FlaB-tFomA.

Limitation

There are some limitations to our study. First, we did not collect patient samples to detect the expression of oral microbial-related markers of Alzheimer's disease. Second, we did not perform relevant in vitro experiments to verify our findings.

CONCLUSION

The various microbial populations in the oral cavity can directly cause damage to the nervous system by breaching the blood-brain barrier, triggering immune cascade reactions. The overall body health is closely related to biological habits. Patients with Alzheimer's disease can regulate the oral microbial population through lifestyle adjustments and cultivation. The oral microbial population can also be modulated through various methods such as medication, lactoferrin, and vaccines to achieve the effect of inhibiting microbial toxins and treating periodontal diseases.

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