Alternative Treatment for Alzheimer's Disease: *Porphyromonas gingivalis* Inhibitors

ARTICLE INFORMATION

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ABSTRACT

Porphyromonas gingivalis (*P. gingivalis*), has been identified as a primary pathogen in causing chronic periodontitis, or gum inflammation. *P. gingivalis* was also isolated in brain samples of patients suffering from Alzheimer's disease. A virulence factor of *P. gingivalis* called gingipains, releases proteases responsible for neurodegeneration and has been identified in the brain of patients suffering from Alzheimer's. Studies show that mice infected with *P. gingivalis* demonstrate an increase in amyloid plaque deposition in brain samples. Further investigation identified gingipains as a neurotoxic agent, both *in vivo* and *in vitro*, which impacts the structure of tau protein, responsible for the normal functioning of neurons. Small-molecule inhibitors targeting gingipains are utilized to prevent the neurotoxic effects of gingipains and facilitate neuronal regeneration. Inhibition of this virulence factor reduced the overall bacterial load, blocked amyloid-beta production, prevented neuroinflammation, and allowed for neuronal recovery. These findings provide a new outlook for the onset of Alzheimer's disease and elucidate a much-needed potential treatment for the condition.

Keywords: Alzheimer's disease, *Porphyromonas gingivalis*, gingipains, inhibitor, periodontal disease, virulence factor

Alzheimer's disease (AD) is a neurodegenerative disease that induces dementia and is associated with the accumulation of two proteins, amyloid-beta (AB) and tau, in the brain. The aggregation of these proteins in the brain interferes with normal neuronal function and leads to degradation of neurons over time. Based on this, researchers proposed the "Amyloid Hypothesis", which states that the defective regulation of such protein can lead to a toxic plaque formation within the brain, which induces the impairment of cognitive functioning exhibited in AD.[1] This model has been accepted for many years, however, novel research has proposed an alternative hypothesis. These new findings suggest that plaque deposits may be a protective response against bacteria that find their way to the brain. In 2016, Kumar et al. discovered that amyloid seems to function as a sticky defence mechanism against bacteria that cross the blood-brain barrier.[2] They found that this protein can act as an antimicrobial compound that kills bacteria; when bacteria were injected into the brains of mice engineered to make tau and amyloid proteins, plaques developed around the bacterial cells overnight.[2]

Porphyromonas gingivalis is an oral pathogen that primarily inhabits the human oral cavity. It is also the prime agent in causing periodontal diseases, such as gum disease.[3] This pathogen is a non-motile, gram-negative anaerobic bacterium, meaning it has a thin peptidoglycan wall and prefers an environment that lacks oxygen. *P. gingivalis* produces a wide variety of virulence factors, which are molecules produced by bacteria that allow them to invade the host and initiate an immune response. The main virulence factor is *gingipain*, which not only plays a role in gum disease, but has also been linked to AD.[4] Although *P. gingivalis* is mainly found in individuals with gingival and periodontal diseases, in twenty-five percent of the cases, individuals without oral disease test positive for the bacteria.[5] Further research into *P. gingivalis* also demonstrated that it may be a risk factor for AD as it has been found to invade the brain and cause inflammation.[6]

Novel research conducted by Dominy et al. (2019) has validated the previous findings for the association between AD and *P. gingivalis*.[7] However, the most significant finding of this research is that inhibiting gingipains leads to therapeutic effects in preventing neuronal degradation and facilitating recovery.[7] This finding may revolutionize AD in the medical community due to countless potential future applications for the prognosis and treatment of this disease.

Comparative Assessment of Bacterial Load in AD and Control Brain Samples

Assessment of brain samples of Alzheimer's-affected patients was matched based on sex and age. This comparative analysis of the brain tissue samples demonstrated a significantly higher level of gingipain antigens compared to control brain core samples of healthy patients. Additionally, as Figure 1 illustrates, *P. gingivalis* bacteria were also found in the cerebrospinal fluid of 70 percent of affected patients. This helps validate the role of *P. gingivalis* in infecting the central nervous system and contributing to neuronal degradation. Overall, these findings help link gingipains to AD pathogenesis, even in cases with optimal dental care. Interestingly, gingipain antigens were also present in patients with AD pathology in the absence of dementia. This finding helps establish the presence of gingipains as an early occurrence in AD progression for middle-aged individuals in the absence of cognitive decline. The significance of this is the potential use of gingipains as a marker for identifying the onset of AD.

Role of P. gingivalis and Gingipains Inhibition in AD Pathogenesis in Mice

Dominy et al. (2019) demonstrated evidence that *P. gingivalis* and the virulence factor gingipains play a crucial role in AD pathogenesis. In mice, the researchers illustrated reduced host AB response following the administration of small-molecule gingipain inhibitors. The inhibitors facilitated a decrease in the overall gingipain load in the brain of mice and impacted the progression of AD by blocking the gingipain-induced neurodegeneration. Figure 1 illustrates the findings when two different strains of *P. gingivalis* (RgpB and Kgp) were used to mimic *P. gingivalis* infection in neuroblastoma cells in mice through intravenous injection of gingipains. The neuroblastoma samples were stained with Fluoro-Jade C (FJC), a fluorescent stain used to visualize degenerating neurons. The researchers found that not only does gingipain induce neurodegeneration, but also the small inhibitory molecules of gingipains (COR271 and COR286) can facilitate neuronal recovery. This was further demonstrated by the significantly higher number of FJC-positive cells after exposure to gingipains, in comparison to much lower neurodegeneration with low numbers of FJC-positive cells in saline control and gingipain-inhibited trials. These results are significant as they demonstrate the efficacy of gingipain inhibitors as a treatment for AD in mice, a commonly used model organism for humans. As a result, further investigation in the field of pharmacology may allow for the treatment against gingipain-induced Alzheimer's in humans.

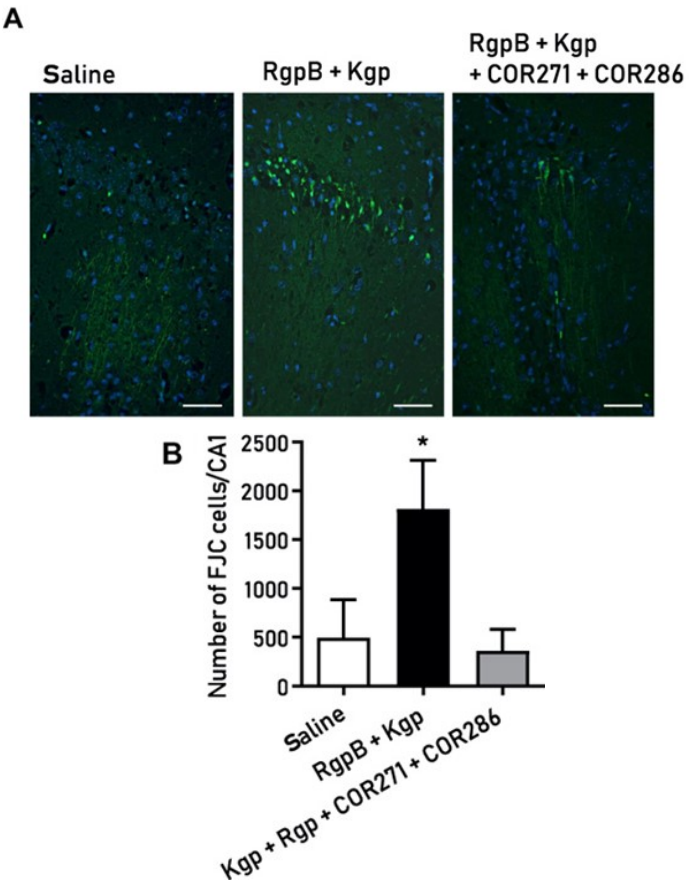

Figure 1. Neuronal protection of small-molecule gingipain inhibitors. A) Fluoro-Jade C (FJC) staining (green) of hippocampal neurons in mice following injection with saline (control), RgpB+Kgp (gingipains), and RgpB+Kgp+COR271+COR286 (gingipains and small-molecule inhibitors). Scale bars, 50 μm. **B)** Total number of FJC-positive cells in an entire hippocampus. There is significant reduction in FJC-positive cells following injection of gingipains and inhibitors (*P<0.05, n=14). Graph with SEM error bars. Reprinted from "Porphyro-monas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors", by Dominy, SS., 2019, Sci Adv, 5, 1–22. Copyright 2019 by American Association for the Advancement of Science (CC by 4.0).

CONCLUSION

This study has also demonstrated the neuroprotective mechanism behind the use of small-molecule inhibitors against gingipains. This provides a novel method for preventing AD pathogenesis and slowing disease

progression prior to the onset of dementia. Results have established gingipains as an early event in AD pathogenesis. This may allow for the utilization of gingipains as a marker for AD progression. For these concepts to be applied for detecting and treating AD, supplemental research is required. Currently, the research in this field has focused on model organisms, with mice being the main source for ample literature. Additional research is required in humans in order to truly identify the efficacy of using inhibitors against gingipains for treating AD in the future. There are also several limitations for this paper that are worth noting. There are various strains of *P. gingivalis* and this paper was not able to identify specifically which strain plays a prominent role in AD progression. This research also overlooked other potential sources of gingipain transmission in the absence of oral infection. However, the novel findings help support an alternative hypothesis for AD involving the role of AB plaques as antimicrobial peptides. The neuroprotective role of gingipain inhibition provides potential treatment for AD in the future.

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