Oral *Treponema denticola* infection induces Aβ_{1-40} and Aβ_{1-42} accumulation in the hippocampus of C57BL/6 mice

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Research Article

Abstract

Accumulation of amyloid-β (Aβ) in the brain is a central component of pathology in Alzheimer's disease. A growing number of evidences demonstrate close associations between periodontal pathogens including *Porphyromonas gingivalis* (*P. gingivalis*) and *Treponema denticola* (*T. denticola*) and AD. However, the effect and mechanisms of *T. denticola* on accumulation of Aβ remain to be unclear. In this study, we demonstrated that *T. denticola* was able to enter brain and act directly on nerve cells resulting in intra and extracellular $Aβ_{1-40}$ and $Aβ_{1-42}$ accumulation in the hippocampus of C57BL/6 mice by selectively activating both β-secretase and γ-secretase. Furthermore, both KMI1303, an inhibitor of β-secretase, as well as DAPT, an inhibitor of γ- secretase were found to be able to inhibit the effect of *T. denticola* on Aβ accumulation in N2a neuronal cells. Overall, it is concluded that *T. denticola* increases the expression of $Aβ_{1-42}$ and $Aβ_{1-40}$ by its regulation on beta-site amyloid precursor protein cleaving enzyme-1 and Presenilin 1.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is characterized by a cerebral accumulation and aggregation of amyloid-β (Aβ) peptides and tau hyperphosphorylation, the main components of plaques and tangles respectively (Scheltens et al. 2016). Aβ oligomers induce AD-like lesions, such as tau phosphorylation or synaptic loss, and accumulate in brain regions related to memory and cognitive function, resulting in related dysfunction (Viola et al. 2015) and recent evidences indicate a role for Aβ as an antimicrobial peptide (Gosztyla et al. 2018). $Aβ_{1-42}$ and $Aβ_{1-40}$ are the most common toxic subtypes in human body (Wirths et al.2019), which are believed to play a key role in neuronal loss and cognitive dysfunction in AD (Lesné et al. 2006). Aβ is a series of short peptides of 38−43 amino acids, produced by degradation of amyloid precursor protein (APP), which is highly expressed in the central nervous system. APP is first hydrolyzed into β - N-terminal fragment and β - C-terminal fragment under the action of β - secretase 1 (BACE1), and subsequently hydrolyzed by γ − secretase of which catalytic active center is Presenilin. Presenilin 1 (PS1) and Presenilin 2 (PS2) are two subtypes of presenilin with similar biological functions. The abnormal expression of BACE1, PS1 and PS2 can affect the cleavage of APP, thus affecting the production of Aβ.

Chronic periodontitis (CP) has been identified as a significant risk factor for the development of AD (Kamer et al.2015). The probability of cognitive impairment in the elderly with alveolar bone resorption is 2.4 times higher than that of those without alveolar bone resorption (Shin et al. 2016). It has been found that the degree of cognitive impairment in patients with severe periodontitis is three times higher than that of patients with mild periodontitis or without periodontitis (Gil-Montoya et al. 2017). The more alveolar bone absorption in the elderly with normal cognitive function, the greater the amount of Aβ deposition in brain tissue. Periodontal pathogens such as *P. gingivalis* or *T. denticola* could cause chronic periodontitis and possibly contribute to the clinical onset of AD (Sochocka et al. 2017), with evidence revealing that oral *P. gingivalis* infection in mice led to brain colonization and increased production of

amyloid plaque component $A\beta_{1-42}$ (Dominy et al. 2019). *T. denticola*, one of the important pathogenic bacteria of CP with a detection rate of 56.8% in CP patients (Sparks et al. 2012) and a member of spirochetes, was detected significantly more frequently in brain samples of AD patients (14 of 16), when compared with that of healthy controls (4 of 18) (Riviere et.al 2002, Poole et al. 2013). Thus, we speculate that *T. denticola* can promote Aβ deposition and pathological process of AD similar to *P. gingivalis*. However, there is a lack of research about the effect and underlying mechanisms of *T. denticola* in AD.

To verify the aforementioned assumption, oral *T. denticola* infection was induced in C57BL/6 mice and examined the effect of *T. denticola* on Aβ in those mice with *P. gingivalis* as positive control and PBS as negative control. Our study demonstrated that oral *T. denticola* infection induces $A\beta_{1-42}$ and $A\beta_{1-40}$ accumulation in the hippocampus of C57BL/6 mice by up regulating BACE1 and PS1. Here, we also reported that both KMI1303 and N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester (DAPT) could inhibit the effect of *T. denticola* on Aβ in N2a neuronal cells.

2. Materials And Methods

2.1. Bacterial strains and growth

P. gingivalis ATCC33277 cultures were routinely grown in Brain Heart Infusion (BHI) medium containing 37 g/L Brain Heart Infusion (Becton, Dickinson and Company), L-cysteine hydrochloride (0.5 mg/mL), and hemin (5 µg/mL) under anaerobic conditions;*T. denticola* ATCC 35405 was grown under anaerobic conditions in new oral spirochete (NOS) broth, as described previously (Fenno et al. 2005). All growth media were incubated in anaerobic conditions for at least 18 h prior to use.

2.2. Mice and treatments

The male mice used in this study (The Animal experiment center of SichuanUniversity, Chengdu, China) were of a C57BL/6 background, and at 8 weeks of age. All the mice were divided into three groups randomly, including the experimental group, the positive control group and the blank control group. The experimental group and the positive control group were separately treated with a mixture of 25U 2%CMC and 25U *T. denticola* or *P. gingivalis* fluid for 24 weeks at a frequency of three times a week (Chukkapalli et al. 2014, Ilievski et al. 2018),while the blank control group received an equal volume of phosphate buffer saline (PBS) three times a week. One week after the final treatment , mice were anaesthetized. After the collection of blood, mice were quickly perfused intracardiacally with chilled PBS (0.1 M, pH 7.3),then hippocampi, trigeminal ganglionweres and aortas were removed, and the hippocampi were dissected. All trigeminal ganglionweres, aortas and some of the hippocampi were flash frozen in liquid nitrogen and stored at -80°C, the other half of hippocampi were fixed with 4%paraformaldehyde. Relative guidelines were followed in our animal experiment.

2.3. Polymerase Chain Reaction

To confirm the spread of periodontal pathogens from the mouth to the brain of mice, genomic DNA was isolated from the tissues samples with the DNeasy Blood & Tissue Kit (Qiagen, Germany). DNA amplification was performed using a PCR amplification kit (TAKARA, Japan) according to the manufacturer's instructions. Briefly, the PCR mixture contained 12.5-μL Taq PCR Master, 0.5-μL (10 μg/mL) DNA samples, 1-μL forward primer, 1-μL reverse primer, and 10-μL sterilized ddH2O. The primers used for amplification were as follows: *P. gingivalis,* 5'-AGGCAGCTTGCCATACTGCG-3' (forward) 5'-ACTGTTAGCAACTACCGATGT-3' (reverse) and *T. denticola*, 5'-TAATACCGAATGTGCTCATTTACAT-3' (forward) 5'-CTGCCATATCTCTATGTCATTGCTCTT-3'(reverse) (TSINGKE, China). The sequencing parameters were an initial denaturation step at 94∘C for 4 min and 35 cycles involving

(94°C for 30 s), annealing (55°C for 5 s), and elongation(72°C for 10 s) . The PCR products were analyzed using agarose gel electrophoresis (1.5%) under 100 V for 23 min with *P. gingivalis* ATCC 33277 DNA and *T. denticola* ATCC 35405 DNA as the positive control and a blank reaction system as the negative control. The specific bands of the samples were compared with the positive group to determine whether the samples contained *P. gingivalis* ATCC 33277 and *T. denticola* ATCC 35405.

2.4. A$\beta_{1\text{-}40}$ and A$\beta_{1\text{-}42}$ ELISA

Protein of mice hippocampi was extracted with total protein extraction kit (PE001, Signalway Antubody, USA). The homogenate was centrifuged and the supernatant was collected to detect the levels of Aβ_{1-40} and Aβ_{1-42} ,which were quantified with beta Amyloid (1−42) Monoclonal antibody (1:500, GT622, Thermo Fisher Scientific, USA), Human Amyloid beta 1−40 ELISA Kit (ab193692, Abcam,Cambridge, MA, USA) according to the manufacturer's specifications.

2.5. Immunohistochemistry

The tissues of the mice hippocampi fixed by 4% paraformaldehyde were embedded in paraffin and sliced coronally into 5-μm sections using a vibratome (Leica, Germany) . The sections were performed for antigen repair at 99.9 ° C for 30 min after deparaffination and then incubated for 10 min in 3% H2O2, and incubated with primary rabbit polyclonal antibodies against Aβ_{1-40} (bs-0877R, 1:100, Boiss, China), primary Rabbit monoclonal antibodies against Aβ_{1-42} (ab224275, 1:200, Abcam, Cambridge, MA, USA) overnight at 4 °C. Then the sections were incubated with biotinlabeled secondary antibodies (1:300, 865002, R&D Systems) for 30 min at 37 °C and the Cell and Tissue Staining Kit (CTS005, Anti-Rabbit HRP-DAB System, R&D Systems) was used to detect the positive staining area. The images were captured by a camera (Nikon, 90i, Tokyo, Japan). And 3 sections per mouse were being counted in a blind manner.

2.6. Western blot analysis

Hippocampi were lysed using lysis buffer containing 20mM Tris, pH 7.5, 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate,50mM NaF, 1mM Na3VO4 , 1mM PMSF, and a protease inhibitor (200612▢ Signalway Antibody, USA). The BCA method was used to qualify protein concentration. The supernatant

of hippocampus extract was mixed with SDS-PAGE protein loading buffer (5x) (Beyotime, China) in the ratio of 4:1 and then boiled. After SDS-PAGE, the proteins were transferred onto a polyvinylidene difluoride membrane ,blocked with 5% non-fat milk in TBST buffer (20mM Tris-HCl, pH 7.4, 137mM NaCl, and 0.1% Tween-20) for 1h at 37°C, and incubated overnight at 4°C with primary rabbit polyclonal antibodies against GAPDH (41549,1:3000, Signalway Antibody, USA),APP, BACE1, PS1 (38604, 24100, 33474, 1:1500, Signalway Antibody, USA) and PS2 (DF7809, 1:1000, Affinity Biosciences, USA). After extensive wash, the membrane was incubated with the appropriate HRP-conjugated secondary antibody (L3012, 1:5000, Signalway Antibody, USA) and then developed using the Super ECL Plus reagents. The relative band intensities were quantified by densitometry using the ImageJ software (1.41V, US National Institutes of Health).

2.7. Quantitative PCR

Total RNA was extracted from mice hippocampi with the RNApure total RNA fast isolation kit (BioTeke, China), and subsequently reverse transcribed with Evo M-MLV RT kit with gDNA Clean for qPCR II (Accurate Biology, China) according to the manufacturer's instructions. The resultant cDNA was used for the template for quantitative PCR analyses with gene-specific primers (TSINGKE, China). The sequences of the primers were as follows: BACE-1, 5'-CAGTGGGACCACCAACCTTC -3' (forward) and 5'-GCTGCCTTGATGGACTTGAC -3' (reverse); APP, 5'-TCCGAGAGGTGTGCTCTGAA-3' (forward) and 5'-CCACATCCGCCGTAAAAGAATG-3' (reverse); PS-1 , 5'-GGTGGCTGTTTTATGTCCCAA-3' (forward) and 5'-CAACCACACCATTGTTGAGGA -3' (reverse);andPS-2⬚5'-GAAGACTCCTACGACAGTTTTGG-3' (forward) and 5'-CACCAGGACGCTGTAGAAGAT -3' (reverse). Real-time PCR reactions were performed with the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System in the presence of 0.8 μL for each primer, 2 μL of cDNA, 6μL Nuclease-Free water, 0.4 μL ROX Reference Dye II and 10 μL of TB Green Primix Ex Taq II(TAKARA, Japan) for a total volume of 20 μL. The following PCR conditions were used for all samples: 95 °C for 30sec, and then 40 cycles of 95 °C for 5 s and 60 °C for 30sec. The fluorescence intensity was monitored at the end of each amplification step.Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was served as the internal reference. The method of$2^{-\Delta\Delta C_t}$ was adopted to calculate the relative mRNA expression of the target genes.

2.8. Cell culture and Treatment

N2a neuronal cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1 U/Ml penicillin, and 1 m g/mL streptomycin in a humidified (5% CO 2 , 37 °C) incubator. After confirming the cell status, the N2a cells were first seeded in 24-well tissue culture plates at a density of 10^5 cells per well in DMEM culture medium with 10% fetal bovine serum. After cells adhesion, N2a cells were treated with 1 μmol/L DAPT (ApexBio Technology, USA) which was used as γ-secretase inhibitor or treated with 1 μmol/L KMI1303(WaKo, Japan) for 6 h, or cultured with no treatment. After that, cells were infected with *T. denticola* or *P. gingivalis* at a density of 10^7 CFU per well for 2h.

2.9. Immunofluorescence

N2a neuronal cells fixed with 4%paraformaldehyde were permeated by 0.5% TritonX-100 in PBS, and then blocked in 2% BSA at 37∘C for 1 h and then incubated with β-Amyloid (1-40 Specific) (D8Q7I) Rabbit mAb (12990, 1 : 600, Cell Signaling Technology, USA), Rabbit monoclonal antibodies against $A\beta_{1-42}$ (ab224275, 1: 200, Abcam, Cambridge, MA, USA) for 1 day at 4∘C. After washing with PBS, the cells were incubated with Goat Anti-Rabbit IgG H&L FITC L30113, 1:200; Signalway Antibody, USA at room temperature for 1h in the dark. After washing with PBS, the sections were incubated with DAPI solution (Solarbio, Beijing, China) at room temperature for 5min and mounted in the Vectashield anti-fading medium (Solarbio, Beijing, China). Fluorescent images were captured by LEICA DMi8 and quantified by the ImageJ software (1.41V, US National Institutes of Health).

2.10. Statistical Analysis

Data were presented as the mean ± standard deviation and analyzed by SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was applied to analyze statistical differences. The level of significance was set at $p < 0.05$ (*).

3. Results

3.1. *T. denticola* 16S rDNA was detected in aorta, trigeminal ganglionwere and hippocampus of mice

Since Spirochetes like Treponema pallidium are capable of entering brain tissue and pathologies suggest a relationship with AD (Miklossy et al. 2016). Besides, *P. gingivalis* was identified in the brain of Alzheimer's disease patients (Dominy et al. 2019). Thus, after 24 weeks of oral injection, Polymerase Chain Reaction was employed to detect whether *T. denticola* and *P. gingivalis* entered the hippocampus. As presented in Fig.1, *T. denticola* and *P. gingivalis* were detected significantly more frequently in hippocampus (7 of 10), compared with blank control samples (0 of 10). To detect how *T. denticola* and *P. gingivalis* entered the hippocampus, the aortas and trigeminal ganglion were subjected to detection. *T. denticola* was found in 7 aorta samples, while *P. gingivalis* was found in 9 samples. Further, 3 trigeminal ganglion samples were revealed to contain *T. denticola*

3.2. *T. denticola* induced $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation in hippocampus

We wondered whether *T. denticola* or *P. gingivalis*, which entered the hippocampus, had an effect on Aβ, so we subjected to ELISA analysis and immunohistochemistrym using the PBS group as blank control and the *P. gingivalis* group as positive control. Our data revealed that oral *T. denticola* or *P. gingivalis* infection induced $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation in the hippocampus of mice, and there was no significant difference between the two groups (Fig.2b and c). Overexpressed $A\beta_{1-40}$ and $A\beta_{1-42}$ mainly accumulated in the cytoplasm and intercellular substance.

3.3. *T. denticola* induced $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation by directly acting on mouse nerve cells

The above experiments revealed that *T. denticola* and *P. gingivalis* entered the hippocampus and the expression of $A\beta_{1-40}$ and $A\beta_{1-42}$ increased in the hippocampus of mice. We speculated that *T. denticola* and *P. gingivalis* entering the hippocampus directly could act on mouse nerve cells and cause $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation. To test our hypothesis, we stimulated N2a neurons with *T. denticola* and *P. gingivalis* in vitro. The immunofluorescence results confirmed our hypothesis. As shown in Figure.3, the expression of $A\beta_{1-40}$ and $A\beta_{1-42}$ in N2a cells co cultured with *T. denticola* or *P. gingivalis* was significantly increased compared with the blank control group.

3.4. *T. denticola* induced Aβ accumulation by up regulating BACE1 and PS1 , KMI-1303 and DAPT inhibited the effect of *T.denticola* on Aβ

For the purpose of further exploring the mechanism of *T. denticola* on Aβ, total RNA extracted from the brain tissues was used for real-time PCR. It was shown that the expressions of BACE1, PS1 and PS2 increased in *T. denticola* and *P. gingivalis* groups. The expression level of BACE1 and PS1 in *T. denticola* group was significantly higher than that in the blank control group. Next, we subjected to Western blot analysis. The results of Western blot showed that the expression levels of BACE1 and PS1 in *T. denticola* and *P. gingivalis* groups were significantly higher than those in blank control group. In addition to the effect on β − secretase and PS1, *P. gingivalis* had an effect on PS2, a subtype of presenilin with similar biological functions to PS1. In order to further confirm the mechanism of *T. denticola* inducing Aβ accumulation, β-secretase inhibitor and γ-secretase inhibitor were used to test and verify the results above in vitro. KMI-1303, the inhibitor with an IC50 value of 9 nM, has the same effect and mechanisms as KMI429 (Asai et al. 2006) on inhibiting β-secretase activity accoding to the manufacturer. PS1 and PS2 are two subtypes of presenilin and have similar biological functions, and the effect of *T. denticola* on PS1 was more significant. Thus we choose DAPT , a potent γ-secretase inhibitor which can lead to a decreased flexibility of key PS1 regions related to the recognition and internalization of γ-secretase substrates (Aguayo-Ortiz et al. 2019) to confirm the mechanisms of *T. denticola* on Aβ. As presented in Fig. 4 the expression of $A\beta_{1-42}$ in the inhibitor group is significantly lower than that in the coculture group.

4. Discussion

CP is known to trigger several human diseases including AD (Kamer et al.2015), Atherosclerosis (Arabi et al. 2018) etc. The periodontal pathogens such as *P. gingivalis* and *T. denticola* have been found in human post-mortem brain tissues of Alzheimer's Disease patients (Riviere et.al 2002, Poole et al. 2013). Many attempts have been made to clarify the role of periodontal pathogens in systemic diseases. Some studies have shown that *P. gingivalis* infection induces Aβ accumulation (Dominy et al. 2019) , tau hyperphosphorylation (Tang et al. 2020) and neuroinflammation (Tang et al. 2020, Liu et al. 2020). The viewpoint that spirochetes are involved in the possible etiology of AD was first proposed by Miklossy (Miklossy et al. 1993). Moreover, in a more recent compended analysis of brain and blood samples from 495 samples from AD patients, 91% were positive for spirochetes compared to 0% for 185 controls (Miklossy et al. 2011). However, the effect and mechanisms of *T. denticola*, a periodontal pathogen as

well as a spirochete in AD was not clearly understood. Here, we used *P. gingivalis* oral infected mouse model (Dominy et al. 2019, Liu et al. 2020) as a positive control to study the effect of *T. denticola*. Our experiment demonstrated that *P. gingivalis* and *T. denticola* could enter the brain mainly through the blood and cause Aβ aggregation to the same extent . Besides, *T. denticola* 16S rDNA was also detected in a small number of trigeminal ganglion samples. Hence we speculate *T. denticola* may directly enter the hippocampus via blood and trigeminal nerves to induce $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation.

Cerebral accumulation and aggregation of Aβ peptides is a characteristic pathological marker of AD patients. Soluble Aβ oligomers are believed to represent key structures that produce cytotoxicity, contribute to synaptic deficits and initiate the detrimental cascade involved in the pathology of AD (Salahuddin et al. 2016, Larson et al. 2012, Klein et al. 2006). BACE1 cleavage of APP is the rate-limiting step along the amyloidogenic processing pathway (O'Brien et al. 2011) and PS1 is involved in γ-secretase activity and influences $A\beta_{1-40}$ and $A\beta_{1-42}$ production. Knockout of BACE1 completely blocks the generation of Aβ (Cai et al. 2001). About 90% of mutations in human PS1 by individually lead to reduced production of $A\beta_{1-40}$ and $A\beta_{1-42}$ (Sun et al. 2017). Our data indicate that *T. denticola* increases $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation in the hippocampus of C57BL/6 mice by upregulating BACE1 and PS1. Besides, we have verified the above results with β-secretase inhibitor and γ-secretase inhibitor in N2a neuronal cells.

In summary, we present data obtained by PCR, ELISA, IHC, qPCR, Western blot and IF to support the hypothesis that *T. denticola* in oral cavity can enter the hippocampus via the blood and trigeminal ganglion and act directly on nerve cells resulting in intra and extracellular $A\beta_{1-40}$ and $A\beta_{1-42}$ accumulation in the hippocampus of C57BL/6 mice. The up-regulation of BACE1 and PS1 is the mechanism of $A\beta_{1-40}$ and $A\beta_{1-42}$ increase induced by *T. denticola* is.

Declarations

Author Contributions All authors contributed to the study conception and design. Hongkun Wu was responsible for conceiving, designing, and supervising the present study, and revising the manuscript. Material preparation, data collection, and analysis were performed by Xinyi Su , Zhiqun Tang, Yuqiu Liu, Wanzhi He, Jiapei Jiang and Yifan Zhang. The first draft of the manuscript was written by Xinyi Su. All authors commented on previous versions of the manuscript and approved the final manuscript.

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Data Availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Code Availability Not applicable.

Compliance with Ethical Standards Conflict of Interest The authors declare that they have no competing interests.

Ethics Declarations All animal experiments were conducted at State Key Laboratory of Oral Diseases and were licensed by Research Ethics Committee of West China Hospital of Stomatology (No. WCHSIRB-D2019-013).

Consent to Participate Not applicable.

Consent for Publication Not applicable.

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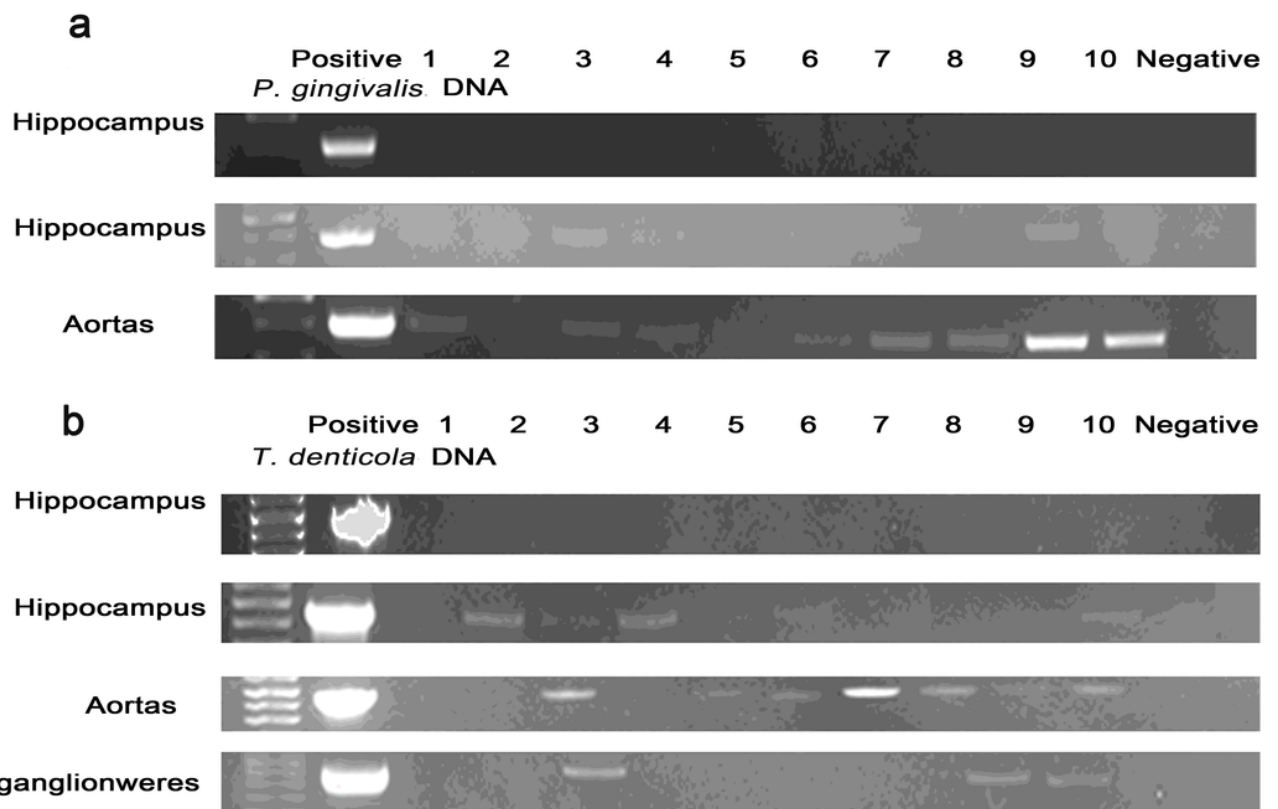
Figures

Figure 1

T. denticola and P. gingivalis are detected in the samples of mice following oral application . (a) From top to bottom, the hippocampus of the PBS group, hippocampus of the P. gingivalis group and aorta of the P. gingivalis group are shown in turn. (b) The hippocampus of the blank control group, hippocampus of the T. denticola group, aorta of theT. denticola group and trigeminal ganglionwere are shown in turn. All results above have been verified more than three times.
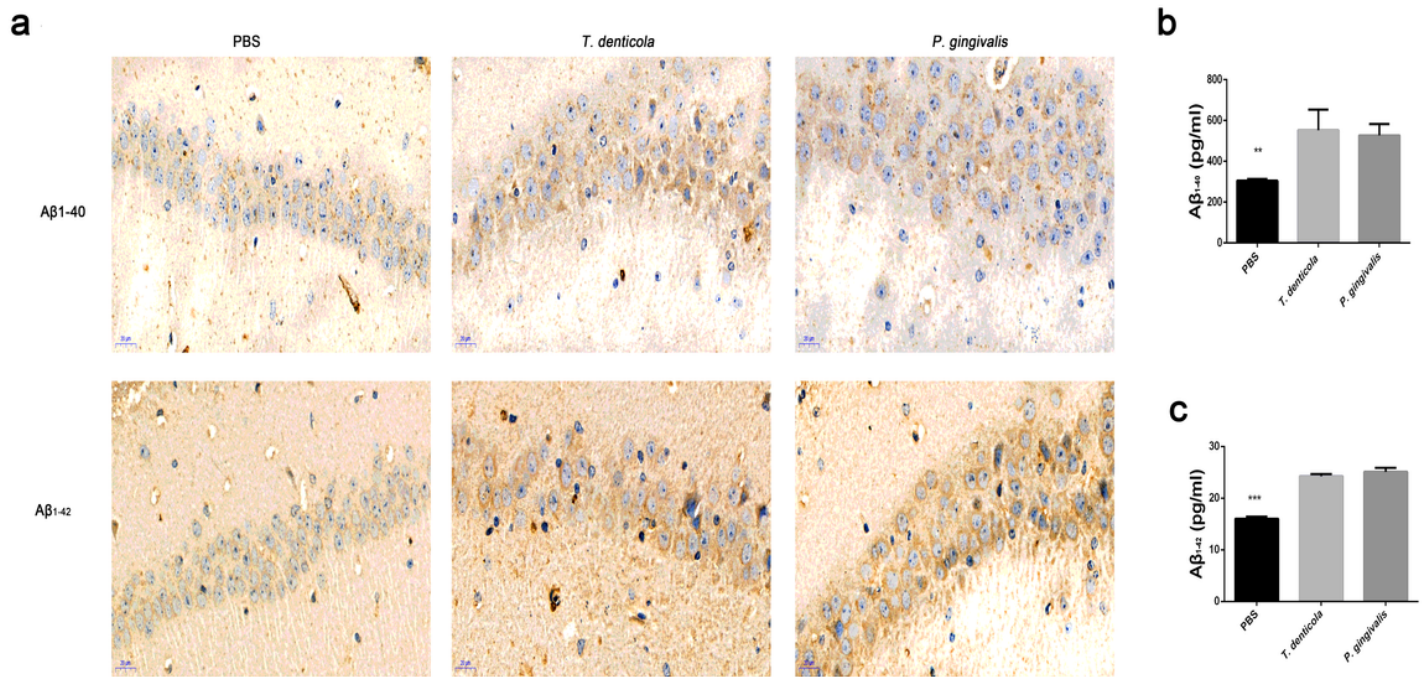
Figure 2

T. denticola increases the expression of Aβ1-40 and Aβ1-42. (a) Immunohistochemistry was used to detect the location of Aβ1-40 and Aβ1-42 deposition in. Images are representative of N = 5 for the PBS group , N = 5 for the T. denticola group and N = 5 for the P. gingivalis group. The brown yellow substance in the cytoplasm and stroma of neurons is the deposition site of Aβ1-40 and Aβ1-42 in hippocampus. (scale bar = 20 μm) (b.c) The bar graph represents the expression levels of Aβ1-40 and Aβ1-42 in hippocampus. The values are shown as the mean ± SD of three independent experiments. **$p < 0.01$, ***$p < 0.001$, N = 5 mice per group.
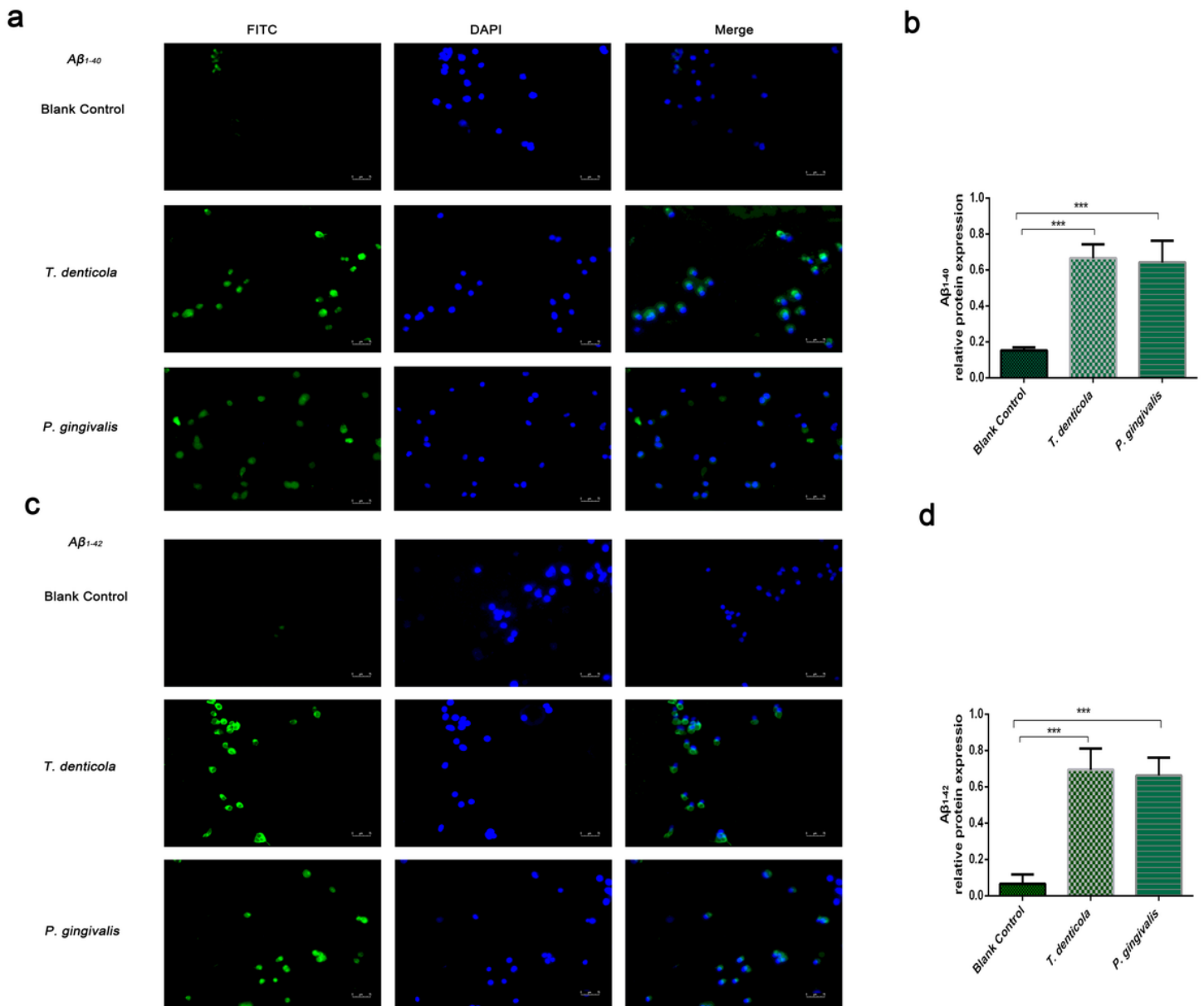
Figure 3

T. denticola directly induces Aβ1-40 and Aβ1-42 accumulation in mouse nerve cells. (a.c) Images represent the results of three repeated experiments. N2a cells were cocultured with T. denticola or P. gingivalis for 2 h , and used for immunofluorescence with anti- Aβ1-40 antibody and anti- Aβ1-42 antibody. Green fluorescent displays Aβ1-40 and Aβ1-42. Counterstaining with DAPI, blue color, suggests cell nuclei. (b.d) Results of IF are expressed as means ± Std. Dev., ***$p < 0.001$.
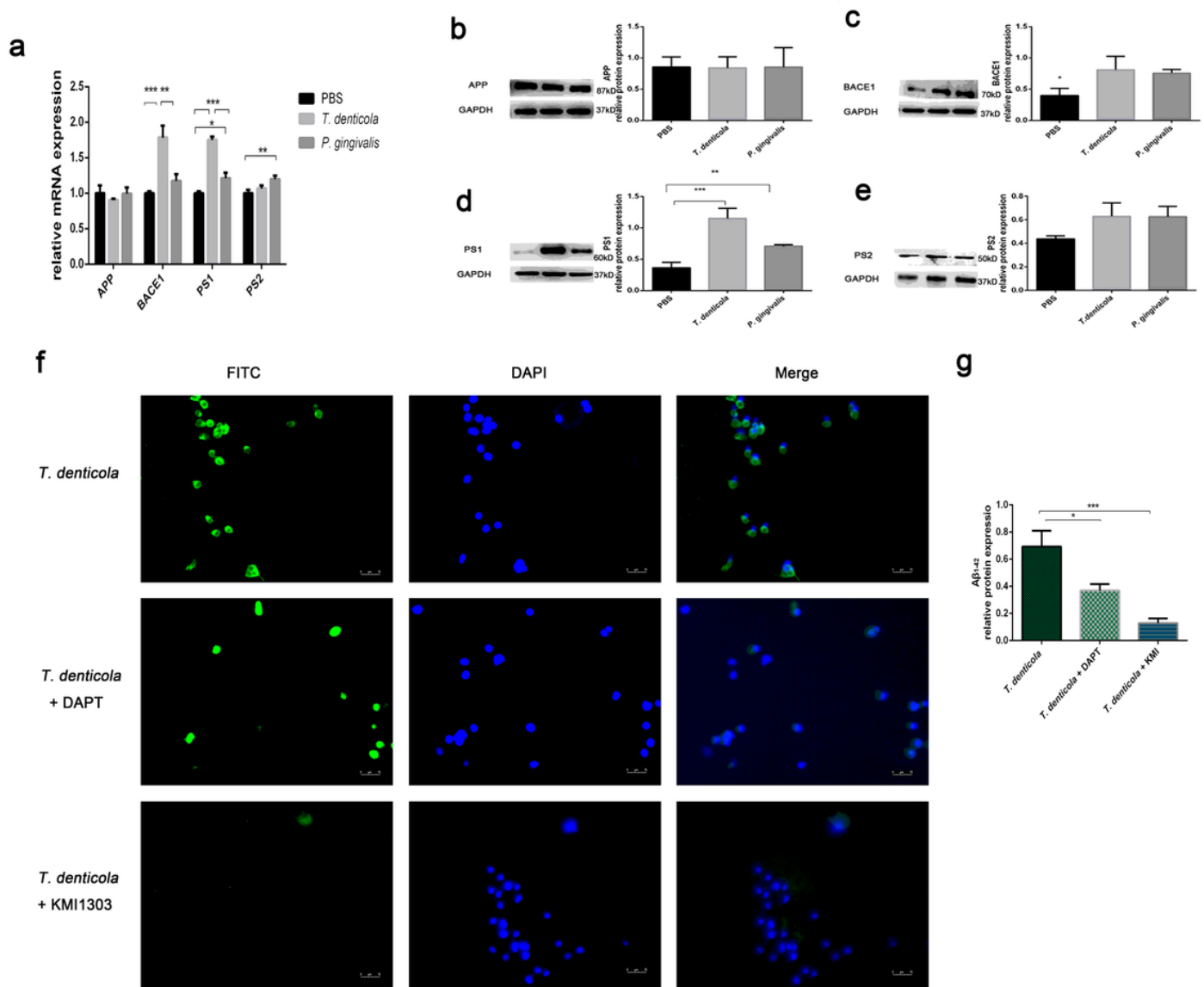
Figure 4

T. denticola induces Aβ accumulation by up regulating BACE1 and PS1 , KMI-1303 and DAPT can inhibit the effect of T. denticola on Aβ. (a) RNA was extracted from mouse hippocampus, and the expression of genes related to Aβ accumulation was detected by qPCR. The values are shown as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001, N = 4 mice per group. (b.c.d.e) Protein expression levels of APP, BACE1,PS1 and PS2 in the hippocampus were examined by western blotting. The results of the quantitative analysis of the blots; results are presented as means ± Std. Dev,*p < 0.05,

**p < 0.01, ***p < 0.001, N = 4 mice per group. (f) Images represent the results of three repeated experiments. N2a cells were treated with 107 CFU T. denticola or 1 μmol/L DAPT and 107 CFU T. denticola or 1 μmol/L KMI1303 and 107 CFU T. denticola. T. denticola -infected N2a cells were stained by an anti- Aβ1-42 protein antibody and viewed under a fluorescence microscope. (g) The results of IF are expressed as means ± Std. Dev., *p < 0.05, ***p < 0.001.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- APPWB.tif
- BACEWB.tif
- IF.tif
- PS1WB.tif
- PS2wb.tif