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Original Article

Mycoplasma hyorhinis markedly degrades β-amyloid peptides *in vitro* and *ex vivo*: a novel biological approach for treating Alzheimer's disease?

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Abstract: Accumulation of amyloid-β (Aβ) peptides (predominantly $A\beta_{40, 42}$) and their aggregation into plaques in the brain are thought to be the one of the major causes of Alzheimer's disease (AD). Originally discovered in our Chinese hamster ovary (CHO) cell line stably expressing human wild-type amyloid precursor protein (APP) (CHO/APPwt) cultures devoid of Aβ production, we found that *Mycoplasma* selectively degrades soluble Aβ in a time and dose (colony forming unit) dependent manner. Moreover, we fully characterized the *Mycoplasma* species as *Mycoplasma hyorhinis* (*M. hyorhinis*) by genetic and colony morphological analyses by light microscopy. Most interestingly, we attenuated the pathogenicity of *M. hyorhinis* by γ irradiation (3.5 Gy), and found that its ability to degrade Aβ was retained. On the other hand, heated and sonicated *M. hyorhinis* failed to retain this ability to degrade Aβ, suggesting that this degradation requires viable cells and likely a biologically active signaling pathway. In addition, we found that *M. hyorhinis* can degrade Aβ produced in AD model mice (PSAPP mice) *ex vivo*. Finally, we found that irradiated (non-pathogenic) *M. hyorhinis* also can degrade Aβ produced in PSAPP mice *in vivo*. These studies suggest that irradiated (non-pathogenic) *M. hyorhinis* can be a novel and alternative biological strategy for AD treatment.

Keywords: *Mycoplasma*, Alzheimer's disease, amyloid-β peptide, amyloid precursor protein

Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders worldwide, manifested by memory loss followed by progressive dementia. Characteristic AD pathology includes the presence of "senile" plaques, composed of the particularly highly neurotoxic 42 amino acid form of Aβ ($A\beta_{42}$), as well as hyper-phosphorylated forms of tau protein in various regions of the brain [1]. An array of cellular, animal, and clinical studies have provided extensive characterization of how these toxic structures form deep inside the brain, but their precise etiology remains unclear. Moreover, a number of clinical trials, including anti-amyloid therapy such as Aβ vaccination [2, 3], are ongoing worldwide for better prognosis and treatment of this devastating disease [4]. However, none of these trials have resulted in any meaningful translatable treatment that improves cognition to any significant degree.

One approach to enhance removal of Aβ pathology might involve a typical part of the human microbiome, specifically involving parasites known as *Mycoplasma*. Recently, Zhao and colleagues reported that *Mycoplasma* from a cell culture contamination efficiently and rapidly degrades extracellular Aβ released into the conditioned media by HEK293 cells stably transfected with the Swedish mutant form of human APP695 [5]. Notably, abolition of the *Mycoplasma* contaminant by quinolone-based antibi-

otics restored extracellular Aβ accumulation in these cells. Further studies indicate that *Mycoplasma* can reduce Aβ-mediated cellular toxicity by enhancing the calpain inhibitor calpastatin, reducing calpain activity as well as calpain-mediated cellular apoptosis [6, 7].

Mycoplasmas are among the simplest self-replicating bacteria and the consequences of an infection for the host cell are variable, ranging from no apparent effect to induction of apoptosis [8]. They are characterized phenotypically from other bacteria by their minute size, with a diameter of 0.2-0.4 μm, minute genome, and lack of a cell wall. Since they are widespread in nature, including as parasites of humans [8], it is important to further characterize its role in the pathogenesis of AD. The present study was undertaken to assess the role of *Mycoplasma* in Aβ degradation both *in vitro* and *in vivo*. Further studies were undertaken to assess whether or not its pathogenicity and ability to break down Aβ could be differentiated by treatment with γ irradiation. These studies could be the basis for development of future *Mycoplasma*-based AD treatment.

Materials and methods

Reagents and antibodies

$A\beta_{40, 42}$ and sAPPα ELISA kits and synthetic $A\beta_{40, 42}$ peptides were purchased from Invitrogen (Carlsbad, CA). For immunoblotting (IB), we used mouse monoclonal anti-N-terminal $A\beta_{1-17}$ antibody (6E10, Covance Research Products, Emeryville, CA) and β-actin antibody (Sigma-Aldrich, St Louis, MO). *M. hyorhinis* DNA was extracted by DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) and Real Time PCR (RT-PCR) was performed using the MycoSensor QPCR Assay Kit (Stratagene, LaJolla, CA). The restriction endonucleases HpaII, PflFI, XbaI, HaeIII, and BstBI were purchased from New England Biolabs (Ipswich, MA, UK). Penicillin and streptomycin were purchased from Invitrogen and *Mycoplasma* Removal Agent (MRA) was purchased from MP Biomedicals, Inc. (Solon, OH).

Cell culture

Mycoplasma-free Chinese hamster ovary (CHO) cell line stably expressing human wild-type APP (CHO/APPwt) was kindly provided by Dr. Stefanie Hahn (University of Heinrich Heine, Düsseldorf, Germany) and cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% fetal bovine serum (FBS), 1 mM sodium pyruvate, and 100 units/ml of penicillin and streptomycin for 24 h. $A\beta_{40, 42}$ peptides and sAPPα protein concentrations (200 pg/ml, 500 pg/ml, and 2,800 pg/ml, respectively) in CHO/APPwt-media were then determined by ELISA kits and the media was stored at -80°C for future study. For dose and time dependent analyses regarding degradation of $A\beta_{40, 42}$ peptides by *M. hyorhinis*, CHO/APPwt-media, containing natural $A\beta_{40, 42}$ peptides, was incubated with *M. hyorhinis* at 0, 0.8, 1.7, 3.25, and 7.5 X 10^6 CFU for 120 min. Moreover, CHO/APPwt-media was incubated with 3.25 X 10^6 CFU *M. hyorhinis* over 0, 30, 60, 120, and 240 min at 37°C.

Mycoplasma-infected (3.25 X 10^6 colony forming units (CFU) of *M. hyorhinis*) murine neuroblastoma N2a cells were cultured in DMEM containing 5% FBS in the absence or presence of MRA. After 24 h incubation at 37°C and 5% CO_2, the media was collected, centrifuged at 20,000 x g and the pellet was resuspended in 200 μl PBS per dish. These samples were used for *Mycoplasma*-infected media (Myco-media) and MRA containing *Mycoplasma* infected media (Myco-media/MRA), respectively. In addition, some of Myco-media were filtered through 0.2 μm filters (Myco-media/Filtered). All of these conditioned media were aliquoted and kept at -80°C until the further study. All experiments using *Mycoplasma* were conducted in compliance with protocols approved by the University of South Florida (USF) Institutional BioSafety Committee (IBC).

PCR

For *Mycoplasma* species identification, infected N2a cells were cultured for one week without antibiotic and supernatant was collected after centrifugation at 20,000 g for 3 min. DNA extraction (DNeasy Blood and Tissue Kit, Qiagen) and PCR analysis (MycoSensor PCR Assay Kit, Stratagene) were both performed according to the protocol described by Uphoff and Drexler [9]. To further confirm our identification, *Mycoplasma* colonies were seeded on solid agar plates, incubated for 9 d at 37°C and colonies were identified by light microscopy based on their typical and characteristic appearance on the agar media, as described previously [10, 11].

M. hyorhinis growth and titration

M. hyorhinis (ATCC 17981-TTR) was obtained from the American Type Culture Collection (ATCC) and grown statically at 37°C with 5% CO_2 in *Mycoplasma* medium (ATCC® Medium 243) as described by Edward and Freundt [12] for 1 w. The media was then centrifuged at 20,000 g for 3 min followed by separation of the cell supernatant. Agar plates were prepared with the same medium containing 1% purified agar (Sigma-Aldrich). Ten microliters of the *M. hyorhinis* containing supernatant were serially diluted with *Mycoplasma* medium, seeded in the agar plates and incubated for 9 d at 37°C [10]. *M. hyorhinis* colonies were observed and counted by light microscopy.

Irradiation of M. hyorhinis

To irradiate *M. hyorhinis,* we utilized the modified protocol of Bender and colleagues [13] at the H. Lee Moffitt Cancer Center irradiation facility (Tampa, FL). *M. hyorhinis* was exposed to 3.5 Gy γ-rays for 19 h. Its DNA was then extracted and analyzed by RT-PCR, to determine whether the irradiation procedure was successful in eliminating the bacteria pathogenicity *via* DNA damage.

Immunoblotting analysis

Cells in culture were washed three times with ice-cold PBS and lysed with cell lysis buffer (Cell Signaling Technology Inc., Danvers, MA). Both supernatant and cell lysates were cryopreserved at -80°C for IB analysis until testing. $A\beta_{40, 42}$ peptides secreted from cells or present in brain homogenates were analyzed by IB using 6E10 antibody according to our previous methods [14].

Mice

Four and Six-month-old female doubly transgenic PSAPP mice, bearing mutant human APP and mutant human presenilin 1 (PS1) transgenes, were purchased from the Jackson Laboratory (Bar Harbor, ME). Intracerebroventricular (i.c.v.) injection of *M. hyorhinis* was performed as described previously [15, 16]. All mice were kept and maintained in the Morsani College of Medicine Animal Facility at USF (Tampa, FL) and all animal model experiments were conducted in compliance with protocols approved by the USF Institutional Animal Care and Use Committee.

Tissue preparation

Mice were euthanized with isoflurane anesthesia followed by transcardial perfusion with ice-cold PBS. Brain tissues were isolated rapidly, divided into left and right hemispheres at the level of the longitudinal fissure of the cerebrum and then coronally sliced in 3 mm thickness using a mouse brain slicer (Muromachi Kikai, Tokyo, Japan). The left hemisphere was incubated with 18 X 10^7 CFU of *M. hyorhinis* (Myco-media) and right hemisphere was incubated with clean media free of *M. hyorhinis* over 8 h at 37°C and 5% CO_2. Soluble $A\beta_{40, 42}$ levels were measured in homogenates of each sample by ELISA and protein expression was examined by IB according to our previous studies [15-17].

Statistical analysis

All data were normally distributed; therefore, in instances of single mean comparisons, Levene's test for equality of variances followed by *t* test for independent samples was used to assess significance. In instances of multiple mean comparisons, one-way analysis of variance (ANOVA) was used, followed by *post hoc* comparison using Bonferonni's method. Levels were set at 0.05 for all analyses. The Statistical Package for the Social Sciences, release IBM 10.0.5 SPSS (IBM, Armonk, NY) was used for all data analyses.

Results and discussion

Mycoplasma degrades natural $A\beta_{40, 42}$ peptides produced by CHO/APPwt cells

To assess the effect of *Mycoplasma* on $A\beta_{40, 42}$ degradation, conditioned media from CHO/APPwt cells (CHO/APPwt-media), was mixed with (1) media from clean N2a cells (Clean media), (2) media from N2a cells infected with *Mycoplasma* (Myco-media), (3) filtered Myco-media (Myco-media/Filtered), or (4) Myco-media containing MRA (Myco-media/MRA), each respectively in four different ratios (1:0, 1:1/4, 1:1/2, and 1:1). The resulting media was then analyzed after 2 h incubation by ELISA and IB for $A\beta_{40, 42}$ and sAPPα. $A\beta_{40, 42}$ levels were significantly decreased in CHO/APPwt-media treated with Myco-media at 1:1/4, 1:1/2, and

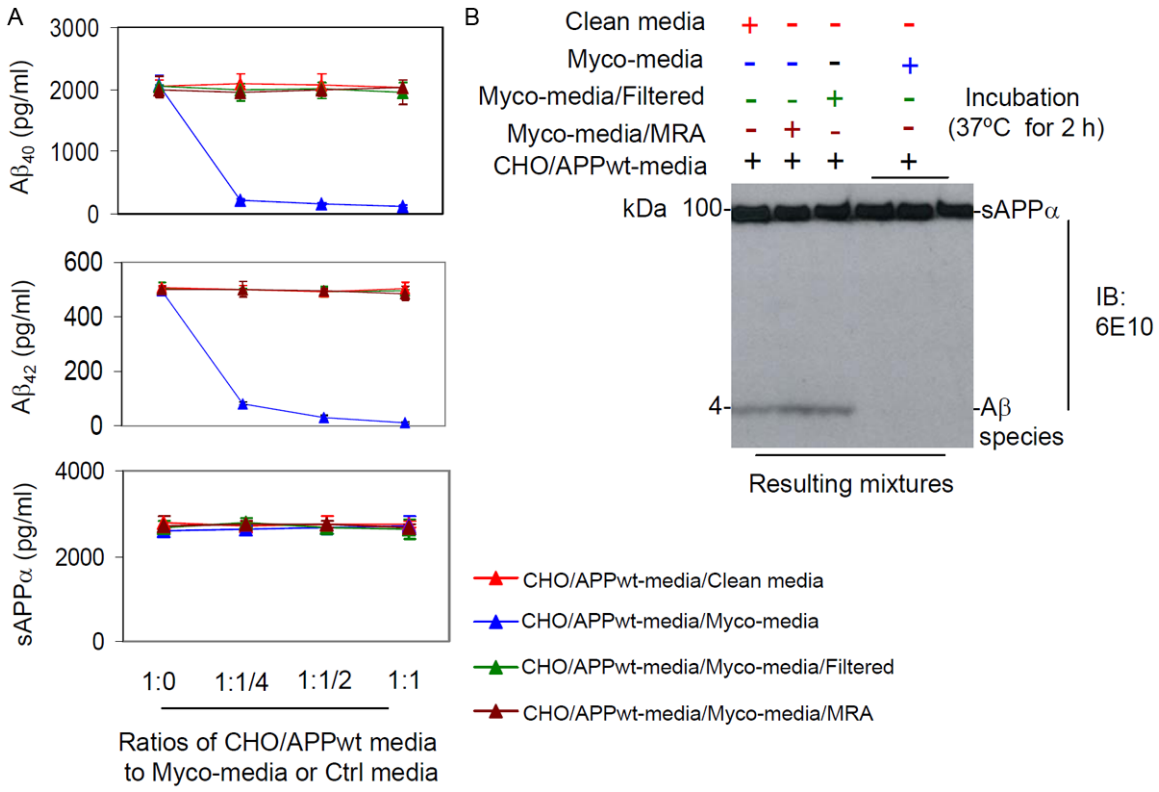

Figure 1. *Mycoplasma* selectively degrades naturally produced Aβ peptides. Conditioned media collected after 24 h culture of CHO/APPwt cells were incubated with (1) media from clean N2a cells (Clean media), (2) media from N2a cells infected with *Mycoplasma* (Myco-media), (3) filtered Myco-media (Myco-media/Filtered), or (4) Myco-media containing MRA (Myco-media/MRA) each respectively in four different ratios (1:0, 1:1/4, 1:1/2, and 1:1) at 37°C for 2 h. $Aβ_{40, 42}$ and sAPPα levels in the media were then determined by (A) ELISA (presented as mean ± SD), and (B) IB using an anti-N-terminal $Aβ_{1-17}$ antibody (6E10). These results are representative of four independent experiments with three replicates for each condition.

1:1 compared with 1:0 dilutions (**Figure 1A**). By contrast, $Aβ_{40, 42}$ levels were unchanged in CHO/APPwt-media treated with clean, filtered, or MRA containing Myco-media. These results were confirmed with IB analysis using 6E10 antibody (**Figure 1B**). *Mycoplasma* did not alter sAPPα levels irrespective of the treatment condition used, suggesting that *Mycoplasma* selectively degrade Aβ peptides. In addition, *Mycoplasma* did not alter either amyloidogenic or non-amyloidogenic APP processing as evidenced by IB analysis of C-terminal fragment of APP (data not shown). Furthermore, we incubated Myco-media with the conditioned media from murine splenocytes challenged with concanavalin A (ConA, 5 μg/ml) and containing IL-2, IL-6, or IFNγ and assessed cytokine levels by ELISA. *Mycoplasma* did not degrade other peptides, such as IL-2, IL-6, or IFNγ (data not shown).

Dose and time dependent degradation of $Aβ_{40, 42}$ peptides by M. hyorhinis

By restriction pattern digestion, we determined the responsible organism as *M. hyorhinis* for $Aβ_{40, 42}$ peptide degradation (**Figure 2A**). To further confirm this result, *M. hyorhinis* colonies were detected on microbiological media by light microscopy (**Figure 2B**). To assess the minimum dose of *M. hyorhinis* that can completely degrade $Aβ_{40, 42}$ peptides, CHO/APPwt-media, containing natural $Aβ_{40, 42}$ peptides, was incubated with *M. hyorhinis* at 0, 0.8, 1.7, 3.25, and 7.5 X 10^6 CFU for 120 min. Near complete degradation of $Aβ_{40, 42}$ occurred with *M. hyorhinis* at 3.25 X 10^6 CFU (**Figure 2C**). To define the minimum time required for $Aβ_{40, 42}$ peptide degradation, CHO/APPwt-media was incubated with 3.25 X 10^6 CFU *M. hyorhinis* over 0, 30, 60, 120, and 240 min at 37°C. ELISA results indicate that 120 min is the minimum time period

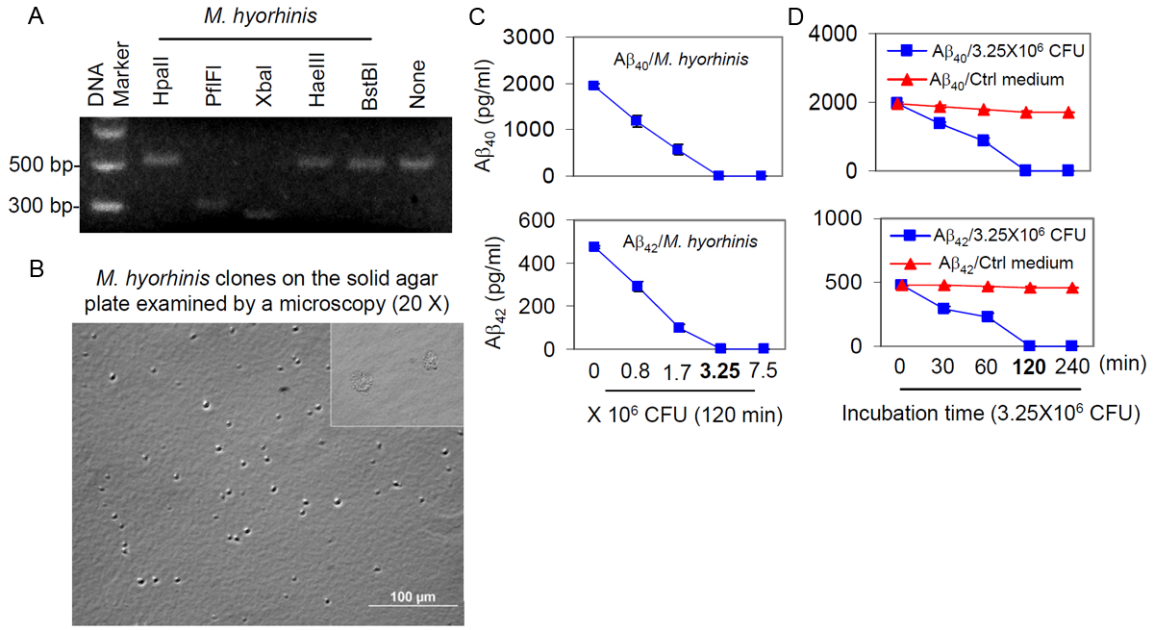

Figure 2. Identification of *M. hyorhinis* and characterization of $A\beta_{40, 42}$ degradation. A: *Mycoplasma* genomic DNA from infected N2a cell cultures was extracted, amplified and analyzed by restriction fragment length polymorphism (RFLP) using five different restriction endonucleases. The digestion pattern observed identified the *Mycoplasma* as *M. hyorhinis.* B: *M. hyorhinis* colonies were observed on solid agar plate by light microscopy (20X). C: CHO/APPwt media was incubated with 0, 0.8, 1.7, 3.25, or 7.5 X 10^6 colony forming units (CFU) of *M. hyorhinis* at 37°C for 120 min. D: CHO/APPwt media was incubated with 3.25 X 10^6 CFU of *M. hyorhinis* or clean *Mycoplasma* media (Ctrl Medium) for 0, 30, 60, 120 and 240 min at 37°C. $A\beta_{40}$ and $A\beta_{42}$ were then determined in the media by ELISA and presented as mean ± SD. These results are representative of three independent experiments with three replicates for each condition.

required for complete degradation of $A\beta_{40, 42}$ peptides (**Figure 2D**).

$A\beta_{40, 42}$ peptides in both natural and synthetic forms are degraded by M. hyorhinis in a similar manner

To further assess the ability of *M. hyorhinis* to degrade $A\beta_{40, 42}$ peptides, we compared the ability of *M. hyorhinis* to degrade both natural and synthetic $A\beta_{40, 42}$ peptides, contained in CHO/APPwt media, and synthetic $A\beta_{40, 42}$ peptides by IB using 6E10 antibody. *M. hyorhinis* at 0, 1.7, and 3.25 X 10^6 CFU decreased both natural (**Figure 3A**) and synthetic $A\beta_{40, 42}$ peptides significantly in a similar dose dependent pattern (**Figure 3B**). As expected, sAPPα bands (100 kDa) were absent in **Figure 3B**, as we used synthetic $A\beta_{40, 42}$ peptides.

Degradation of $A\beta_{40, 42}$ peptides by irradiated M. hyorhinis

Since *M. hyorhinis* is considered to be a pathogenic parasite, we sought to determine whether irradiated (non-pathogenic) *M. hyorhinis* could retain its ability to degrade $A\beta_{40, 42}$ peptides. CHO/APPwt-media were incubated at 37°C for 120 min with PBS or 3.25 X 10^6 CFU of (1) cryopreserved (Myco Ctrl), (2) fresh, non-irradiated (Non-irradi Myco), (3) irradiated (3.5 Gy, Irradi Myco), (4) heated (56°C X 10 min; Heat Myco), or (5) sonicated *M. hyorhinis.* (Soni Myco). The resulting mixtures were then analyzed for $A\beta_{40, 42}$ peptides by ELISA and IB using 6E10 antibody. The irradiated *M. hyorhinis* was able to significantly degrade $A\beta_{40, 42}$ peptides, similar to that observed with the cryopreserved (Myco Ctrl) or fresh, nonirradiated (Non-irradi Myco) *M. hyorhinis* (**Figure 3C** and **3E**). As expected, we found that heated or sonicated *M. hyorhinis* failed to degrade $A\beta_{40, 42}$ peptides. In addition, RT-PCR analysis of DNA extracted from *M. hyorhinis* after irradiation showed that this treatment almost completly eliminated the proliferative capacity of *M. hyorhinis* (**Figure 3D**).

Degradation of brain tissue-derived $A\beta_{40, 42}$ peptides of PSAPP mice by M. hyorhinis

We have shown that *M. hyorhinis* can degrade both natural and synthetic $A\beta_{40, 42}$ peptides.

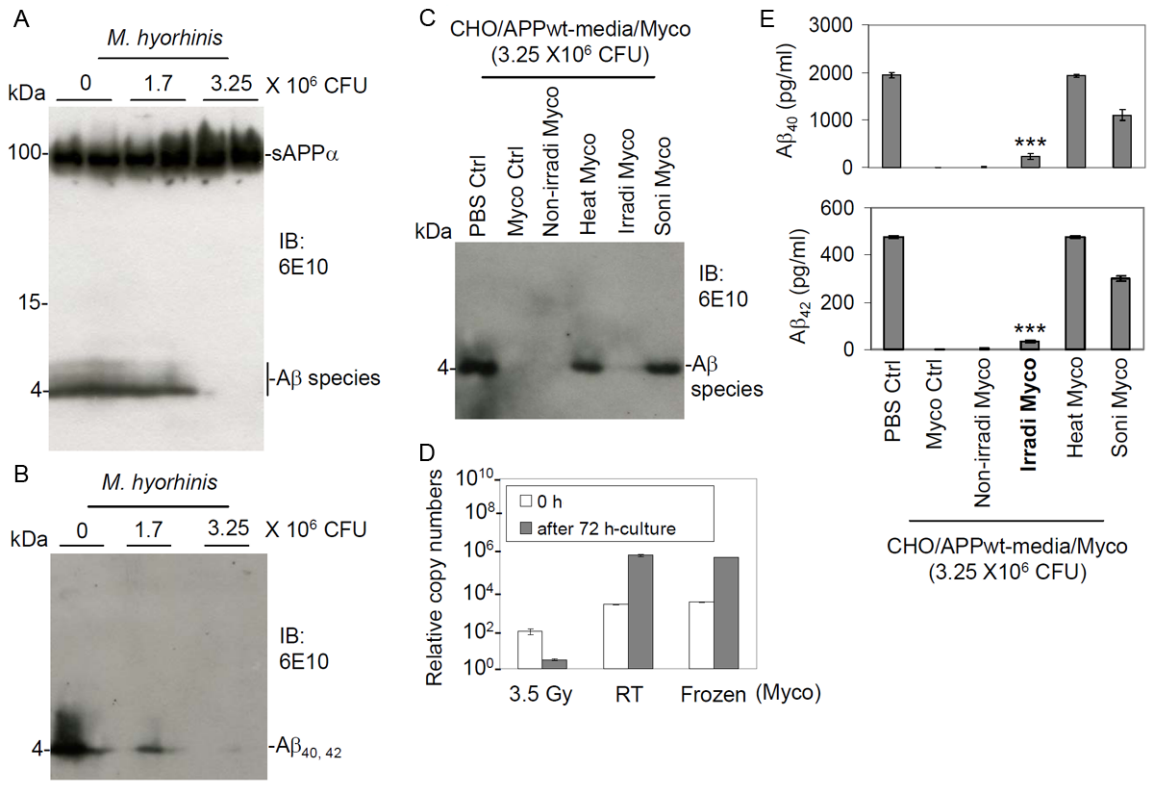

Figure 3. Degradation of $A\beta_{40, 42}$ peptides using irradiated *M. hyorhinis*. *M. hyorhinis* at 0, 1.7, and 3.25 X 10^6 CFU were incubated with (A) CHO/APPwt-media or (B) synthetic human $A\beta_{40, 42}$ peptides (each peptide at 500 ng/ml) at 37°C for 120 min. Aβ and sAPPα levels were then determined by IB using 6E10 antibody. These results are representative of two independent experiments. In addition, CHO/APPwt-media were incubated with PBS or 3.25 X 10^6 CFU of (1) cryopreserved (Myco Ctrl), (2) fresh, non-irradiated (Non-irradi Myco), (3) irradiated (Irradi Myco), (4) heated (56°C for 10 min, Heat Myco), or (5) sonicated *M. hyorhinis* (Soni Myco) at 37°C for 120 min. The resulting mixtures were then subjected $A\beta_{40}$ and $A\beta_{42}$ analysis by (C) IB analysis and (E) ELISA (presented as mean ± SD). Both $A\beta_{40}$ and $A\beta_{42}$ levels were significantly decreased by Myco Ctrl, Non-irradi Myco, and Irradi Myco (***$p < 0.001$). (D) RT-PCR results of irradiated (3.5 Gy), fresh (kept in room temperature, RT) and cryopreserved *M. hyorhinis* (Frozen, -80°C) at 0 and 72 h incubation. These results are representative of two independent experiments with three replicates for each condition.

Furthermore, we wished to determine whether this organism could degrade $A\beta_{40, 42}$ peptides *ex vivo*. We removed brain tissue of six-month-old female PSAPP transgenic mice and divided them into left and right hemispheres. Coronal slices (3 mm in thickness) from the left and right hemispheres were incubated with 1.8 X 10^7 CFU of *M. hyorhinis* or clean media for 8 h respectively. The levels of $A\beta_{40, 42}$ were significantly reduced in samples containing *M. hyorhinis* compared with those in clean media as determined by ELISA (**Figure 4A**) and confirmed by IB analysis using 6E10 antibody (**Figure 4B**). Finally, we attempted to determine whether this organism could degrade $A\beta_{40, 42}$ peptides *in vivo*. We analyzed soluble $A\beta_{40, 42}$ peptides in four-month-old female PSAPP transgenic mice following i.c.v. injection of irradiated *M. hyorhinis*. The levels of $A\beta_{40}$ and $A\beta_{42}$ were significantly reduced in PSAPP transgenic mice i.c.v. injected with irradiated *M. hyorhinis* compared to those injected with PBS as determined by ELISA (**Figure 4C**) and confirmed by IB analysis using 6E10 antibody (**Figure 4D**).

Discussion

The present study explored a new approach for removing Aβ from the brain involving *Mycoplasma*. We show that *Mycoplasma* can degrade Aβ, both naturally produced by CHO/APPwt cells and synthetic, in a dose and time dependent fashion and that the *Mycoplasma* species mediating this effect is *M. hyorhinis*. This degradation appears to be selective, since *Mycoplasma* did not alter the levels of sAPPα or

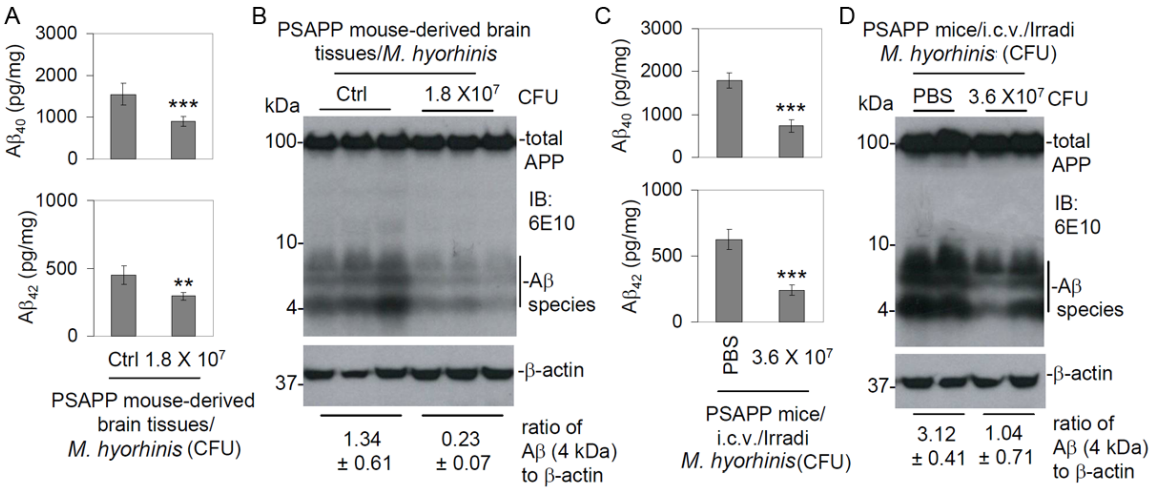

Figure 4. Degradation of $A\beta_{40, 42}$ peptides of PSAPP mice *ex vivo* and *in vivo* by *M. hyorhinis*. Six-month-old transgenic PSAPP mice were euthanized and brain tissue was removed from each mouse and divided into left and right hemispheres. The left hemisphere of each mouse was incubated with 1.8 X 10^7 CFU *M. hyorhinis* and the right hemisphere was incubated with clean media (Ctrl, without *M. hyorhinis*) for 8 h respectively. After incubation, brain tissue was homogenized and soluble $A\beta_{40, 42}$ peptides were measured individually by (A) ELISA (presented as mean ± SD, n = 3) and confirmed by (B) IB analysis using 6E10 antibody. *Mycoplasma* significantly decreased both $A\beta_{40}$ and $A\beta_{42}$ peptides. Furthermore, PSAPP mice at 4 months of age were intracerebroventricular (i.c.v.) injected with irradiated *M. hyorhinis* at 3.6 X 10^7 CFU/mouse (n = 5) and euthanized 48 h after the injection. Mouse brain homogenates were prepared (the right half of brain tissues (the non-injection side)) and subjected to soluble $A\beta_{40, 42}$ peptide ELISA (C) and IB analysis (D). The ratio of Aβ to β-actin for brain tissues incubated with *M. hyorhinis* or clean media, as well as that prepared from PSAPP mice i.c.v. injected with *M. hyorhinis* or PBS, are shown below IB, indicating that *M. hyorhinis* indeed degraded Aβ (**$p < 0.01$, ***$p < 0.001$).

degrade other peptides such as IL-2, IL-6, or IFNγ. In addition, *M. hyorhinis* retained its ability to reduce Aβ after γ-ray irradiation, a treatment which substantially reduced its pathogenicity. However, its ability to reduce Aβ was eliminated by filtration, sonication, heat or treatment with MRA, indicating that this effect is not mediated by a direct molecular interaction of Aβ with released or cytoplasmic constituents but required intact viable cells and possibly a signal transduction process. Finally, we showed that *M. hyorhinis* can reduce Aβ *ex vivo* in cortical brain slices isolated from PSAPP mice, an AD mouse model, as well as *in vivo* in PSAPP transgenic mice.

These results confirm and extend those previously reported by Zhao et al. [8], who showed that *M. hyorhinis* can reduce Aβ naturally produced by SH-SY5Y cells overly expressing the Swedish mutant form of APP and that this effect was eradicated by treatment of the *Mycoplasma* with a quinolone-based antibiotic. Subsequent studies showed that *M. hyorhinis* can reduce Aβ-mediated cellular toxicity by enhancing levels of the calpain inhibitor, calpastatin, thereby reducing calpain activity and calpain-mediated apoptosis [6]. This effect was further shown to be due to a membrane lipoprotein in *M. hyorhinis* which induces nuclear translocation of the transcription factor, nuclear factor-kappa B(NF-κB) [7].

Taken together, these results implicate *M. hyorhinis*, a natural part of the human microbiome, as a potentially effective and novel treatment for AD. Another potential known benefit of *Mycoplasma* is their propensity to consume fatty acids and cholesterol for their own membrane synthesis [18]. However, the major disadvantage with using *Mycoplasma* as a potential therapeutic agent is its disease causing properties. *Mycoplasma hyorhinis* was first isolated from the respiratory tract of young pigs, and has been implicated in pleuritis, peritonitis, pericarditis, arthritis, and otitis media of swine [19, 20]. The interest in *M. hyorhinis* has recently increased after the detection of this organism in human beings [21], where it can affect membrane properties and cellular functions related to the immune system [22]. Additional studies implicate *M. hyorhinis* in the pathogen-

esis of cancer, such as melanoma [23, 24]. However, by treatment such as irradiation, it may be possible to reduce its pathogenicity while retaining its ability to reduce Aβ. Given the simple genome of *Mycoplasma*, it may also be possible to harness the natural properties of this bacterium by genome modification, thereby reducing its pathogenicity while enhancing its beneficial effects for the treatment of AD and other neurological diseases. As there is yet no effective treatment for neurological diseases such as AD, this possibility merits further investigation.

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Disclosure of conflict of interest

None.

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