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RHOA TARGETING BY PROBIOTICS AS STRATEGY TO INTERFERE THE CRITICAL LINK TO MAJOR HALLMARKS OF ALZHEIMER'S DISEASE

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ABSTRACT

Alzheimer's disease (AD) is the commonest form of dementia characterised by aggregation of amyloid beta (Aβ) plaque. RhoA, which is being increasingly recognised for its role in AD pathogenesis through the amyloidogenic pathway, may serve as a potential therapeutic target. Preliminary screening of MRS broth fermented with lactic acid bacteria (LAB) yielded strain-dependent inhibition of RhoA activation *in vitro*. LAB also significantly inhibited Aβ produced by SK-N-SH transfected with amyloid precursor protein (APP) gene. A strong correlation was found between inhibition of RhoA and Aβ. LAB-derived supernatant were also presented with increased organic acids which included lactic acid (>100%), acetic acid (≥15.3%), butyric acid (≥34.5%) and propionic acid (≥29.1%). The findings were validated using lipopolysaccharide (LPS)-challenged rats with neuroinflammation that mimic AD. Sprague Dawley rats (male, 3 months) were divided (n=6/group) into wild-type, control, vehicle controls and treatment groups [10^9 CFU/ mL LAB6/ *Pediococus pentasaceus* or LAB12/ *Lactobacillus plantarum* and 10 mg/kg ibuprofen (positive control) for 31 days]. Except for wild-type, all rats were injected (*i.p.*) with 0.25 mg/kg LPS for 4 days starting from day 28. The rodents were then subjected to the Morris Water Maze Test. Brains were harvested and subjected to Western Blot, immunohistostaining and biochemical analyses. LAB-fed LPS-challenged rats exhibited significantly (p<0.05) reduced escape latency and escape distance. They remained longer in the platform quadrant (≥9.8±0.5 sec) when compared to control (≤5.1±1.9 sec). The improved memory was accompanied by significantly (p<0.05) decreased Aβ (≤31.4%) and RhoA activity (≤22.5%), augmented BDNF (≥15.4%) and ACh (≥32.3%), reduced AChE (≤40.1%) and NO (≤30.3%) levels. LAB-fed rats also showed increased IL-10 (≥32.1%) and decreased IL-1β (≤39.5%). The LAB was then investigated using Aβ-induced rats that mimic Aβ plaque aggregation in AD. Sprague Dawley rats (male, 3 months) were divided (n=7/group) into wild-type, sham, control, vehicle controls and treatment groups. Except for wild type and sham, all rats were subjected to intracranial injection with 5 µg/µL Aβ 1-42 peptide. Treatment groups (day 4) were administered with either 10^9 CFU/mL LAB6, LAB12 or 10 mg/kg ibuprofen for 30 days. The Aβ-induced rats were assessed for parameters similar to those of LPS-challenged rats. LAB-fed rats exhibited significantly (p<0.05) reduced escape latency, escape distance and remained longer in platform quadrant. The improved memory was accompanied by reduced Aβ (≤31.4%) and RhoA activity (≤35.7%), increased BDNF (≥18.3%) and ACh (≥30.3%), reduced AChE (≤28.8%) and NO (≤33.6%). LAB-fed rats also exhibited up-regulation of IL-10 (≥33.3%) and down-regulation of IL-1β (≤41.6%). The present findings indicated that LAB-induced neuroprotection could be mediated via inhibition of RhoA-Aβ generated neuroinflammation and accompanied by increased production of organic acid metabolites, restored BDNF, reduced degradation of acetylcholine and down-regulation of pro-inflammatory as well as up-regulation of anti-inflammatory cytokines.

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CHAPTER ONE
INTRODUCTION

Alzheimer's disease (AD) is the commonest form of dementia. It is termed the "21st century plague" given the drastic increase of this "protein misfolding disorder" over the past few decades (Knowles *et al.*, 2014), especially amongst the aging population (Alzheimer's Association, 2017). At present, approximately 40 million people worldwide are suffering from AD and this number is projected to rise to about 135 million by 2050, amongst which 70% of AD patients are expected to come from low- or middle-income nations (Alzheimer's Disease International, 2015). It is apparent that AD is no longer a neurodegenerative disease confined to wealthy nations but is on the verge of becoming a global issue. In Malaysia, the actual number of AD patients is often underestimated as AD symptoms are considered as part of ageing. In 2010, there were about 50,000 Malaysians who suffered from AD. Today, the actual number may have amounted to 60,000 (Alzheimer's Disease Foundation Malaysia, 2016). Generally, the increased global incidence of AD is attributable to the increased number of people over the age of 65, whereby AD affects between 1% and 2% of the population. The incidence of AD further rises to between one-third and one-half amongst those living to the age of 85 (Alzheimer's Association, 2016).

There is no cure for AD just yet. This is mainly due to the lack of understanding with regard to mechanisms underpinning the pathogenesis of this neurodegenerative disease. Existing anti-AD drugs treat only signs and symptoms of AD. There is no evidence that these treatments can significantly alter the progression of AD (Schneider *et al.*, 2011). The current therapeutic strategies against AD revolves around four major drugs that have been approved by the Food and Drug Administration (FDA) for AD treatment and they include three acetylcholinesterase (AChE) inhibitors (i.e. donepezil, galantamine and rivastigmine) and an N-methyl-D-aspartate (NMDA) antagonist (i.e. memantine) (Karthivashan *et al.*, 2018).A previous study showed that the combination of memantine and cholinesterase inhibitors yielded a statistically significant but clinically marginal improvement in cognitive function and global assessment of dementia (Raina *et al.*, 2008). AChE inhibitors block the action of AChE which in turn prevent ACh deficits in the brain during onset of AD (Birks, 2012). On the other hand, excessive amounts of glutamate neurotransmitters can lead to excitotoxicity. In this