

## Bitter Components in Beer Regulate Microglial Inflammation and Prevent Cognitive Decline

Yasuhisa Ano<sup>1\*</sup>, Akihiko Takashima<sup>2</sup> and Hiroyuki Nakayama<sup>3</sup>

<sup>1</sup>Research Laboratories for Health Science & Food Technologies, Kirin Company Ltd, 1-13-5 Fukuura Kanazawa-ku, Yokohama-shi, Kanagawa 236-0004, Japan

<sup>2</sup>Faculty of Science, Gakushuin University, Toshima-ku, Tokyo 171-8588, Japan

<sup>3</sup>Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

\*Corresponding author: Yasuhisa Ano, Central Laboratories for Key Technologies, Kirin Company Ltd, 1-13-5 Fukuura Kanazawa-ku, Yokohama-shi, Kanagawa 236-0004, Japan, E-mail: [Yasuhisa\\_Ano@kirin.co.jp](mailto:Yasuhisa_Ano@kirin.co.jp)

Received date: May 12, 2017; Accepted date: June 13, 2017; Published date: June 19, 2017

Copyright: © 2017 Ano Y, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

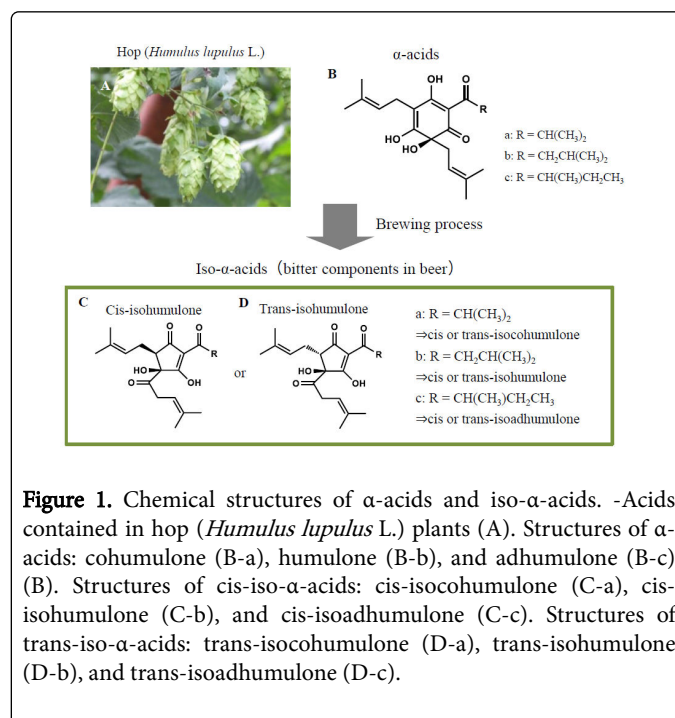
Epidemiological studies suggest that moderate consumption of alcoholic beverages can reduce the risk of dementia. Recent work has demonstrated that intake of iso- $\alpha$ -acids, the bitter components in beer; prevent Alzheimer's disease pathology by regulating microglial function. In a transgenic mouse model of Alzheimer's disease, iso- $\alpha$ -acid intake led to a significant reduction of A $\beta$  and inflammatory cytokines. In addition, iso- $\alpha$ -acids enhanced microglial phagocytosis of A $\beta$  and induced microglia to an anti-inflammatory M2 type. These findings suggest that iso- $\alpha$ -acid consumption in daily life may be beneficial in preventing dementia.

**Keywords:** Alzheimer's disease; Inflammation; Microglia

### Introduction

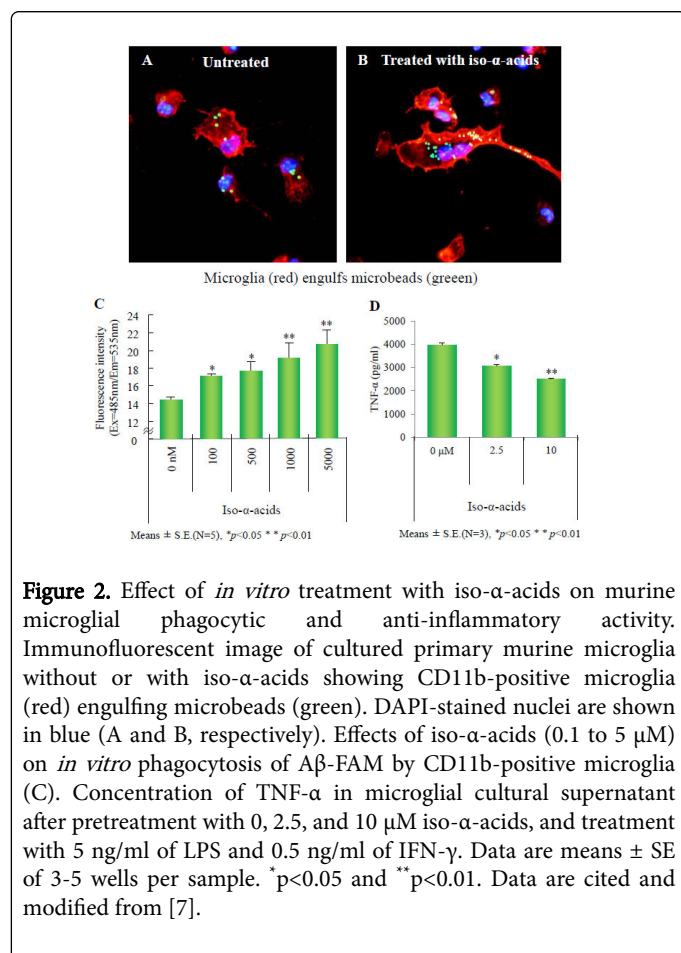
With the rapid growth of aging populations worldwide, cognitive decline and dementia are becoming an increasing burden not only on patients and their families, but also on national healthcare systems. Because of the lack of a disease therapy for dementia, preventive approaches are receiving increasing attention. Dementia is a collective term, encompassing Alzheimer-type dementia, vascular dementia, Lewy body dementia and frontotemporal dementia, and approximately 60% of dementia is classified as Alzheimer-type dementia. In Alzheimer's disease,  $\beta$ -amyloid (A $\beta$ ) becomes aggregated and gets deposited as the affected individual ages. The A $\beta$  deposits induce inflammation and neurologic deficits in the brain and result in cognitive decline [1,2]. Because A $\beta$  is slowly produced in the brain for more than 10-20 years before cognitive decline are manifest, preventive approaches before rather than remedy after disease onset represents a promising therapeutic avenue.

Etiological reports suggest that low-to-moderate consumption of alcoholic beverages, such as wine and beer, reduce the risk of dementia. Individuals who consume low-to-moderate levels of alcoholic beverages on a daily basis have been shown to have a significantly lower risk of the development of cardiovascular and neurodegenerative disease relative to those who abstain from alcohol beverages or drink heavily [3-5]. Aside from the effects of alcohol itself, various reports have shown that resveratrol, a polyphenol in red wine, has a preventive effect on dementia and cognitive decline [6-8]. On the other hand, even though it is the most-consumed alcoholic beverage, no constituents of beer had been demonstrated to have preventive effects on dementia. However, a recent study using a rodent model has now shown that intake of ingredients in beer has preventive effects on Alzheimer's disease pathology [9]. These ingredients are iso- $\alpha$ -acids, the hop-derived bitter components of beer.



Hops, the female inflorescences of the hop plant (*Humulus lupulus* L. Figure 1A), are essential materials in brewing that give beer its fresh bitterness and bright flavor. Hops have also been traditionally used as a medicinal plant, and several health benefits have been reported including appetite improvement, induction of sound sleep, stress relief, osteoporosis prevention, suppressing high-blood pressure, and obesity improvement. Various ingredients contained in hops or beer, such as humulone, lupulon, xanthohumol and eudesmol, in addition to iso- $\alpha$ -acids derived from  $\alpha$ -acids (humulone, Figure 1B), are well known to

impart the bitter taste to beer.  $\alpha$ -Acids from hops are isomerized into iso- $\alpha$ -acids during the brewing process (Figure 1C and 1D). The concentrations of iso- $\alpha$ -acids are dependent on the type of beer; for example, ordinal beer contains iso- $\alpha$ -acids at 10-30 ppm. Iso- $\alpha$ -acids are especially rich in India Pale Ale (IPA)-type beers and are present in non-alcoholic beverages with a beer flavor. A previous study using rodents and humans demonstrated the effects of iso- $\alpha$ -acids on anti-obesity and metabolic syndrome improvement [10,11].

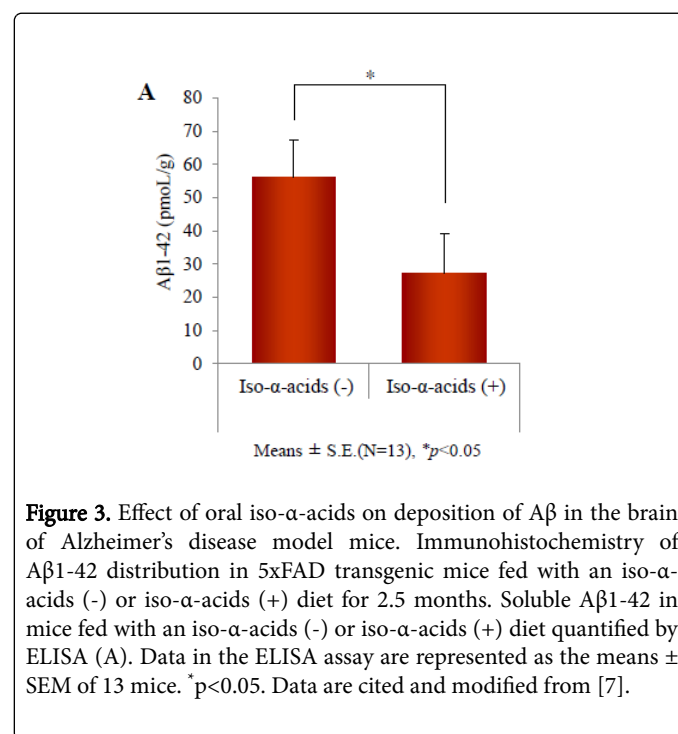


**Figure 2.** Effect of *in vitro* treatment with iso- $\alpha$ -acids on murine microglial phagocytic and anti-inflammatory activity. Immunofluorescent image of cultured primary murine microglia without or with iso- $\alpha$ -acids showing CD11b-positive microglia (red) engulfing microbeads (green). DAPI-stained nuclei are shown in blue (A and B, respectively). Effects of iso- $\alpha$ -acids (0.1 to 5  $\mu$ M) on *in vitro* phagocytosis of A $\beta$ -FAM by CD11b-positive microglia (C). Concentration of TNF- $\alpha$  in microglial cultural supernatant after pretreatment with 0, 2.5, and 10  $\mu$ M iso- $\alpha$ -acids, and treatment with 5 ng/ml of LPS and 0.5 ng/ml of IFN- $\gamma$ . Data are means  $\pm$  SE of 3-5 wells per sample. \* $p$ <0.05 and \*\* $p$ <0.01. Data are cited and modified from [7].

More recently, iso- $\alpha$ -acids were identified as novel components regulating microglia [9], a type of immune cell in the nervous systems. As a regulator of the immune response in the brain, microglia have been recently attracting increasing attention because numerous reports suggest that inflammation in the brain severely induces cognitive decline and exacerbates Alzheimer's disease pathology. Microglia play an important role in removing waste products such as A $\beta$ , but their function declines alongside the aging process, when, in contrast, they can induce inflammation in the brain. Iso- $\alpha$ -acids were identified as a component in beer that can enhance microglial phagocytic activity (Figure 2A and 2B) and suppress microglial inflammatory responses [9]. Iso- $\alpha$ -acids enhanced both microglial phagocytosis of A $\beta$  and expression of CD36, a scavenger receptor for A $\beta$  (Figure 2C) [12]. Simultaneously, iso- $\alpha$ -acids suppressed the production of TNF- $\alpha$  in response to LPS stimulation and induce microglia towards a CD206-positive M2 anti-inflammatory cell type (Figure 2D) [13].

After the evaluation in primary microglia, the preventive effects of iso- $\alpha$ -acids on Alzheimer's disease were evaluated by using a rodent

model. Iso- $\alpha$ -acids were fed to 5xFAD transgenic mice overexpressing mutant human APP (695) with the Swedish (K670N, M671L), Florida (I716V), and London (V717I) Familial Alzheimer's Disease (FAD) mutations, along with human PS1 harboring two FAD mutations, M146L and L286V. These model mice rapidly develop severe amyloid pathology [14]. Immunohistochemical analysis revealed that intake of iso- $\alpha$ -acids reduced A $\beta$  deposition by 30%-50% in the cerebral cortex and hippocampus. Quantification by ELISA showed that iso- $\alpha$ -acids also suppressed the accumulation of soluble and insoluble A $\beta$  (Figure 3). The levels of inflammatory cytokines and chemokines such as TNF- $\alpha$ , IL-1 $\beta$ , and MIP-1 $\alpha$ , which are induced after A $\beta$  accumulation, were also significantly reduced. Analysis of phagocytic activity and cell-surface marker expression in microglia in the brain of 5xFAD mice also demonstrated that intake of iso- $\alpha$ -acids significantly ameliorated the decline in phagocytosis of A $\beta$  in 5xFAD mice and enhanced CD36 expression. In addition, a novel object recognition test, a standard behavioral pharmacological evaluation [15], revealed that iso- $\alpha$ -acids prevent cognitive decline and memory impairment in 5xFAD mice. Collectively, these results demonstrate that iso- $\alpha$ -acids have preventive effects on Alzheimer's disease pathology and cognitive decline by improving microglial phagocytic activity and inflammatory response.



**Figure 3.** Effect of oral iso- $\alpha$ -acids on deposition of A $\beta$  in the brain of Alzheimer's disease model mice. Immunohistochemistry of A $\beta$ 1-42 distribution in 5xFAD transgenic mice fed with an iso- $\alpha$ -acids (-) or iso- $\alpha$ -acids (+) diet for 2.5 months. Soluble A $\beta$ 1-42 in mice fed with an iso- $\alpha$ -acids (-) or iso- $\alpha$ -acids (+) diet quantified by ELISA (A). Data in the ELISA assay are represented as the means  $\pm$  SEM of 13 mice. \* $p$ <0.05. Data are cited and modified from [7].

In summary, a new beneficial aspect of iso- $\alpha$ -acids, hop-derived bitter components in beer, has been identified. It is widely known that alcoholic beverages should be consumed in moderation; in addition, more elderly individuals tend to refrain from alcoholic intake. Thus, a new tool making it possible to consume iso- $\alpha$ -acids comfortably in daily life is desired.

## References

- Mandrekar-Colucci S, Landreth GE (2010) Microglia and inflammation in Alzheimer's disease. CNS Neurol Disord Drug Targets 9: 156-167.
- Wyss-Coray T, Rogers J (2012) Inflammation in Alzheimer disease-a brief review of the basic science and clinical literature. Cold Spring Harb Perspect Med 2: a006346.

3. Matsui T, Yoshimura A, Toyama T, Matsushita S, Higuchi S (2011) Preventive effect of moderation in drinking on dementia. *Nihon Rinsho* 2: 217-222.
4. Neafsey EJ, Collins MA (2011) Moderate alcohol consumption and cognitive risk. *Neuropsychiatr Dis Treat* 7: 465-484.
5. Horvat P, Richards M, Kubinova R, Pajak A, Malyutina S, et al. (2015) Alcohol consumption, drinking patterns, and cognitive function in older Eastern European adults. *Neurology* 84: 287-295.
6. Arntzen KA, Schirmer H, Wilsgaard T, Mathiesen EB (2010) Moderate wine consumption is associated with better cognitive test results: a 7 year follow up of 5033 subjects in the Tromsø Study. *Acta Neurol Scand Suppl* : 23-29.
7. Porquet D, Griñán-Ferré C, Ferrer I, Camins A, Sanfeliu C, et al. (2014) Neuroprotective role of trans-resveratrol in a murine model of familial Alzheimer's disease. *J Alzheimers Dis* 42: 1209-1220.
8. Witte AV, Kerti L, Margulies DS, Flöel A (2014) Effects of resveratrol on memory performance, hippocampal functional connectivity, and glucose metabolism in healthy older adults. *J Neurosci* 34: 7862-7870.
9. Ano Y, Dohata A, Taniguchi Y, Hoshi A, Uchida K, et al. (2017) Iso-alpha-acids, Bitter Components of Beer, Prevent Inflammation and Cognitive Decline Induced in a Mouse Model of Alzheimer's Disease. *J Biol Chem* 292: 3720-3728.
10. Yajima H, Ikeshima E, Shiraki M, Kanaya T, Fujiwara D, et al. (2004) Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor alpha and gamma and reduce insulin resistance. *J Biol Chem* 279: 33456-33462.
11. Obara K, Mizutani M, Hitomi Y, Yajima H, Kondo K (2009) Isohumulones, the bitter component of beer, improve hyperglycemia and decrease body fat in Japanese subjects with prediabetes. *Clin Nutr* 28: 278-284.
12. Yu Y, Ye RD (2015) Microglial A $\beta$  receptors in Alzheimer's disease. *Cell Mol Neurobiol* 35: 71-83.
13. Cherry JD, Olschowka JA, O'Banion MK (2014) Neuroinflammation and M2 microglia: the good, the bad, and the inflamed. *J Neuroinflammation* 11: 98.
14. Oakley H, Cole SL, Logan S, Maus E, Shao P, et al. (2006) Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. *J Neurosci* 26: 10129-10140.
15. Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdóttir HN, et al. (2013) The rodent hippocampus is essential for nonspatial object memory. *Curr Biol* 23: 1685-1690.

This article was originally published in a special issue, entitled: "**Neuroinflammatory Diseases**", Edited by Michael C. Levin, University of Tennessee Health Science Center, USA