

Probiotic Mixture KF Attenuates Age-Dependent Memory Deficit and Lipidemia in Fischer 344 Rats

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Received: May 6, 2015
Accepted: May 14, 2015

First published online
May 14, 2015

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pISSN 1017-7825, eISSN 1738-8872

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To investigate the memory-enhancing effect of lactic acid bacteria, we selected the probiotic mixture KF, which consisted of *Lactobacillus plantarum* KY1032 and *Lactobacillus curvatus* HY7601 (1×10^{11} CFU/g of each strain), and investigated its antilipidemic and memory-enhancing effects in aged Fischer 344 rats. KF (1×10^{10} CFU/rat/day), which was administered orally once a day (6 days per week) for 8 weeks, significantly inhibited age-dependent increases of blood triglyceride and reductions of HDL cholesterol ($p < 0.05$). KF restored age-reduced spontaneous alternation in the Y-maze task to 94.4% of that seen in young rats ($p < 0.05$). KF treatment slightly, but not significantly, shortened the escape latency daily for 4 days. Oral administration of KF restored age-suppressed doublecortin and brain-derived neurotrophic factor expression in aged rats. Orally administered KF suppressed the expression of p16, p53, and cyclooxygenase-2, the phosphorylation of Akt and mTOR, and the activation of NF- κ B in the hippocampus of the brain. These findings suggest that KF may ameliorate age-dependent memory deficit and lipidemia by inhibiting NF- κ B activation.

Keywords: Aging, lipidemia, memory deficit, *Lactobacillus plantarum*, *Lactobacillus curvatus*

Excessive oxidative stress is generated in the human body primarily as a result of redox imbalance between systems that produce oxidants and antioxidant defense mechanisms. Chronic excessive oxidative stress causes low-grade inflammation by activating the nuclear factor-kappa B (NF- κ B) signaling pathway, which accelerates the aging process [1, 3, 21]. Oxidative stress contributes to chronic inflammatory diseases, such as osteoarthritis and atherosclerosis, as well as many age-related degenerative diseases, such as Alzheimer's disease (AD) [5, 11] and Parkinson's disease [7].

Lactic acid bacteria (LAB) are gram-positive, acid-tolerant, lactic acid-producing microorganisms [14]. Among the beneficial LAB, the *Lactobacillus* spp. can restore disrupted gut microbiota [17], induce nonspecific activation of the host immune system in humans and animals [2], and show anti-colitic [8, 9] and memory-enhancing effects in mice [9, 20]. However, the effects of LAB in age-related lipidemia and

degenerative dementia have not been thoroughly studied.

In the present study, we investigated whether the probiotic mixture KF, consisting of a 1:1 ratio of *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 [16], can inhibit age-dependent lipidemia and memory deficit in aged Fischer 344 rats.

Each of the bacterial components in KF was cultured using previously reported methods [20]. The probiotics *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 were cultured briefly in MRS broth (10 L), centrifuged at $10,000 \times g$ for 30 min, washed with phosphate-buffered saline, and freeze-dried. Each strain (1×10^{11} CFU/g) was resuspended in 1% glucose solution in 50 mM sodium bicarbonate buffer (GSB) and used for the *in vivo* study.

All experiments were carried out in accordance with the Kyung Hee University guideline for University Laboratory Animals Care and Use and were approved by the Committee for the Care and Use of Laboratory Animals at

Kyung Hee University.

Male Fischer 344 rats (18 months old) obtained from Harlan (Indianapolis, IN, USA) were provided with water and food *ad libitum*, and maintained in a ventilated room at an ambient temperature of $22 \pm 1^\circ\text{C}$ with $50 \pm 10\%$ humidity and a 12 h diurnal light cycle (lights on 07:00–19:00) for 4 weeks prior to the experiment. Twenty-four rats were randomly distributed into four groups. All behavioral experiments were conducted in a room adjacent to the housing room under similar ambient conditions.

To measure the effect of KF on memory deficit in aged Fischer 344 rats, KF (1×10^{10} CFU/rat/day) or rapamycin (1 mg/kg/day, used as a positive control) was orally given once a day for 6 days each week; this was continued for 8 weeks. Y-maze and Morris water maze tasks were performed sequentially. Next, the hippocampus of the brain was removed and prepared for immunoblotting and enzyme-linked immunosorbent assay (ELISA).

For the measurement of memory-related behavioral responses, the Y-maze task was carried out, as previously reported [20], 1 h after the final oral administration of KF (1×10^{10} CFU/rat) or vehicle (GSB). The Morris water maze task was carried out for 4 days as previously reported [20], 1 h after oral administration of KF (1×10^{10} CFU/rat) or vehicle on each consecutive day.

For the ELISA, the hippocampus was homogenized in 1 ml of ice-cold RIPA lysis buffer containing 1% phosphatase inhibitor cocktail and 1% protease inhibitor cocktail as previously reported [9]. TNF- α , IL-1 β , and IL-6 levels were assayed using commercial ELISA kits (Pierce Biotechnology, Inc., Rockford, IL, USA).

For the immunoblot analyses, the hippocampus homogenate supernatant was used for the immunoblotting. Levels of p16, BDNF, DCX, p-FOXO3a, p-Akt, p-mTOR, COX-2, iNOS, p-p65, and β -actin were assayed as previously described [20].

Blood triglyceride, total cholesterol, and HDL cholesterol were analyzed using a commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

All data are presented as the mean \pm standard deviation (SD), with statistical significance analyzed by one-way ANOVA and Turkey's multiple comparison tests (12.0K for Windows; SPSS, Inc., Chicago, IL, USA) ($p < 0.05$).

To identify the potential inhibitory effect of KF on age-dependent lipidemia, we measured the blood TG, total cholesterol, and HDL cholesterol levels in young and aged rats (Fig. 1). Although aging slightly, but not significantly, increased the body, liver, and epididymal weights, there was a significant increase in blood TG level and decrease in

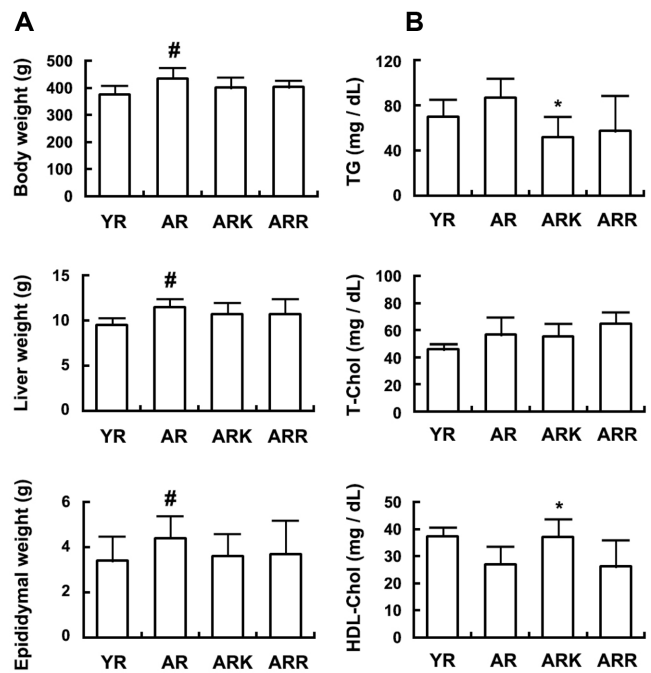


Fig. 1. Effect of KF on body, liver, and epididymal pad weights (A) and blood triglyceride, total cholesterol, and HDL cholesterol levels (B) in the aged rats.

Blood triglyceride, total cholesterol, and HDL cholesterol were analyzed using a commercial kit. KF (1×10^{10} CFU/rat, p.o.) or vehicle was administered for 8 weeks (YR, young Fischer 344 rats treated with vehicle; AR, aged rats treated with vehicle; ARK, aged rats treated with KF; ARR, aged rats treated with rapamycin (1 mg/kg, p.o.)). Data represent the mean \pm SD ($n = 6$ per group). # indicates a significant difference compared with the YR group ($p < 0.05$). *Indicates a significant difference compared with the AR group ($p < 0.05$).

blood HDL cholesterol. KF showed slight, but not significant, inhibition against the body and epididymal pad weights. Furthermore, KF significantly inhibited the age-dependent increase in blood TG and reduction in blood HDL cholesterol.

Next, we assessed the memory deficit-ameliorating effect of KF and rapamycin, an mTOR-inhibiting lifespan extender [15], in aged Fischer 344 rats. In the Y-maze task, spontaneous alternation in aged rats was attenuated more than that of young rats (Fig. 2A). However, oral administration of KF (1×10^{10} CFU/rat) significantly reversed the age-dependent reduction of spontaneous alternation to 94.4% of that in young rats ($p < 0.05$). We also investigated the effect of KF on spatial memory by the Morris water maze task (Fig. 2B). The escape latencies of the aged rats increased more significantly throughout the training period than those of the young rats ($p < 0.05$). Oral administration of KF (1×10^{10}

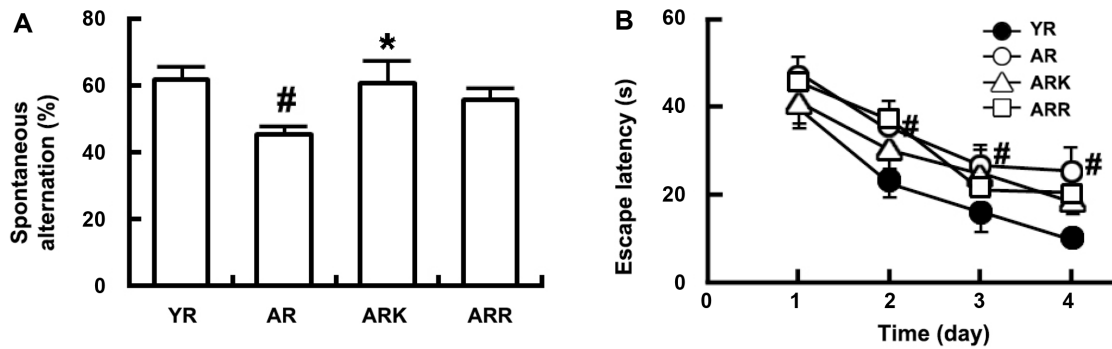


Fig. 2. Effect of KF on the age-dependent memory deficit in rats.

(A) Effect of KF and rapamycin in the Y-maze task. (B) Effect of KF and rapamycin in the Morris water maze task. Acquisition trials were performed 1 h after the final administration of test agents. KF (1×10^{10} CFU/rat) or vehicle was orally administered for 8 weeks (YR (closed circle), treated with vehicle alone in young Fischer 344 rats; AR (closed square), treated with vehicle alone in aged rats; ARK (closed triangle), treated with KF in aged rats; ARR (closed diamond), treated with rapamycin in aged rats (1 mg/kg)). Data represent the mean \pm SD ($n = 6$ per group). #Significantly different compared with YR ($p < 0.05$). *Significantly different compared with AR ($p < 0.05$).

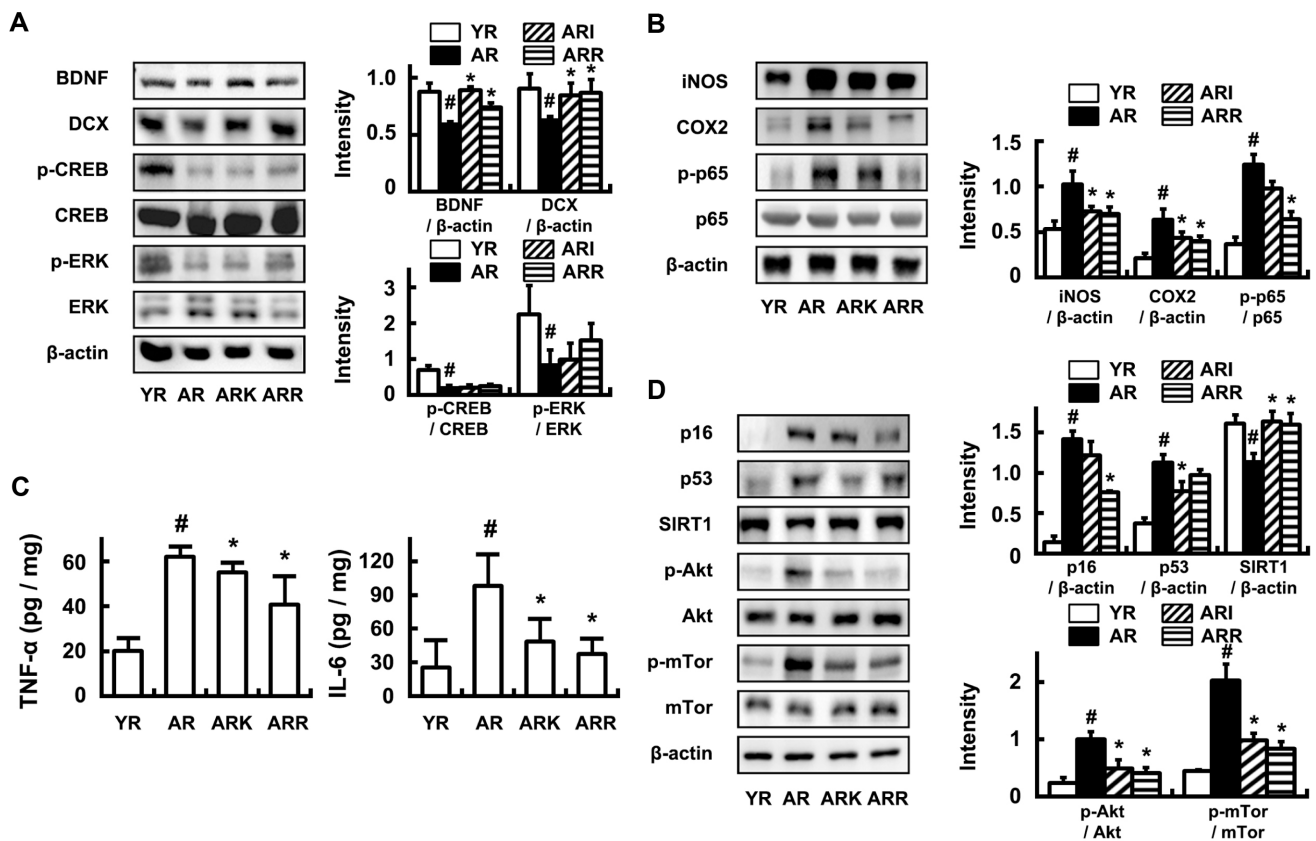


Fig. 3. Effect of KF on the DCX and BDNF expression and CREB phosphorylation (A), iNOS and COX2 expression, and NF- κ B activation (B), TNF- α and IL-6 expression (C), and p16, p53, and SIRT1 expression, and Akt, mTOR, and FOX3a phosphorylation (D) in the hippocampi of aged rats.

KF (1×10^{10} CFU/rat) or vehicle was orally administered for 8 weeks (YR, treated with vehicle alone in young Fischer 344 rats; AR, treated with vehicle alone in aged rats; ARK, treated with KF in aged rats; ARR, treated with rapamycin in aged rats (1 mg/kg)). TNF- α and IL-6 were analyzed by ELISA. Other protein expression was assessed by immunoblotting. Data represent the mean \pm SD ($n = 3$ per group). #Significantly different compared with YR ($p < 0.05$). *Significantly different compared with AR ($p < 0.05$).

CFU/rat, p.o.) shortened the escape latency slightly but not significantly. KF and rapamycin had no effect on general locomotor behavior (data not shown).

We also measured the effect of KF on hippocampal CREB phosphorylation and DCX and BDNF expression, which are associated with cognitive function in aged rats [6, 10] (Fig. 3A). The DCX and BDNF expression and CREB phosphorylation were more down-regulated in aged rats than in young rats. However, oral administration of KF in aged rats increased the aging-suppressed expression of DCX, BDNF, and phosphorylation of CREB. The restorative effect of KF was stronger than that of rapamycin.

We also measured the expression of the inflammatory markers COX-2 and iNOS and the activation of their transcription factor NF- κ B, which is generally activated in response to ROS [4]. In aged rats, COX-2 and iNOS expression and NF- κ B activation were more pronounced than in young rats (Fig. 3B). Treatment with KF suppressed aging-dependent COX-2 and iNOS expression and NF- κ B activation. KF treatment also inhibited the aging-induced expression of TNF- α and IL-6, which are the representative proinflammatory cytokines [13]. The inhibitory effects of KF were comparable to that of rapamycin. Furthermore, treatment of aged rats with KF inhibited the age-induced expression of p16 and p53, as well as phosphorylation of Akt, mTOR, and FOX3a more potently than rapamycin treatment (Fig. 3C).

Aging increases reactive oxygen species generation, which continuously stimulates NF- κ B activation, ultimately leading to inflammation [4, 21]. Continuous inflammation is considered a major factor in accelerating the degenerative process in AD [12]. AD observed in advanced age is significantly associated with a deficit in central nervous system cholinergic function and down-regulated expression of hippocampal BDNF and DCX [18, 19]. In the present study, we also found that aging suppressed BDNF and DCX expression and memory impairment in aged rats. KF treatment increased DCX and BDNF expression and CREB phosphorylation in the hippocampi of aged rats and ameliorated age-dependent memory impairment. Therefore, KF may attenuate memory deficit by inducing DCX and BDNF expression and activating CREB. KF treatment also inhibited the aging-dependent activation of NF- κ B, expression of proinflammatory cytokines, and expression of senescence markers p16 and p53. These results suggest that KF may ameliorate inflammatory and senescence responses by inhibiting the NF- κ B signaling pathways. Moreover, KF inhibited age-dependent lipidemia, particularly by increasing TG and reducing HDL cholesterol.

Thus, KF may ameliorate age-dependent dementia and lipidemia by inhibiting NF- κ B activation.

Acknowledgments

This study was supported by grants from the Bio & Medical Technology Development Program (2013M3A9B6076413) of the National Research Foundation (NRF) funded by the Korean government (MSIP).

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