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Effect of LTA isolated from bifidobacteria on D-galactose-induced aging

Zheng-Jun Yi [a,1], Yu-Rong Fu [b,*,1], Meng Li [a], Kun-Shan Gao [a], Xu-Guang Zhang [a]

[a] Department of Laboratory Medicine of Clinical Faculty, Weifang Medical University, Weifang, China
[b] Department of Medical Microbiology, Weifang Medical University, Weifang, China

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ABSTRACT

Background: Bifidobacteria are a natural part of the bacterial flora in the human body and have a symbiotic bacteria–host relationship with human beings. Aging is associated with reduced number of beneficial colonic bifidobacteria and impaired immunity. Lipoteichoic acid is a major constituent of the cell wall of bifidobacteria which is important for bacterial survival, growth, and function. The possible anti-aging effects of lipoteichoic acid isolated from bifidobacteria is presently unknown.
Objective: The aim of the present study was to investigate possible anti-aging effects of lipoteichoic acid isolated from bifidobacteria on senescent mice artificially induced by chronic injection of D-galactose and explore potential anti-aging's mechanisms.
Methods: Mice were artificially induced senescence by consecutive injection of D-galactose (100 mg/kg) once daily for 7 weeks and lipoteichoic acid from bifidobacterium bifidum, was simultaneously administered to them once a week by intraperitoneal infusion. Mice were sacrificed, blood and other samples were collected at the indicated time. Anti-oxidation activity in brain, histology of tissue, gene expression, lymphocyte's DNA damage and cytokine production of lymphocytes in vitro and in vivo were measured.
Results: Lipoteichoic acid could significantly improve general appearance of the aging model mice, improve anti-oxidation activity in brain, increase IL-2 level and decrease TNF-α level in vitro and in vivo, respectively. Besides, LTA remarkably inhibited DNA damage in the both splenic lymphocytes and circulating lymphocytes. Moreover, LTA could decrease p16 expression while increase c-fos expression in the D-galactose treated mice.
Conclusion: Taken together, the results indicated, for the first time, that LTA could suppress the aging process via the following several mechanisms, including enhancement of anti-oxidation activity in brain, improvement of immune function and alteration of gene expression.

1. Introduction

Bifidobacteria are a natural part of the bacterial flora in the human body and have a symbiotic bacteria–host relationship with human beings. Bifidobacteria are involved in some physiological functions, including anti-infection action (Ewaschuk et al., 2008; Lomax and Calder, 2009; Graul et al., 2009), anti-tumor action (Capurso et al., 2006; Hidaka et al., 2007; Wei et al., 2007) and anti-aging action (Hopkins and Macfarlane, 2002; Hébuterne, 2003). Aging is associated with reduced number of beneficial colonic bifidobacteria and impaired immunity (He et al., 2001; Woodmansey, 2007). Nevertheless, little is currently known about the molecular basis for the effects of bifidobacteria on aging.

Lipoteichoic acid (LTA) is a major constituent of the cell wall of gram-positive bacteria which is important for cell wall remodeling, bacterial survival, growth and function. Many gram-positive bacteria possess common-type LTA consisting of a 1,3-1inked poly (g1ycerophosphate) chain which is bound through a phosphodiester linkage to a membrane glycolipid. However, LTA from bifidobacterium bifidum consists of a 1,2-linked polyglycerophosphate chain (instead of the usual 1,3-), a polysaccharide chain (1,6-linked β-D-glucan or 1,5-linked β-D-galactofuranan) and a glycolipid moiety.

To date, little is known about the role of LTA from bifidobacteria. Although several reports suggested that LTA from bifidobacteria was involved in anti-tumor action and anti-aging action (Yue et al., 2007a,b; Yanhua et al., 2009), these studies have been too under-powered to draw definitive conclusion. Rodent chronically injected with D-galactose has been used as an artificially induced animal aging model for anti-aging research (Song et al., 1999). The aim of the present study was to make use of the artificially induced aging model mice to further investigate potential anti-aging effects of LTA from bifidobacteria and explore underlying anti-aging molecular mechanisms.

* Corresponding author. Tel.: +86 0536 8068955.
E-mail address: yifuyurong@163.com (Y.-R. Fu).
[1] These authors contributed equally to this work.

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2. Material and methods

2.1. Isolation and purification of LTA

Bifidobacteria bifidum (ATCC 86321) were purchased from microbiology institute of Chinese academy of sciences. LTA was isolated from disintegrated bacteria with aqueous phenol and purified by nuclease digestion and consecutively different chromatography. The purity of LTA was checked by nuclear magnetic resonance and mass spectrometry analysis according to the procedure (Morath et al., 2001; Lynch et al., 2004). The purity of the LTA was greater than 99%.

2.2. Experiment animals

Animal housing and all experimental procedures followed the requirements of the *Provisions and General Recommendations of Chinese Experimental Animal Administration Legislation.* Seven-week-old female Kun-ming mice (20 ± 2 g) were purchased from the Experimental Animal Center of Weifang Medical University and housed under standard pathogen free condition. During the entire experiment process, they had free access to food and water. After 1-week acclimatization to the home cage, the mice were randomly divided into three groups of 20 each; mice of group one were artificially induced senescence by subcutaneous (s.c.) injection of D-galactose (100 mg/kg) (Sigma) once daily (Wei et al.,2005), and normal saline was simultaneously administered to them by intraperitoneal (i.p.) injection once a week (the aging model mice); mice of group two were treated by i.p. injection of LTA (4 mg/kg) once a week after s.c. injection of D-galactose once daily (the LTA treatment mice); mice of group three were treated by i.p. injection of normal saline once a week and by s.c. injection of normal saline once daily (the young control mice). The mice were sacrificed after treatment for 7 weeks.

2.3. Observation of general appearance, measurement of body weight, organ index and pathological examination

During the entire experiment process, general appearance was observed. After treatment for 7 weeks, the mice were sacrificed. The brains, spleens, thymus glands, kidneys and livers were weighted and their weights relative to the final body weight (organ index) were calculated.

The thymus glands and kidneys were fixed in neutral-buffered 10% formalin, sectioned and stained with hematoxylin and eosin (HE). For the thymus glands, histopathological examination was observed under a light microscope; for the kidneys, glomeruli were counted and glomerular volume was assessed according to the procedure (Boubred et al., 2007).

2.4. Anti-oxidant measurements in brain

Superoxide dismutase (SOD), malondialdehyde (MDA), nitric oxide (NO) and nitric oxide synthase (NOS) kits were purchased from Nanjing Jiancheng Biological Technology Company, China. The activities of SOD and NOS, as well as levels of NO and MDA, were determined according to the instructions.

Briefly, the brains were separated into two parts for determination of enzymatic activity, NO and MDA levels, respectively. The samples for enzymatic activity analysis were washed and homogenized in phosphate-buffered saline (PBS) (pH 7.4) using Homogenizer. The homogenized samples were then sonicated for 1.5 min (30-s sonications interrupted with a 30-s pause on ice). The samples were then centrifuged at 17,000*g* for 15 min, and the supernatants, if not used for enzyme assays immediately, were kept in the deep freeze at −80 °C. The samples for MDA and NO analysis were washed with ice-cold 0.9% NaCl solution and homogenized in 1.15% KCl. The samples were then centrifuged and supernatants were assayed for NO and MDA levels.

The protein content in the brain homogenates was measured by the Lowry method using bovine serum albumin (Sigma, USA) as a standard. SOD and NOS activities were expressed as nU/mg protein and U/mg protein, respectively. MDA and NO levels were expressed as nmol /mg protein.

2.5. Measurement of DNA damage in lymphocytes and cytokines production in vitro and in vivo

The spleens were sterilely homogenized and lymphocytes were prepared by ficoll separating medium (Yi et al., 2007). The splenocytes (5×10^5 cells/well) were stimulated with ConA (5 μg/ml) in a 96-well round bottom plate for 72 h (Wang et al., 2007), and level of IL-2 in the culture supernatant was determined by ELISA (Camarillo, CA).

The peripheral blood lymphocytes were prepared by ficoll separating medium and diluted to 1×10^5 cells/ml. Comet assay was used to determine DNA damage in both circulating lymphocytes and above prepared splenocytes as described (Singh et al., 1988). Under fluorescence microscope, cell morphology was observed and percentage of cells presenting DNA damage was assessed.

Studies showed that aging is characterized by a pro-inflammatory state that contributes to the onset of disability and age-related diseases. TNF-α is an important pro-inflammatory mediator that influences host defense against infection and cancer. In the study, TNF-α level in serum was measured using enzyme-linked immunosorbent assay (ELISA) kits (Camarillo, CA) according to the instructions.

2.6. Western blot analysis of gene expression

The tissue proteins were separated by 12% SDS–PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and then blotted onto nitrocellulose membrane using a semidry transfer system (Huang 2008; Kurien and Scofield, 2009). Following blocked with 4% nonfat milk, the membranes were probed sequentially with primary mouse-anti human p16, c-fos antibodies and HRP (Peroxidase, Horseradish)-labeled goat-anti mouse secondary antibodies (Santa cruz), and immunoreactive bands were detected with 3,3′-diaminobenzidine (DAB) staining. β-actin was served as protein loading control.

2.7. Statistical analysis

Data were presented as mean ± standard deviation (SD). ANOVA tests or student's *t* tests were used for statistical analysis. The data were regarded as significantly different at $P < 0.05$.

3. Results

3.1. Changes of general appearance, organ index and morphological structures

During the entire experiment process, the aging model mice were habituated to s.c. injection with D-galactose at dose of 100 mg/kg each day and general appearance was observed. The mice were sacrificed at the indicated time and tissues were collected for organ index and histology examination. Results showed that, compared with that of young control mice, hair of the aging model ones gradually lost elasticity and got brittle; skin became thin and sag little by little; body weight, organ indexes, including