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Journal of Ethnopharmacology 98 (2005) 61–65
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

The Chinese traditional medicine 'Bushen Yinao Pian' increased the level of ageing-related gene *LRPAP-1* expression in the cerebral tissue of accelerated senescence-prone mouse 8/Ta

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Received 8 June 2004; received in revised form 9 November 2004; accepted 23 December 2004

Abstract

The molecular mechanism of the Chinese traditional medicine 'Bushen Yinao Pian' (a complex prescription used for clinical anti-ageing in China for over 20 years) is elusive. In this study, the cDNA of low-density lipoprotein related-receptor associated protein-1 (*LRPAP-1*), an ageing-related gene, which functions as a chaperon or escort protein in the intracellular transport of low-density lipoprotein related-receptor, a transporter of amyloid beta protein (AβP), had been cloned by screening cDNA library based on analyzing the gene expression in cerebral tissue between the test and the control accelerated senescence-prone mouse 8/Ta (SAMP8/Ta). The result shows that this complex prescription increased the expression level of *LRPAP-1*. It indicated that the Chinese traditional medicine 'Bushen Yinao Pian' plays an important role in anti-ageing by increasing *LRPAP-1* expression level.

Keywords: Chinese traditional medicine; SAMP8/Ta; Anti-ageing; Gene expression; DDRT-PCR

1. Introduction

Ageing is a progressive physiological change in an organism that leads to senescence, or decline of biological functions and of the organism's ability to adapt to metabolic stress. SAMP8/Ta mice are used as a murine model for ageing studies, which have short life span and ageing-related deficits in learning and cognitive abilities, emotional disorder, abnormal circadian rhythms and impaired immune response (Takeda et al., 1991). In China, the traditional medicine 'Bushen Yinao Pian' (a complex prescription used for anti-ageing) had been used for over 20 years and known to be effective in clinic. This complex prescription includes 15 Chinese medicines, most of those had been proved to be anti-ageing (Chen and Li, 1993; Xiao et al., 1993). Pharmacological studies of mice showed that the prescription could significantly increase the concentration of haemoglobin and the amount of erythrocyte in post-bleeding mice, promote the pleuripotent hematopoietic stem cell proliferation and differentiation, decrease the walking time and error times significantly in T-maze tests, and in conditional avoidance tasks (datum in publishing). But the molecular mechanism of its anti-ageing is still unclear. In this study, we applied mRNA

Abbreviations: AD, Alzheimer's disease; Amp, ampicillin; AβP, amyloid beta protein; cDNA, complementary DNA; CNS, central nervous system; CSF, cerebrospinal fluid; dCTP, deoxycytidine triphosphate; DDRT-PCR, differential display reverse transcription PCR; DNA, deoxyribonucleic acid; dNTP, deoxynucleoside triphosphate; IPTG, isopropyl-1-thio-β-galactopyranoside; LB, Luria-Bertani medium; LDL, low-density lipoprotein; LRP, LDL receptor-related protein-1; LRPAP-1, low-density lipoprotein related-receptor associated protein-1; M-MuLV, moloney murine leukemia virus; PCR, polymerase chain reaction; RAP, receptor associated protein; RNA, ribonucleic acid; RT, reverse transcription; SAMP8/Ta, accelerated senescence-prone mouse 8/Ta or senescence-accelerated prone mouse 8/Ta; X-Gal, 5-bromo-4-chloro-3-indolyl-β-galactopyranoside

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doi:10.1016/j.jep.2004.12.023

differential display reverse transcription PCR (DDRT-PCR) to analyze the effect of the 'Bushen Yinao Pian' on the gene expression changes in the cerebral tissue of SAMP8/Ta mice.

2. Material and methods

2.1. Materials

2.1.1. Chinese traditional medicine

'Bushen Yinao Pian', a complex prescription used for anti-ageing in China, was offered by Lingtai Bichen Medical Technology Co. Ltd. (Mudangjiang City, China). This complex prescription is composed of 15 Chinese medicines, which are Ginseng root (*Panax ginseng* C.A. Mey.), Pilose Anter (*Cervus nippon* Temminck), Tuckahoe (*Poria cocos* (Schw.) Wolf.), rhizome of common Yam (*Dioscorea opposita* Thunb.), prepared rhizome of adhesive Rehmannia (*Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. et Mey.), root of Chinese Angelica (*Angelica sinensis* (Oliv.) Diels), rizome of Chuanxiong Ligusticum (*Ligusticum chuanxiong* Hort.), fruit of Malaytea scurfpea (*Psoralea corylifolia* L.), root of common Epipremnum (*Achyranthes bidentata* Bl.), fruit of Hairystamen Wolfberry (*Lycium barbarum* L.), root of Ningpo Figwort (*Scrophularia ningpoensis* Hemsl.), root of Creeping Liriope (*Liriope spicata* (Thunb.) Lour.), fruit of Chinese Magnoliavine (*Schisandra chinensis* (Turcz.) Baill.), seed of Spine Date (*Ziziphus jujuba* Mill. Var. Spinosa (Bunge) Hu ex H. F. Chou), and Cinnabar (*Cinnabaris*).

2.1.2. Animal feed

Senescence-accelerated prone mouse 8/Ta (SAMP8/Ta) were offered by Kyoto University of Japan and housed in the experimental animal center of our Institute under the same condition; in a clean facility on a 12-h light/dark cycle. The control group mice were given a standard commercial pellet diet (Feed-Processing Plant of the Experimental Animal Center, the Academy of Military Medicine Sciences, Beijing, China) and the test group mice given the medicine appended pellet diet, which is added 1% 'Bushen Yinao Pian' (a complex prescription of Chinese traditional medicine for anti-ageing) in the standard commercial pellet diet (Feed-Processing Plant of the Experimental Animal Center, the Academy of Military Medicine Sciences, Beijing, China), and tap water ad libitum.

2.1.3. Chemical reagents

200 IU/μl M-MuLV reverse transcriptase (New England Biolabs Inc., Hertfordshire, England, UK); RNeasy Mini Kit (Qiagen Inc. Valencia); 10 mM dNTP Mix, Glycogen (Sigma-Aldrich Fine Chemicals, St. Louis, Missouri); RQ1-DNase, RNasin, Taq DNA polymerase pGEM-T vector system, X-Gal, IPTG (Promega Co., Madison); [α-^{32}P]dCTP (activity is 7×10^{10} Bp/mmol) (Beijing Yahui Corporation, Beijing, China); agarose (Biowest, distributed by Shanghai Yito Enterprise Co. Ltd., Shanghai, China); powerscript reverse transcriptase, Advantage 2 PCR kit, SMART cDNA library construction kit (BD Biosciences. Clontech Inc., Palo Alto); random primer DNA labeling kit (TaKaRa Biotechnology Co. Ltd., Dalian, China); UltraPure plasmid DNA mini purification kit (SBS, Beijing, China); the primers were synthesized by Shanghai Sangon Biotech Corporation (Shanghai, China); other reagents are all analytical grade.

2.2. Methods

2.2.1. Total RNA isolation

After 3 months of feeding, the male mice used for the experiment were decapitated, and the cerebral tissues were taken out. About 30 mg cerebral tissue of each mouse were cut out; then according to the protocol of the RNeasy mini kit to isolate total RNA, the total RNA samples were treated with RQ1 RNase-Free DNase and stored at −20 °C before using.

2.2.2. DDRT-PCR

To detect gene expression change between the test group and the control group, samples from the same litter mice were compared according to the modified protocol of Liang and Pardee (1998). Briefly, it is as follows: in each PCR tube (Axygen Scientific Inc., Union city), add 2 μl of total RNA (1 μg), 2 μl of 10× RT buffer (attached to the enzyme), 1.6 μl of 2.5 mM dNTPs, 2 μl of one of three DDRT 3′ primers (the sequences are AAGCTTTTTTTTTTTA, AAGCTTTTTTTTTTTC, and AAGCTTTTTTTTTTTG, respectively), 0.5 μl of RNasin ribonuclease inhibitor (40 IU/μl), and add double distilled water to 19 μl. 65 °C for 5 min, 42 °C for 10 min, then in each tube add 1 μl of M-MuLV reverse transcriptase (200 IU/μl); 37 °C for 50 min, 75 °C for 5 min on Thermocycler (Eppendof-Netheler-Hinz GmbH, Hamburg, Germany). Set up the PCR system for amplification of the RT products as follow: 2 μl of RT product, 2 μl of 10× Taq DNA polymerase buffer (attached to the enzyme), 2 μl of 25 mM $MgCl_2$, 1.6 μl of 2.5 mM dNTPs, 2 μl of the 5′ primer (2 μM) (the sequences are ACAGAGCACA, CACAGTTTGC, CCACAGAGTA, GGAACTCCGT, GGCAAGTCAC, and AGGACCGCTA), 2 μl of 3′ primer (2 μM), 0.4 μl of Taq DNA polymerase (5 IU/μl), 0.5 μl of α-^{32}p-dCTP (10 μCi/μl), add water to 20 μl. The parameters of PCR are as follows: 94 °C for 10 min, 94 °C for 1 min, 40 °C for 2 min, 72 °C for 1 min, 40 cycles, 72 °C for 5 min on thermocycler. After electrophoresis on 6% urea denature polyacrylamide gel, the was gel exposed to an X-ray film (Fuji Photo Film Co. Ltd., Tokyo, Japan) for 72 h at −20 °C.

2.2.3. Recovery and amplification of cDNA fragment

Located bands of interest and cut out the located bands from the gel. Soaked it in 100 μl double distilled water