Gene expression patterns of hippocampus and cerebral cortex of senescence-accelerated mouse treated with Huang-Lian-Jie-Du decoction

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ABSTRACT

Alzheimer's disease (AD) is a progressive, neurodegenerative disease, which primarily affects the elderly. Clinical signs of AD are characterized by the neuron loss and cognitive impairment. At gene and protein levels, the senescence-accelerated mouse/prone 8 (SAMP8) is a suitable animal model to investigate the fundamental mechanisms of age-related learning and memory deficits. Huang-Lian-Jie-Du decoction (HL), a well-known traditional Chinese medicinal prescription, has been employed in the treatment of wide range of disease conditions. Modern pharmacological studies have showed that HL possesses many effects, which include amelioration of learning and memory function of CNS. This paper investigated the gene expression patterns of hippocampus and cerebral cortex of SAMP8, which were treated with HL employing the cDNA microarray and real time quantitative RT-PCR techniques. The results showed that HL has the significant modulating effects on age-related changes of the gene expressions in the hippocampus and cerebral cortex in SAMP8, which include genes that involved in signal transduction (Dusp12, Rps6ka1, Rab26, Penk1, Nope, Leng8, Syde1, Phb, Def8, Ihpk1, Tac2, Pik3c2a), protein metabolism (Ttc3, Amfr, Prr6, Ube2d2), cell growth and development (Ngrn, Anln, Dip3b, Acrbp), nucleic acid metabolism (Fhit, Itm2c, Cstf2t, Ddx3x, Ercc5, Pcgfr6), energy metabolism (Stub1, Uqcr, Nsf), immune response (C1qb), regulation of transcription (D1ertd161e, Gcn5l2, Ssu72), transporter (Slc17a7, mt-Co1), nervous system development (Trim3), neurogila cell differentiation (Tspan2) and 24 genes whose biological function and process were still unknown. It was suggested by the changes of the 62 genes with HL treatment that the ameliorating effect of HL on the cognitive impairments of SAMP8 might be achieved by multi-mechanism and multi-targets.

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Alzheimer disease (AD) is the most common form of progressive dementia in the elderly. It is a neurodegenerative disorder characterized by the neuropathologic findings of intracellular neurofibrillary tangles (NFTs) and extracellular amyloid plaques that accumulate in vulnerable brain regions. Genes of amyloid precursor protein (APP), APOE4, presenilin-1 (PSEN1), PSEN2, cystatin-3 (CST3) [11], PAXIP1 and NOS3 [19], etc. have been identified as causes of AD, but for this multi-factorial diseases, there could be many more unknown genes which play very important roles in AD.

The current drugs only provide limited or transient benefit to many patients [27]. In the past, drug discovery merely based on a single-target-directed strategy, such as β-amyloid, AChE inhibitor, NMDA-receptor antagonists, neurotrophic factor, etc. The method seems inefficient for the treatment of complex diseases which have multiple pathogenic factors [2]. In fact, the traditional Chinese medicine and their effective components have been proven their

own inimitable predominance with multi-factorial, multi-target and multi-functional action, such as modulation of cholinergic systems, anti-oxidant, anti-amyloid, and anti-inflammatory [1].

Huang-Lian-Jie-Du decoction (HL) is a very recognized traditional Chinese medicinal prescription, which consists of *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex* and *Gardeniae fructus*. The main effects of HL, described in traditional Chinese medicine, are "Purging the fire and detoxifying". It has been used for treating many diseases conditions over the century. Modern pharmacological studies demonstrate that HL possesses wide effects, including the therapies of cerebrovascular disease [13], gastritis [18], inflammation [24], etc., especially used for the therapies of many kinds of dementia in clinic in China and Japan. Studies showed HL plays a crucial protective role in ischemia-induced brain injury [12] and increases cerebral blood flow in the areas around margins of ischemia and reduces the size of the infarction [16]. It improves the microcirculation through lipid and protein metabolisms, and is useful for the treatment of cerebral vascular attack in human [21]. Moreover, investigation showed that HL can improve the disruption of spatial cognition induced by cerebral ischemia and central

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cholinergic dysfunction in rats [10], and showed HL prolonged the step-down latency significantly and decreased the step-down errors in the passive avoidance task, as well as shortened the latency of escaping markedly onto the platform in the training trial and increased the percentage of crossing the former platform quadrant in the probe trial in the Morris water maze test [26]. In addition, another study showed that HL significantly improved the learning behaviors in AD rats, which suggested that HL ameliorates age-related deterioration of learning and memory [8]. Although the studies showed HL against the impairment of learning memory might be associated with the prevention of a decrease in acetylcholine contents or amelioration immune function and reduction of oxidative stress, the active mechanism of HL on enhancing the cognitive ability is still unknown.

The senescence-accelerated mouse/prone 8 (SAMP8) strain, a substrain of SAM, has been proposed as a suitable animal model of age-related cognitive decline with relevance to alterations of the gene expression and protein abnormalities in AD [3,6], with the features of an increase in hyperphosphorylated forms of tau in the brain [4], age-related increases in the level of hippocampal Aβ-peptide, learning and memory deficits, and a shorter lifespan than their controls, SAM/resistant1 (SAMR1) [17].

Original SAMR1 and SAMP8 mouse were kindly provided by Dr. T. Takeda at Kyoto University (Kyoto, Japan) and maintained at Beijing Institute of Pharmacology and Toxicology under conditions of natural light–dark cycle (12-h light; 12-h dark), temperature ($25 \pm 1\,^\circ$C) and relative humidity (50 ± 5%). All procedures were carried out according to the Care and Use guide of laboratory animals by the NIH. Only 6-month-old male SAMP8 and SAMR1 were employed.

Traditional Chinese medicinal herbs, *C. rhizoma*, *S. radix*, *P. cortex* and *G. fructus* were purchased from Beijing Tong-Ren-Tang drug store and subjected to pharmacognosy identification before preparing the decoction. HL decoction was prepared with decocting *C. rhizoma* and *P. cortex* in the ratio of 3:2 (dry weight), keeping on 30 min in 75–80 °C water each time and three times in total. Meanwhile *S. radix* and *G. fructus* were decocted with 75–80 °C water keeping on 50 min each time and three times in total, according to the ratio of 2:3 (dry weight). These two decoctions were filtered, concentrated, sterilized and made to the final concentration of 1 g/ml d.w. This process was done according to the preparing method of Chinese traditional medicine, respectively and put together partes aequales before treatment. To ensure homogenicity of HL extract, coldspray ionisation-mass spectrometry (CSI-MS) technique was applied to detect the active components of HL extract including coptisine, berberine, palmatine, baicalin, wogonoside and as a quality control method (Fig. 1).

Eight SAMP8 were administrated by gastric intubation 5 g/kg mouse body weight of HL suspended in distilled water every day for 1 month as HL-treated group. Eight SAMR1 and 24 SAMP8 were used for comparison with same volume distilled water as above group every day for 1 month as normal animal control group and model control group, respectively.
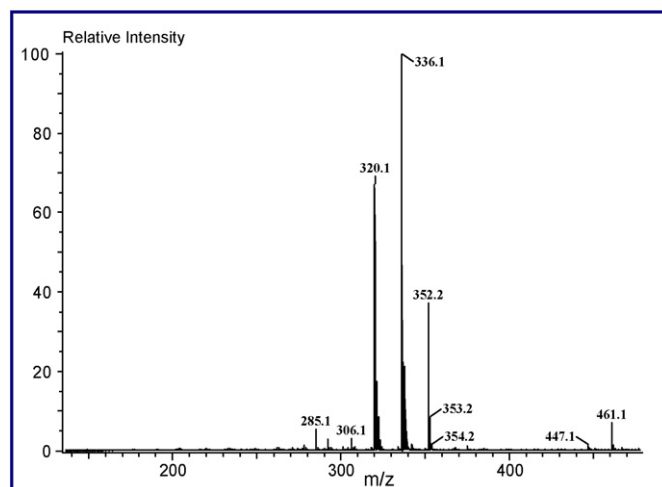

Fig. 1. The active components of HL analyzed by (+) CSI-MS spectrum. m/z 320, m/z 336, m/z 352, m/z 447, m/z 461 in the figure represent coptisine, berberine, palmatine, baicalin, wogonoside of the five active components in Huang-Lian-Jie-Du decoction, respectively.

Total RNA was purified from the hippocampus and cerebral cortex tissue of each group using TRIZOL Reagent (Invitrogen Cat. No. 15596-026). The integrity of total RNA was detected by agarose gel; the purity and concentration were detected by the spectrophotometer (NanoDrop, ND-1000).

Fifty micrograms of total RNA from hippocampus and cerebral cortex of each group were used, respectively for reverse transcription according to standard protocols using SUPERSCRIPT III reverse transcriptase (Invitrogen Cat. No. 18080-044) and aa-dUTP (Amersham Pharamacia Biotech). Subsequently, aa-cDNA first strand was purified using QIAquick PCR purification kit (QIAGEN Cat. No. 28104). The purified aa-dUTP of control group and treated group were labeled with cy5 or cy3 monofunctional dye (Amersham Pharamacia Biotech), respectively. The corresponding fluorescent labeled with cy3 and cy5 cDNA were combined, purified using MinEluteTM Reaction Cleanup Kit (QIAGEN Cat. No. 28204) and lyophilized in the nucleic drier (SPD1010 SpeedVac).

Amino-groups on the spotted slide [7] were blocked at room temperature through washing with NaBH$_4$/PBS/ethanol. Pre-hybridization in hybridization box was performed for 1 h at 42 °C plating with buffer containing 5× SSC, 0.1% SDS, 1% BSA under glass cover-slip in hybridization chamber (Robbins Scientific Co.). After pre-hybridization, the slide was washed and dried immediately. The above-mentioned dried fluorescent labeled cDNA was resolved with 15 μl hybridization buffer, denatured at 98 °C for 5 min, centrifuged at 12,000 rpm for 5 min and then the whole supernatant was pipetted and spread on the slide. The procedure of hybridization was same to the pre-hybridization except 18-h hybridization time. Subsequently, the slide was washed and dried immediately.

Table 1
The primer sequence of seven genes for real-time quantitative PCR

Gene name	Forward (5′→3′)	Reverse (5′→3′)	Size (bp)
Rab26	AGAAGCTGGCCAAGGAGTATG	TCGACCCTCCCTCTTAACGTA	170
Slc17a7	CCCCACCCTTAGAACGGAGT	GGAGACGAGCAGCCAGAACA	176
CaMKII	CCTACACGAAGATGTGCGACC	ATCAGGTGGATGTGAGGGTT	163
Stub1	GAGGCCAAGCACGATAAATAC	GTGATACCACTGGGTGTAATGC	152
Ube2d2	TTCTACGGTCACAGTGGTCTC	CGCATACTTCTGAGTCCATTCC	176
Ngrn	ACCATTGGCCACTTTGGAGTA	CAACAGAGAGACCACCAAGCA	151
Ttc3	GGAAGATGCTGTCTGCTCAC	TGGTGGAAGGATAAGGAAGGAG	173
β-Actin	TTGCTGACAGGATGCAGAAGGAG	GTGGACAGTGAGGCCAGGAT	127