

Effect of ginsenoside on IL-1 β and IL-6 mRNA expression in hippocampal neurons in chronic inflammation model of aged rats¹

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KEY WORDS ginseng; saponins; aging; inflammation; interleukin-1; interleukin-6; gene expression; hippocampus

ABSTRACT

AIM: To study the effects of ginseng root saponins (GRS) on interleukin-1 β (IL-1 β) and interleukin-6 (IL-6) expression in hippocampal neurons in chronic inflammation model of aged rats. **METHODS:** Chronic inflammation was induced by consecutive intraperitoneal injections of bacterial lipopolysaccharides (LPS) in aged rats. The semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) was used for determination of IL-1 β and IL-6 mRNA in hippocampal neurons of aged rats. **RESULTS:** No marked expression of IL-1 β and IL-6 mRNA was found in hippocampal neurons of young rats (3 months). In adult rats (9 months) the levels of IL-1 β and IL-6 expressed were 1.54 ± 0.21 and 1.63 ± 0.21 , respectively; in aged rats (24 months) treated with LPS the levels were 1.98 ± 0.21 and 1.91 ± 0.11 respectively, significantly higher than those of adult rats. After treatment with GRS (10, 20, 40 mg/kg), the IL-1 β and IL-6 mRNA content in aged rats decreased markedly. **CONCLUSION:** The levels of IL-1 β and IL-6 mRNA in hippocampal neurons increased with aging. GRS exerted inhibitory effects on IL-1 β and IL-6 gene expression in chronic inflammation model of aged rats.

INTRODUCTION

Elevated amounts of two pro-inflammatory cy-

tokines, interleukin-1 β (IL-1 β) and interleukin 6 (IL-6), have been described in neurodegenerative diseases such as Alzheimer's disease (AD) and Down's syndrome. Recent epidemiological studies have shown that antiinflammatory drugs may be considered as an antirisk factor for AD^[1]. This suggests that, besides genetic factors, inflammatory processes may be involved in the development of AD. As a possible model for chronic inflammation associated with neuronal degeneration, we studied IL-1 β and IL-6 expression in hippocampal neurons of aged rats induced by consecutive intraperitoneal injections of lipopolysaccharides (LPS). We also studied the dose-response relationship of GRS on IL-1 β and IL-6 mRNA expression in hippocampus of aged rats.

MATERIALS AND METHODS

Reagents GRS was provided by Dr HU Li-Hong, Department of phytochemistry from the same Institute, purity >95%. Bacterial lipopolysaccharides (LPS) was purchased from Sigma (St Louis, MO, USA). Other chemicals were of AR grade.

Rats Sprague-Dawley young male rats (3 months), adult male rats (9 months) and aged male rats (22-24 months) were supplied by the Experimental Animal Center of Shanghai University of Traditional Chinese Medicine (Grade II, Certificate No 01304).

LPS and GRS treatment In aged rats LPS treatment group was administered LPS 50 μ g/kg, once a week, ip, from 9th to 24th month ($n = 15$); GRS treatment group was administered GRS 10, 20, and 40 mg/kg, once a day, ip, from 20th to 24th month ($n = 10$). Control rats were given saline.

Preparation of tissue Rats were decapitated after the last treatment. The hippocampus was quickly removed from the skull and immediately frozen in liquid nitrogen.

Isolation of RNA and cDNA synthesis Total RNA was isolated from hippocampus according to the acid guanidinium/phenol/chloroform (AGPC) method

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described by Chomczynski and Sacchi (1987)^[2]. RNA concentration was determined by spectrophotometry at 260 nm, and quality assessed by agarose gel electrophoresis and ethidium bromide staining. RNA (1 µg) was converted to cDNA utilizing random hexamers pd(N)₆ (100 pmol, Pharmacia) as a primer and MMLV-H reverse transcriptase (RT) (200 u, Gibco BRL) in a buffer, compatible with PCR, containing: ammonium sulfate 16.6 mmol/L, Tris-HCl 6.7 mmol/L, pH 8.8, MgCl₂ 6.7 mmol/L, 2-β-mercaptoethanol 10 mmol/L, EDTA 6.7 µmol/L also containing 400 µmol/L each of dNTP (dATP, dCTP, dGTP and dTTP from Pharmacia), and 20 units of ribonuclease inhibitor (RNA sin, Promega). The reaction mixture was incubated at 42 °C for 30 min, heated to 95 °C for 5 min to denature the MMLV-H reverse transcriptase, cooled on ice for 3 min, and stored at -20 °C.

Oligonucleotide primers The sequences for the external and internal primer pairs are listed in Tab 1. β-actin was used as the "housekeeping gene".

Tab 1. Oligonucleotide primers for the amplifications and detection of rat cytokine sequences.

Molecule	Primer	Sequence (5' to 3')	Predicted Size (bp)
IL-1β ^[3]	forward	ATAGCAGCTTTCGACACTTTGAG	748
	backward	GTCAACTATGCCCGACCATT	
IL-6 ^[4]	forward	TTCCTACTTCCACAAGTC	567
	backward	CTAGGTTTCCCGAGTAG	
β-actin ^[5]	forward	AATGAGCTTCGCGTTGCCCC	800
	backward	GCCCTACCTTAGACGACCGT	

Polymerase Chain reaction amplification An aliquot of the first strand cDNA synthesis mixture (equivalent to 500 ng of total RNA) was used for PCR (amplification of cDNA from specific genes; IL-1β, IL-6, and β-actin). β-actin expression served as a control for the quantity of amplified cDNA. Oligonucleotide primers (0.5 µM) and cDNA were incubated in the PCR buffer with 400 µM each of dNTP and 10 % (vol/vol) dimethyl sulfoxide. After 10 min at 80 °C, 2 units of Taq DNA polymerase and α-³²P-dATP 0.1 µL (10 ci/µL, Amersham) were added and the second strand of cDNA was synthesized. The above was incubated in a Techne-PHC2 thermal cycler for 30 cycles as follows 95 °C 45 S, 55 °C 60 S, 72 °C 45 S. After the 30 cycles were complete, the tubes were maintained at 72 °C for 10 min.

Analysis of the cDNA-PCR amplified products The amplified products were analyzed on 1.5 % agarose gels. The ethidium bromide-stained bands, containing the ³²P-labeled PCR products, were washed first in 2 × SSC and 0.05 % SDS at room temperature and then in 2 × SSC and 0.1 % SDS at 65 °C. The bands were exposed to X-ray film at -80 °C with intensifying screens for the time necessary for the signal to be in a linear range for quantification. The exposure time was the same in all experiments. Densitometric analysis of autoradiograms was done with an IBAS 2 image analyzer integrating the optical density with the area of the bands. The signal associated with the presence of β-actin mRNA was used as an internal standard to normalize IL-1β and IL-6 expression.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and compared with *t*-test.

RESULTS

Total RNA was isolated from the hippocampus of rats. RNA concentration (Absorbance, A) determined by spectrophotometry at 260/280 nm was 1.8 - 2.0. The results of total RNA electrophoresis on 1.8 % agarose gel showed that the bands were clear, ultraviolet light of 28 S was twice as 18 S, and there were no other big molecular bands (Fig 1).

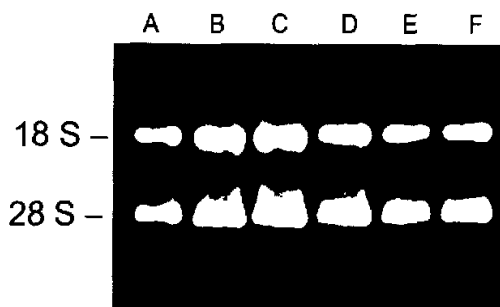


Fig 1. Results of total RNA extracted from a part of sample. A: Young rats; B: Adult rats; C: Aged rats; D: GRS 10 mg/kg; E: GRS 20 mg/kg; F: GRS 40 mg/kg.

The IL-1β and IL-6 mRNA content (A) in hippocampal neurons showed that no marked expression was found in young rats (Fig 2). The IL-1β and IL-6 mRNA contents in aged rats were shown to be significantly higher than those of adult rats (*P* < 0.05). After treatment with GRS (10, 20, 40 mg/kg), the IL-1β and

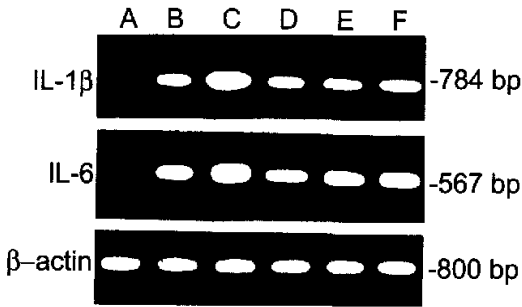


Fig 2. Effect of GRS on expression of IL-1 β and IL-6 mRNA in hippocampal neurons of aged rats. A: Young rats; B: Adult rats; C: Aged rats; D: GRS 10 mg/kg; E: GRS 20 mg/kg; F: GRS 40 mg/kg.

IL-6 mRNA content in aged rats decreased. There were significant differences between GRS groups and aged rats ($P < 0.05$ or $P < 0.01$). The inhibitory effect of GRS on IL-1 β and IL-6 mRNA content was found not to be dose-dependent in the range of 10–40 mg/kg (Tab 2).

Tab 2. Effect of GRS on IL-1 β and IL-6 mRNA expression. β -actin mRNA was used as internal standard. The data are the $\bar{x} \pm s$ of 5–6 determinations. ^b $P < 0.05$, ^c $P < 0.01$ vs adult group. ^e $P < 0.05$, ^f $P < 0.01$ vs aged group.

Group	IL-1 β mRNA/ β -actin mRNA	IL-6 mRNA/ β -actin mRNA
Adult	1.54 \pm 0.21	1.63 \pm 0.21
Aged	1.98 \pm 0.21 ^c	1.91 \pm 0.11 ^b
GRS 10 mg/kg	1.61 \pm 0.20 ^f	1.62 \pm 0.22 ^e
GRS 20 mg/kg	1.59 \pm 0.21 ^f	1.60 \pm 0.21 ^e
GRS 40 mg/kg	1.66 \pm 0.23 ^f	1.64 \pm 0.21 ^e

DISCUSSION

It has been reported that the activity of interleukin-1 and interleukin-6 markedly increases in brain tissues of aged rats as compared to young rats^[6,7]. Our results, using the RT-PCR technique, suggest that the level of IL-1 β and IL-6 mRNA tends to increase with aging in rat hippocampal neurons during chronic inflammation. Our findings are consistent with previous reports. There is evidence that pro-inflammatory cytokines (IL-1, IL-6, IL-10, TNF- α , TGF- β) play important roles in neurodegenerative diseases such as AD. IL-6 is a prototypic cytokine with a spectrum of biological actions^[8], many of

which overlap with those of other cytokines, including IL-1 α/β and tumor necrosis factor α . Expression of IL-1 β and IL-6 in the brain has been documented in a wide range of CNS disorders, including AIDS dementia complex, Alzheimer's disease, and Parkinson's disease^[9]. Localized production of IL-1 β and IL-6 in the CNS may be mediated not only by infiltrating immuno-inflammatory cells, but also by astrocytes and microglia. Thus, IL-1 β and IL-6 can be produced in the CNS during the host response to infection or injury and could potentially initiate or contribute to pathology.

We suggest that IL-6 is synthesized in brain tissue in AD and increases neuronal (and probably also astrocytic) synthesis of α_2 -macroglobulin. It has been reported that IL-6 can induce β -amyloid precursor protein (APP) mRNA expression in neurons and this action seems independent of IL-1. In turn, IL-1 β mRNA is induced by A β fragment 25–35 in astroglial cells. Both inflammatory cytokines appear to be involved in A β production, suggesting a causal role of the immunological response in AD pathogenesis.

GRS, a yellowish brown powder, extracted from the root of *Panax ginseng* CA Meyer, can improve learning and memory in various animal models and has been applied to geronotherapeutics. This study shows that GRS decreases the IL-1 β and IL-6 mRNA content in hippocampal neurons of aged rats. The inhibitory effect of GRS against the LPS-induced IL-1 β and IL-6 gene expression in brain was found not to be dose-dependent in the range of 10–40 mg/kg. The mechanism of pro-inflammatory cytokine mRNA expression in the brain remains to be clarified. Our findings provide an experimental basis for further elucidating the pharmacological effect of GRS and for looking at anti-inflammatory drugs as a new method of treatment for some neurodegenerative diseases such as AD.

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人参皂苷对慢性炎症型老年大鼠海马 IL-1 β 和 IL-6 mRNA 表达的影响¹

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关键词 人参; 皂苷类; 衰老; 炎症; 白介素-1;

白介素-6; 基因表达; 海马

目的: 研究人参皂苷对大鼠海马 IL-1 β 和 IL-6 mRNA 增龄性变化的影响, 为进一步阐明人参皂苷的药理作用及应用于某些神经退行性疾病提供依据. 方法: 应用腹腔注入脂多糖造成慢性炎症刺激的老年大鼠模型, 并以青年和成年大鼠为对照. 用半定量反转录-聚合酶链反应(RT-PCR)方法测定海马 IL-1 β 和 IL-6 mRNA 含量. 结果: 大鼠海马 IL-1 β 和 IL-6 mRNA 含量: 青年组(3月龄)无表达; 成年组(9月龄)分别为 1.54 ± 0.21 和 1.63 ± 0.21 ; 老年组(24月龄)分别为 1.98 ± 0.21 和 1.91 ± 0.11 ; 人参皂苷 10 mg/kg 组分别为 1.61 ± 0.20 和 1.62 ± 0.22 ; 人参皂苷 20 mg/kg 组分别为 1.59 ± 0.21 和 1.60 ± 0.21 ; 人参皂苷 40 mg/kg 组分别为 1.66 ± 0.23 和 1.64 ± 0.21 . 老年组和成年组相比较, $P < 0.01$ 或 $P < 0.05$; 人参皂苷不同剂量组和老年组相比较, $P < 0.05$ 或 $P < 0.01$. 结论: 大鼠海马 IL-1 β 和 IL-6 mRNA 的表达水平随增龄而升高. 人参皂苷能有效地抑制这一表达.

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