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Effects of *Coriolus versicolor* polysaccharide B on monocyte chemoattractant protein 1 gene expression in rat[1]

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KEY WORDS *Coriolus versicolor*; polysaccharides; monocyte chemoattractant protein-1; gene expression; rats

ABSTRACT

AIM: To investigate the effect of *Coriolus versicolor* polysaccharide B (CVPS-B), a new water-soluble component of polysaccharides from the fungus *Coriolus versicolor* (Fr) L on monocyte chemoattractant protein-1 (MCP-1) gene expression in rat splenocytes. **METHODS**: Expression of MCP-1 mRNA in rat splenocytes was examined by reverse transcription-polymerase chain reaction (RT-PCR) with β-actin as an internal standard. Sequencing of RT-PCR products was performed to confirm their specificity in MCP-1 gene composition. **RESULTS**: (1) Without pre-treatment of lipopolysaccharide (LPS), the relative MCP-1 mRNA expression ratios (MCP-1/β-actin) for the saline control group and for CVPS-B groups in 3 different doses (10, 20, and 30 $mg \cdot kg^{-1} \cdot d^{-1}$, ip, for 4 d) were 1.4 ± 0.3, 1.6 ± 0.4, 1.7 ± 0.5, and 1.5 ± 0.4, respectively ($P > 0.05$); (2) LPS (10 $\mu g \cdot kg^{-1}$, ip) enhanced the expression of MPC-1 mRNA by the ratio of 114 %; (3) pre-treatment with CVPS-B of 4 different doses (5, 10, 30, and 50 $mg \cdot kg^{-1} \cdot d^{-1}$, ip, for 4 d) decreased the LPS induced expression of MPC-1 mRNA by the ratios of 51 %, 70 %, 84 %, and 99 %, respectively ($n = 6$). **CONCLUSION**: In a dose-related fashion, CVPS-B inhibited the expression of MCP-1 mRNA induced by LPS in the rat splenocytes, but did not significantly affect the expression of MPC-1 mRNA in the normal rat.

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INTRODUCTION

Recruitment of macrophages into tissues is an important process in inflammation and host defense, and in this process, monocyte chemoattractant protein-1 (MCP-1) is thought to play a significant role. MCP-1 is a novel chemotactic factor specifically attracting and activating monocytes so that the monocytes can pass through the microvascular walls, enter into the tissues, and be transformed into macrophages[1]. In mice, rats, rabbits, and human, previous studies have demonstrated that MCP-1 is produced by various kinds of cells, including monocytes/macrophages, lymphocytes, fibroblasts, endothelial cells, smooth muscle cells, and the cells of certain tumors such as gliomas or malignant fibrous histiocytoma. Human MCP-1 mRNA was found to be expressed at high levels in pathologic foci of pulmonary fibrosis, glomerulonephritis, rheumatoid arthritis, and atherosclerosis[1,2].

Coriolus versicolor polysaccharide is a complicated protein-binding polysaccharide extracted from *Coriolus versicolor* fungus (*Class basidiomycetes*). The effective components of this biological response modifier from *Coriolus versicolor* polysaccharide have been studied for a long time. Recently, a new water-soluble component has been isolated from wild *Coriolus versicolor* (Fr) L and named as *Coriolus versicolor* polysaccharide B (CVPS-B). It has a molecular weight of M_r 39 000 and does not contain any protein, peptide, or ribonucleic acid[3]. We have reported that the polysaccharide krestin (PS-K), a *Coriolus versicolor* polysaccharide, prevented plaque formation in experimentally atherosclerotic rabbits[4,5]. It was found that MCP-1 mRNA expression was not detectable in the normal arteriole intimal membrane. There was, however, expression of MCP-1 mRNA at a high level in macrophage-rich areas of human and rabbit atherosclerotic lesions, suggesting that MCP-1 might both initiate and amplify monocyte recruitment to

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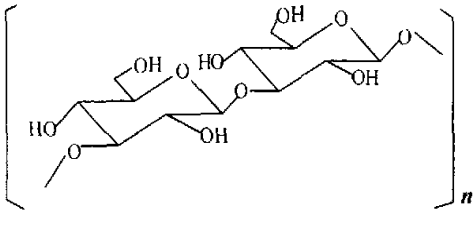
the artery wall during early atherogenesis[6]. In the present study, we intended to investigate the effect of CVPS-B on the expression of MCP-1 mRNA in rat and to elucidate the molecular mechanism by which *Coriolus versicolor* polysaccharides prevented the plaque formation in experimentally atherosclerotic rabbits.

MATERIALS AND METHODS

Rats Male Wistar rats weighing 250 g ± 10 g (Grade Ⅱ, Certificate № 99A060) were obtained from Animal Breeding Center of First Military Medical University, Guangzhou, China.

Drugs and reagents *Coriolus versicolor* polysaccharide B (CVPS-B) was isolated and purified from the natural *Coriolus versicolor* (Fr) L.[3]. Its purity was determined by HPLC. The chromatographic conditions were as follows: (1) chromatographic column: TSK G2000W, 7.5 mm × 300 mm, Pharmacia, LKB; (2) mobile phase: water; (3) flow rate: 1 mL/min; (4) detector: evapolation light scattering detector (ELSD). The HPLC chromatogram for CVPS-B showed only a sharp peak. Its monosaccharide composition and the molar ratio of monosaccharide to another monosaccharide is Glc : Fuc : Ara : Man : Gal = 13.65 : 5.88 : 4.45 : 2.43 : 1.00. According to the analysis of the ^{1}H-NMR and ^{13}C-NMR data from the partial hydrolysate of CVPS-B, its main chain is β-(1-3) polyglucosan and its branch chain contains fucoses, arabinose, mannose, and galactose, but does not contain rhamnose. It merits our attention that CVPS-B contains neither protein, peptide, nor ribonucleic acid[3].



The molecular structure of the main chain for CVPS-B

Reagent Trizol was a product of Gibco/BRL (USA). The PCR primers for rat β-actin and MCP-1 were synthesized by Gibco/BRL (USA). The kit of titam™ for one tube RT-PCR system was a product of Boehringer Mannhein Germany. Lipopolysaccharide (LPS) was purchased from Sigma. Other chemicals were of AR grade.

Treatment of rats Male Wistar rats were kept under standardized animal house conditions (temperature 28 – 31 ℃, photoperiod approximately 12 h light and 12 h dark daily, relative humidity 50 % – 60 %). At least 7 d were allowed for the rats to become acclimatized to animal house conditions and daily handling. The rats were randomly divided into 12 groups, and were treated as described below. LPS or CVPS-B dissolved in physiological saline was ip injected into rat respectively in different concentrations in a fixed volume of 0.2 mL. The same volume of sterilised physiological saline instead of LPS or CVPS-B was injected into the control rat. At the designed time the rats were killed by cervical dislocation. The spleen was dissected and a portion of it was used with reagent Trizol for total RNA extraction.

Reverse transcription-polymerase chain reaction (RT-PCR) The total RNA of the rat spleen tissue was isolated using Trizol reagent, and final RNA pellet was redissolved in water pre-treated with 0.1 % diethylpyrocarbonate and stored at – 70 ℃. The RNA was quantified by measuring the A_{260}. Subsequent RT-PCR was performed in DNA thermal cycler (PE-200, USA). The first strand of cDNA was synthesized from the total RNA using Avian myeloblastosis virus (AMV) reverse transcriptase and random Oligo (dT) 15 primers. The reverse transcription reaction was performed at 42 ℃ for 55 min. At the end of reverse transcription, the mixture was heated at 92 ℃ for 5 min and immediately cooled on ice. For amplification of the desired cDNA, the rat β-actin primers (sense: 5′-ATGGATGACGATATCGCTG-3′; antisense: 5′-ATGAGGTAGTCTGTCAGGT-3′) and the rat MCP-1 primers (sense: 5′-ACAGTTGCTGCCTGTAGCAT-3′; antisense: 5′-CACACTAGTTCTCTGTCATAC-3′) were used. The PCR cycle program was as follows: denaturing at 94 ℃ for 45 s, annealing at 55 ℃ for 120 s, and extending at 72 ℃ for 120 s. A total of 36 cycles was used. In the end, a prolonged elongation time of 5 min at 72 ℃ was given. To ensure that amounts of PCR products obtained were linear in respect to RNA inputted, a kinetic analysis was performed by varying the amounts of RNA inputted, and the amounts of RNA located at the linear area were selected. The PCR products were clearly visible after 2 % agarose gel electrophoresis and ethidium bromide staining. The RT-PCR product amounts of MCP-1 mRNA were standardized relative to that of β-actin mRNA in the same sample (the latter acted as an internal standard) *via* densitometric analysis by autogel analysis

system (STORM860, USA). The results were expressed as the relative level of mRNA expression (ratio of MCP-1/β-actin).

Nucleotide sequences of RT-PCR products The sequencing of RT-PCR products was performed by Takara Biotechnology Co, Ltd (Dalian, China).

Data analysis The data were expressed as $x \pm s$ and statistically compared by ANOVA.

RESULTS

MCP-1 mRNA expression induced by LPS in rat splenocytes The rats were randomized to 7 groups (6 rats for each group): for group 1, each rat was injected ip with saline 0.2 mL·d^{-1} for 4 d in succession; for group 2, group 3, and group 4, the rat was given ip with saline 0.2 mL·d^{-1} for 4 d, and 0.2 mL of LPS was given respectively in a dose of 10, 20, or 30 μg/kg at 23 o'clock of d 4; for group 5, group 6, and group 7, the rat was given ip with CVPS-B respectively in a dose of 10, 30, or 50 mg·kg^{-1}·d^{-1} for 4 d. At 7 o'clock of d 5, all the rats were killed by cervical dislocation and splenocyte homogenates were prepared.

As shown in Fig 1, the products of RT-PCR for β-actin mRNA and MCP-1 mRNA were 596 bp and 418 bp respectively. In control group, a basic expression of MCP-1 in rat splenocytes was shown (Tab 1, Fig 1). LPS enhanced expression of MCP-1 mRNA in rat splenocytes significantly and this inducing effect was obviously enough at a lower dose (10 μg·kg^{-1}), and this dose was selected for the following tests to measure the up- or down-regulation of CVPS-B on the LPS induced effect. This basic expression datum (1.4 ± 0.3, ratio of MCP-1/β-actin) was also used in the later calculations.

Tab 1. Effect of LPS or CVPS-B on the MCP-1 mRNA expression in rat splenocyte analyzed by RT-PCR. $n = 6$. $x \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* **control (group 1).**

Group	LPS/ μg·kg^{-1}	CVPS-B/ mg·kg^{-1}·d^{-1}	Relative level of mRNA (ratio of MCP-1/β-actin)
1	-	-	1.4 ± 0.3
2	10	-	2.9 ± 0.4^c
3	20	-	3.5 ± 0.4^c
4	30	-	4.9 ± 0.4^c
5	-	10	1.6 ± 0.4^a
6	-	30	1.7 ± 0.5^a
7	-	50	1.5 ± 0.4^a

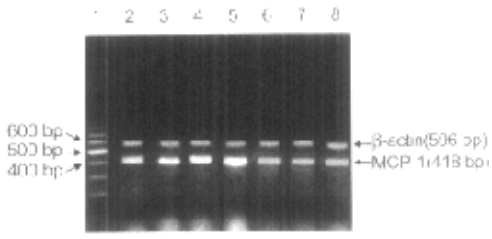


Fig 1. Agarose gel electrophoresis of the RT-PCR products for MCP-1 mRNA and β-actin mRNA. Lane 1: DNA Ladder; Lane 2: group 1 (control); Lane 3: group 2 (LPS 10 μg·kg^{-1}); Lane 4: group 3 (LPS 20 μg·kg^{-1}); Lane 5: group 4 (LPS 30 μg·kg^{-1}); Lane 6: group 5 (CVPS-B 10 mg·kg^{-1}·d^{-1} for 4 d); Lane 7: group 6 (CVPS-B 30 mg·kg^{-1}·d^{-1} for 4 d); Lane 8: group 7 (CVPS-B 50 mg·kg^{-1}·d^{-1} for 4 d). Typical representative of 6 experiments.

Effect of CVPS-B on the MCP-1 mRNA expression in normal rat splenocytes without pre-treatment of LPS Pre-treatment with CVPS-B for 4 d respectively in 3 different doses did not significantly affect on the MCP-1 mRNA expression in normal rat splenocytes. ($P > 0.05$, Tab 1).

Effect of CVPS-B on the MCP-1 mRNA expression induced by LPS in rat splenocytes Thirty rats were randomized to 5 groups. Group 1 was pre-treated with saline as control. Group 2, 3, 4, and 5 were pre-treated respectively with CVPS-B in 4 differential doses (5, 10, 30, and 50 mg·kg^{-1}·d^{-1}, ip for 4 d). At 23 o'clock of d 4, 10 μg·kg^{-1} of LPS was intraperitoneally injected to each rat. At 7 o'clock of d 5, all the rats were killed by cervical dislocation. CVPS-B decreased the expression level of MCP-1 mRNA induced by LPS significantly (Tab 2, Fig 2). Furthermore, the prevently inhibition rates were significantly correlated with the logarithm of its doses in the range from 0 to 50 mg·kg^{-1}·d^{-1} (coefficient of correlation, $R = 0.97$; standardized coefficient, Beta = 0.98; $P < 0.05$). The related data were calculated on the following formula.

The basic expression was 1.4 ± 0.3 (Tab 1).

The LPS induced expression rate = [(the relative level of mRNA for each group − the basic expression)/ the basic expression] × 100 %

The drug induced inhibition rate = [(114 − LPS induced expression rate)/114] × 100 %

Where 114 was the mRNA expression ratio of

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MCP-1/β-actin induced by LPS in control group (the group for zero dose of CVPS-B, Tab 2).

Tab 2. Effect of CVPS-B on the MCP-1 mRNA expression induced by LPS in rat splenocytes. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* control.

CVPS-B/ mg·kg^{-1}·d^{-1}	Relative level of mRNA (ratio of MCP-1/β-actin)	LPS induced expression rate/%	Drug induced inhibition rate/%
0	3.1±0.3	114	0
5	2.3±0.4^b	56	51
10	1.9±0.4^c	34	70
30	1.7±0.3^c	18	84
50	1.5±0.3^c	1	99

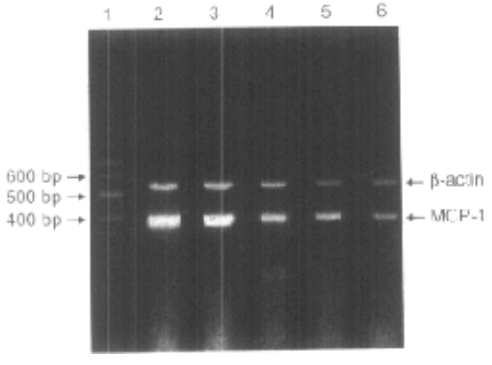

Fig 2. The image of gel electrophoresis for dose-related effect of CVPS-B on the expression of MCP-1 mRNA induced by LPS. Lane 1: DNA Ladder; Lane 2: group 1 (control); Lane 3: group 2 (CVPS-B 5 mg·kg^{-1}·d^{-1} for 4 d); Lane 4: group 3 (CVPS-B 10 mg·kg^{-1}·d^{-1} for 4 d); Lane 5: group 4 (CVPS-B 30 mg·kg^{-1}·d^{-1} for 4 d); Lane 6: group 5 (CVPS-B 50 mg·kg^{-1}·d^{-1} for 4 d). Typical representative of 6 experiments.

Sequencing of RT-PCR products Except for the first 9 bases after the primers, all the other coding were identical to the reported sequence of MCP-1 from gene bank.

DISCUSSION

CVPS-B used in our experiments is different from *Ganoderma Lucidum* polysaccharide (GLP) which is prepared from cultured *Ganoderma Lucidum* mycelia and contained in some commodities named as health foods on Chinese outlet for goods. It is said that GLP could enhance some immunological reactions and present anti-tumor effects[8]. Our CVPS-B was prepared from the hymeniophore of the wild *Coriolus versicolor* (Fr) L collected from Changbai mountain in the northeast China. CVPS-B is a new aqueous composition of polysaccharide, its structural characterization is similar to that of polysaccharide krestin (PS-K) from the cultured *Coriolus versicolor* in Japan, but it contains neither protein, peptide, nor ribonucleic acid[3], and its other biological activities may be worthy for studying further.

In the complex of the different pathogenetic events leading to atherosclerosis, recent data suggest a central role of MCP-1, because mice knockout for MCP-1 or its receptor CC-chemokine receptor 2 was considerably resistant to plaque formation[9]. In this study, we investigated the effects of CVPS-B, a new water-soluble composition of polysaccharides from the natural *Coriolus versicolor* (Fr) L, on MCP-1 gene expression in rat splenocytes. Our studies showed that in a dose-dependent fashion, CVPS-B inhibited the expression of MCP-1 mRNA induced by LPS in the rat splenocytes may be inferred as one of the molecular biological mechanisms how *Coriolus versicolor* polysaccharide prevented the plaque formation in experimentally atherosclerotic rabbits. In another aspect, CVPS-B did not significantly affect the expression of MPC-1 mRNA in the normal rat without pre-treatment of LPS.

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云芝多糖B对大鼠单核细胞趋化蛋白-1基因表达的影响[1]

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关键词 云芝; 多糖类; 单核细胞趋化蛋白-1; 基因表达; 大鼠

目的: 探究野生云芝多糖水溶性新组分 CVPS-B 对大鼠脾细胞单核细胞趋化蛋白-1 (MCP-1) 基因表达的影响. **方法**: 以 β-actin 为内标准物, 用逆转录聚合酶链式反应(RT-PCR)检测 CVPS-B 分别对正常情况下以及脂多糖(LPS)诱导下大鼠脾细胞 MCP-1 基因表达的影响, 并对 RT-PCR 产物进行测序, 以证实其特异性. **结果**: (1) 正常情况下大鼠脾细胞 MCP-1 mRNA 的表达(MCP-1/β-actin 的比值)生理盐水对照组为 1.4 ± 0.3; CVPS-B 三个剂量组(10、30 和 50 $mg \cdot kg^{-1} \cdot d^{-1}$, ip, 连续 4 d)分别为: 1.6 ± 0.4、1.7 ± 0.5 和 1.5 ± 0.4, 后三组与对照组无显著差异 ($P > 0.05$); (2) 大鼠腹腔给药 LPS (10 $\mu g \cdot kg^{-1}$)可使脾细胞 MCP-1 mRNA 的表达增加 114 %. (3) CVPS-B 4 个剂量组 (5、10、30 和 50 $mg \cdot kg^{-1} \cdot d^{-1}$, ip, 连续 4 d)可使 LPS (10 $\mu g \cdot kg^{-1}$, ip) 诱导的脾细胞 MCP-1 mRNA 的表达分别减低 51 %, 70 %, 84 % 和 99 % ($n = 6$). **结论**: CVPS-B 可预防性抑制 LPS 对大鼠脾细胞 MCP-1 基因表达的诱导作用, 且呈剂量依赖性, 但对正常情况下大鼠脾细胞 MCP-1 mRNA 的表达则无明显影响.

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