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Reduction in monocyte chemoattractant protein-1 mRNA expression in peripheral blood mononuclear cells of diamorphine addicts[1]

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KEY WORDS diamorphine; monocyte chemoattractant protein-1; mononuclear cells; habituation (psychophysiology)

ABSTRACT

AIM: To investigate whether there is a significant difference in the monocyte chemoattractant protein-1 (MCP-1) mRNA expression between diamorphine (heroin) addicts and normal volunteers. **METHODS**: Expression of MCP-1 mRNA in the peripheral blood mononuclear cells of diamorphine addicts and normal volunteers 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 the specificity of these products in MCP-1 gene composition. **RESULTS**: The relative MCP-1 mRNA expression ratios (MCP-1/β-actin) in the peripheral blood mononuclear cells of normal volunteers control group and diamorphine addicts group were 0.47 ± 0.12 ($n = 15$) and 0.21 ± 0.09 ($n = 21$), respectively, and there was a significant difference ($P < 0.05$). **CONCLUSION**: The significant reduction of MCP-1 mRNA expression in the peripheral blood mononuclear cells of diamorphine addicts may be one of the mechanisms for the high incidence of severe infectious diseases, including AIDS, among diamorphine addicts.

INTRODUCTION

The abuse of diamorphine (diacetylmorphine, heroin) is well known to be associated with an increased incidence of several types of infections, including HIV. A few studies have assessed whether diamorphine produces pharmacological alterations of immune status that might contribute to the increased rate of infections among diamorphine addicts. In rats, diamorphine produces a suppression of the concanavalin A stimulated-proliferation of T cells, a suppression of lipopolysaccharide (LPS) stimulated-proliferation of B cells, and a reduction of interferon-gamma and cytotoxicity of natural killer (NK) cells in spleen[1]. In contrast, diamorphine does not alter the relative numbers of $CD4^+$ $CD3^+$ T cells, $CD8^+CD3^+$ T cells, $CD45^+$ B cells, and CD11b/c^+ $ED1^+$ monocytes/macrophages in rat spleen[1]. The administration of diamorphine to rats results in a pronounced reduction in LPS-induced expression of inducible nitric oxide synthase (iNOS) and a reduction in the level of plasma nitrite/nitrate, the more stable end-product of nitric oxide degradation[2]. However, in diamorphine addicts, conflicting results, both decreased and increased, have been reported concerning the function of T lymphocytes[3]. Monocyte chemoattractant protein-1 (MCP-1) is a novel chemokine that may initiate and amplify monocyte recruitment to the microvascular walls, and let monocyte enter into the tissues and be transformed into macrophages[4]. Recruitment of macrophages into tissues is an important process in inflammation and host defense. In another word, MCP-1 is thought to play a significant role in inflammation and host defense[4], including the prevention of tumors, because MCP-1 is also an important determinant of macrophage infiltration in tumors[5]. The present study investigated whether there is any alteration of MCP-1 mRNA expression in the peripheral blood mononuclear cells of diamorphine addicts compared to that of the normal volunteers without the history of drug abuse, so as to test the hypothesis that the change of MCP-1 mRNA expression might be involved in the high incidence of infectious diseases among diamorphine users.

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MATERIALS AND METHODS

Subject selection and blood samples This human experiment was in accordance with the ethical standards provided by the responsible committee of Guangzhou public health bureau. The venous bloods of heroin addict group were obtained from 21 patients on the first day who voluntarily entered into the treatment center for opiate addicts in Guangzhou Tonghe Convalescent Hospital. Sixteen of them were male and five of them were female. The mean age was (29 ± 5) a. The time range for taking heroin was 12 months to 6 a. For everyone of them, the morphine screening in blood was positive with the enzymatic immunoassay for qualitative determinations. The control group consisted of fifteen persons with similar age without the history of drug abuse or neuropsychiatric disorders. Toxicologically screening in these control subjects gave negative results. For each subject, 5 mL of venous blood was collected and anticoagulated with heparin. Preparation of peripheral blood mononuclear cells was performed with the buffer for seperation of mononuclear cells (Ficoll). The pellets of peripheral blood mononuclear cells (the mixture of lymphocytes and monocytes) were stored at liquid nitrogen (−280 ℃).

Reagents Reagent Trizol was a product of Gibco/BRL (USA). The PCR primers for human β-actin and MCP-1 were synthesized by Gibco/BRL (USA). The titamTM one tube RT-PCR system was product of Boehringer Mannhein (Germany). Other chemicals were of analytical grade.

Reverse transcription polymerase chain reaction (RT-PCR) The total RNA of the peripheral blood mononuclear cells 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 human MCP-1 primers (sense: 5′-AGGATGAAAGTCTCTGCCGCCCTTCTG-3′; antisense: 5′-ATTAAGGCATAATGTTTCACA-3′) and the human β-actin primers (sense: 5′-CGAGAAGATGACCCAGATCA-3′; antisense: 5′-AGGGGCCGGACTCGTCATAC-3′) were used[6]. 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; and 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's 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.

Statistical analysis The data were expressed as $\bar{x} \pm s$ and statistically compared by ANOVA.

RESULTS

MCP-1 mRNA expression in the peripheral blood mononuclear cells As shown in Fig 1, the products of RT-PCR for human β-actin mRNA and MCP-1 mRNA were 240 bp and 510 bp respectively. In control group, the basic expression of MCP-1 mRNA in the peripheral blood mononuclear cells of normal volunteers was 0.47 ± 0.12 (MCP-1/β-actin); and in diamorphine addicts group, the relative level of MCP-1 mRNA expression was 0.21 ± 0.09 (MCP-1/β-actin) (Tab 1). The latter was significantly lower than the former. In other word, MCP-1 mRNA expression in diamorphine addicts was inhibited.

DISCUSSION

The conflicting results concerning the function of T lymphocytes in diamorphine (heroin) addicts have been reported[3]. One of reasons for this conflicting results might be the subjects selection. Perhaps some authors selected the heroin addicts having taken heroin for a longer time in succession, others might selected the

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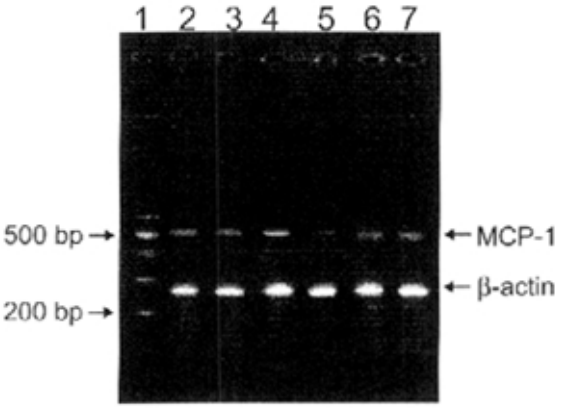

Fig 1. Agarose gel electrophoresis of the RT-PCR products for MCP-1 mRNA and β-actin mRNA. Lane 1 was 100 bp DNA Ladder, Lane 2, Lane 3, and Lane 4 were 3 representative samples among the 15 normal volunteers, and Lane 5, Lane 6, and Lane 7 were 3 representative samples among the 21 diamorphine addicts.

Tab 1. MCP-1 mRNA expression in the peripheral blood mononuclear cells of diamophine addicts and normal volunteers analyzed by RT-PCR. $\bar{x} \pm s$. $^bP < 0.05$ *vs* control.

Group	*n*	Relative level of mRNA expression (ratio of MCP-1/β-actin)
Control	15	0.47 ± 0.12
Diamorphine addicts	21	0.21 ± 0.09[b]

heroin withdrawal subjects having taken heroin for a long time off and on, but for a short time in succession. In the present study, the standard of subjects selected was rather rigid. All the heroin addicts selected had taken heroin at least for 12 months in succession.

Recruitment of macrophages into tissues is an important process in inflammation and host defense, and in this process, MCP-1 is thought to play a significant role. The pharmacological alterations of the level of MCP-1 mRNA expression by drug abuse might induce the alterations of human immune status. This study provides the first evidence that taking heroin for a long time induces an alteration of MCP-1 mRNA expression, and suggests that reduction in MCP-1 mRNA expression may be involved in the increased incidence of severe infectious diseases, including AIDS, among heroin users.

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乙酰吗啡成瘾者外周血单个核细胞中单核细胞趋化蛋白-1 mRNA 表达的抑制[1]

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关键词 乙酰吗啡；单核细胞趋化蛋白-1；单个核细胞；习惯化（心理生理学）

目的：探究乙酰吗啡(海洛因)成瘾者单核细胞趋化蛋白-1 (MCP-1)基因表达状况是否与正常人有区别. **方法**：以 β-actin 为内标准物，用逆转录聚合酶链式反应(RT-PCR)分别检测乙酰吗啡成瘾者和正常志愿者外周血单个核细胞中 MCP-1 mRNA 表达状况，并对 RT-PCR 产物进行测序，以证实其特异性. **结果**：外周血单个核细胞中 MCP-1 mRNA 表达的相对水平，正常志愿者对照组为 0.47 ± 0.12 ($n = 15$)；乙酰吗啡成瘾组为 0.21 ± 0.09 ($n = 21$)，两组间差异显著($P < 0.05$). **结论**：乙酰吗啡成瘾者外周血单个核细胞中 MCP-1 mRNA 的表达显著被抑制；这可能是乙酰吗啡成瘾者群体中包括 AIDS 等严重感染性疾病发病率显著增高的机制之一.

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