Suppression of morphine withdrawal syndrome by interleukin-2 and its gene

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Received 13 December 2004; accepted 13 January 2005

The naloxone-precipitated withdrawal syndrome in mice and rats after intrathecal injection of recombinant human interleukin-2 protein (rIL-2) or its gene was studied. The results showed that rIL-2 could significantly decrease the number of jumps in mice. In rats, rIL-2 significantly suppressed irritating, diarrhea, weight loss, abnormal posture and salivation. Tendencies towards reductions in teeth chewing and dog-shaking were also observed. Furthermore, pcDNA3-IL-2 (8μg DNA) had a similar effect as 1×10^4 IU rIL-2 protein on inhibition of morphine withdrawal syndrome in mice, and the expression of rIL-2 protein in spinal cord could be detected for 6 days. These findings provided further evidence for the neuro-regulatory function of an immunological molecule such as IL-2.

Key words: Interleukin-2; Interleukin-2 gene; Intrathecal injection; Morphine withdrawal syndrome

INTRODUCTION

Many experiments have indicated the existence of cross-talk between the nerve system and the immune system. The nerve system is known to modulate the function of the immune system. However, the molecular basis for the immune system to modulate the nerve system is not well understood. Several cytokines can modulate nociception, but some of the findings are contradictory. Some experiments [1] showed that interleukin (IL)-1β produces hyperalgesia by its actions on the medial preoptic (MPO) and lateral preoptic (LPO) areas and analgesia on the ventromedial hypothalamus (VMH) in the rat. Pain modulatory actions of cytokines may also occur at the spinal cord level, and they play important roles especially in the mediation of neuropathic pain. Some evidence showed that there was interaction between the opioid system and cytokines [2,3].

Our previous studies suggested that IL-2 is an important analgesic molecule in both the central and peripheral nerve system [4,5]. It is interesting to note that IL-2 could still show the antinociceptive effect, and this effect was higher than that of morphine itself while rats were tolerant to morphine [6]. The antinociceptive effect of IL-2 gene (pcDNA3-IL-2) could also be blocked by naloxone [5]. The antinociceptive domain is located at the Phe44, Tyr45, Tyr107 and Phe117 residues of hIL-2, which seem to form an active center in the tertiary structural level and separate from the immune active domain [7].

Because opioid addiction is a serious social problem, several drugs have been used to detoxify opioid addicts [8]. The study of opioid detoxification can lead to pharmacological treatments for addiction and can also provide important information of the neural mechanisms of motivated behaviors. This investigation aims to examine the effect of intrathecal (i.t.) injection of IL-2 on naloxone-precipitated withdrawal in morphine-dependent mice and rats. In order to overcome the short half-life of IL-2 protein, a further study with i.t. delivery of liposome-mediated hIL-2 gene on the effect of naloxone-precipitated withdrawal syndrome was carried out.

MATERIALS AND METHODS

Experimental animals: Male Kunming mice (20–24 g) and male Sprague-Dawley rats (240–260 g) were used (Shanghai Experimental Animal Center of Chinese Academy of Sciences, Shanghai, China). Mice were housed three per cage and rats were housed one per cage at a temperature of 22°C, with food and water ad lib and a 12:12 h light:dark cycle. All animal experiments were approved by the Administrative Committee of Experimental Animal Care and Use of Shanghai, and conformed to the National Institute of Health Guide for the care and use of laboratory animals.

Construction of expression vector: The whole-length cDNA of hIL-2 gene was cloned into a pcDNA3 empty plasmid, which has a cytomegalovirus (CMV) promoter followed by polycloning sites [5].

Lumbar subarachnoid catheterization: A PE-10 catheter (Becton Dickinson, Sparks, Maryland, USA) was inserted into the lumbar subarachnoid space between lumbar

MOLECULAR NEUROSCIENCE NEUROREPORT

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vertebrae 5 (L5) and 6 (L6) using the ‘catheter-through-a-needle’ technique [9]. After surgery, a minimum of 4 days was allowed for recovery before the experiments were carried out.

**Administration of rIL-2 protein and its gene:** The rIL-2 protein and its gene were injected into the subarachnoid in mice according to the method of Hylden and Wilcox [10]. The total volume of injection was 10 μl. An i.t. injection of pcDNA3 or pcDNA3-IL-2 (8 μg DNA) was given 24 h before naloxone administration to precipitate morphine withdrawal syndrome. For rats, the i.t. injection of human rIL-2 protein was by the preimplanted PE-10 catheter with syringe (Bionanalytical System Inc., West Lafayette, Indiana, USA) at 2 μl/min, and the total volume of injection was 30 μl. For the detection of hIL-2 level in cerebrospinal fluid (CSF) and messenger RNA (mRNA) expression in the spinal cord, an i.t. injection of 25 μg pcDNA3 or pcDNA3-IL-2 was administered in rats. DNA and lipofectamine reagent (2 μg/μl, Invitrogen, Carlsbad, CA, USA) were mixed in a ratio of 1:2 (w/w). Before injection, animals were lightly anesthetized with ether to avoid struggling and then rIL-2 and its gene were injected into the subarachnoid.

**Development of morphine dependence and measurement of abstinence signs:** The mice were given subcutaneous injections of morphine hydrochloride (Shenyang First Pharmaceutical Factory, Sheng Yang, China) three times daily (08:30, 14:00 and 20:30) for 6 days with increasing doses on each day (5, 10, 20, 40, 80, 160 mg/kg) [11]. On the seventh day, all mice received a single injection of morphine (160 mg/kg). Three hours after the last injection of morphine, for the group with i.t. injection of rIL-2, mice were anesthetized with ether to avoid struggling and then rIL-2 and its gene were injected into the subarachnoid.

**RESULTS**

**Effect of rIL-2 on inhibition of morphine withdrawal syndrome in mice:** An i.t. injection of rIL-2 caused a powerful and significant suppression of the morphine withdrawal syndrome. rIL-2 significantly reduced the number of jumps in naloxone-precipitated morphine withdrawal mice at a dose of 1 × 10^4 IU. This effect was dose dependent (p < 0.01, r = 0.70). The jump behavior almost disappeared at a dose of 8 × 10^4 IU.

**Effect of rIL-2 gene on inhibition of morphine withdrawal syndrome in mice:** Gene transfection and expression of rIL-2 in vivo were reported in our previous study [5]. The therapeutic effect of i.t. injection of rIL-2 gene on chronic neuropathic pain could be maintained till day 6. Here, the liposome-mediated plasmid for gene therapy of morphine withdrawal syndrome was studied after 24 h i.t. injection of gene. The results showed that i.t. delivery of pcDNA3-IL-2/ lipofectamine complex (8 μg DNA) could significantly suppress the number of jumps in naloxone-precipitated morphine withdrawal mice (Fig. 1) versus pcDNA3/ lipofectamine. The rIL-2 gene had an effect similar to that of
of 1 × 10^4 IU rIL-2 protein on the inhibition of morphine withdrawal syndrome in mice.

**Effect of rIL-2 protein on inhibition of morphine withdrawal syndrome in rats:** In rats, two types of morphine withdrawal scores were used. rIL-2 significantly suppressed the total scores of morphine withdrawal (Table 1). The reduction in nonmotor scores caused by different doses of rIL-2 appeared to be matched by reductions in total scores. Similarly, significant reductions in some motor scores such as abnormal posture and salivation were seen, whereas only a tendency but no statistically significant difference was seen in the reductions of teeth chewing and dog-shaking.

**Assay of rIL-2 level in CSF after i.t. gene delivery:** rIL-2 protein could be detected in pia mater, dorsal root ganglion (DRG), spinal dorsal horn and CSF after i.t. gene delivery [5]. The amount of rIL-2 protein secreted into CSF could indirectly reflect the rIL-2 protein level expressed by hIL-2 gene transfected into the adjacent tissues. So we assayed the rIL-2 protein level in CSF daily to estimate how long the function of injected rIL-2 gene would last. The results showed that rIL-2 was expressed in high levels at 24 h (p < 0.01, 37.8 ± 4.3 ng/ml CSF of pcDNA3-IL-2/lipo group vs. 2.1 ± 0.4 ng/ml CSF of pcDNA3/lipo group) after i.t. gene delivery, and reached the highest level on the third day (p < 0.01, 48.7 ± 8.3 ng/ml CSF of pcDNA3-IL-2/lipo group vs. 3.0 ± 0.7 ng/ml CSF of pcDNA3/lipo group). The IL-2 expression could be maintained at day 6 (p < 0.01, 10.4 ± 2.0 ng/ml CSF of pcDNA3-IL-2/lipo group vs. 1.7 ± 0.3 ng/ml CSF of pcDNA3/lipo group). rIL-2 in CSF was not detected by ELISA after i.t. delivery of vehicle or pcDNA3/lipo. These results suggest that the function of hIL-2 gene could last for 4–5 days.

**IL-2 mRNA expression in spinal cord after i.t. delivery of pcDNA3-IL-2/lipofectamine:** To elucidate the molecular basis of hIL-2 gene therapy, we detected hIL-2 mRNA in the spinal cord of morphine-dependent rat by in-situ hybridization. On the third day after i.t. injection of hIL-2 gene, when the rIL-2 protein level in CSF became highest, hIL-2 mRNA could be detected in spinal matter and paretic parenchyma, which were mainly in the gray matter (Fig. 2). The results indicate that the hIL-2 gene could be transacted into and expressed in spinal cord regions of nervous function modulation.

**DISCUSSION**

Opiates have been shown to influence immune function *in vivo* [15], and clinical observations have shown that opiate addicts have increased susceptibility to infections [16]. Otherwise, cytokines play unique roles as signal molecules in the bi-directional communication between the nerve system and the biodefense system. Cumulative evidence exists that several cytokines can modulate nociception [4,17]. Some evidence suggests that opioid receptors may be involved [2,3]. Our previous studies [4] showed preliminary evidence for the direct action of IL-2 on opioid receptors.

In addition, our previous studies showed that IL-2 had an antinociceptive (analgesic) effect both in the central and peripheral nerve system and a superior antinociceptive effect when delivered i.t. as compared with peripherally [18]. When IL-2 protein or IL-2 gene was i.t. delivered, IL-2

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**Table 1.** Effect of intrathecal (i.t.) injection of human rIL-2 on signs of morphine withdrawal in rats.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Motor scores</th>
<th>Nonmotor scores</th>
<th>Total scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>IL-2 (2.0 × 10^4 IU)</td>
<td>IL-2 (4.0 × 10^4 IU)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Motor scores</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wet dog-shaking</td>
<td>1.27 ± 0.49</td>
<td>1.60 ± 0.75</td>
</tr>
<tr>
<td></td>
<td>Abnormal posture</td>
<td>8.00 ± 0</td>
<td>6.40 ± 1.60</td>
</tr>
<tr>
<td></td>
<td>Salivation</td>
<td>1.82 ± 0.12</td>
<td>1.20 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>Teeth chewing</td>
<td>0.27 ± 0.10</td>
<td>0.20 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>Nonmotor scores</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Diarrhea</td>
<td>7.27 ± 0.49</td>
<td>5.60 ± 0.98</td>
</tr>
<tr>
<td></td>
<td>Irritating</td>
<td>3.54 ± 0.68</td>
<td>1.60 ± 1.60</td>
</tr>
<tr>
<td></td>
<td>Weight loss</td>
<td>11.82 ± 102</td>
<td>10.00 ± 1.58</td>
</tr>
</tbody>
</table>

Four days after lumbar subarachnoid catheterization, rats were made physically dependent on morphine. Naloxone-precipitated morphine withdrawal syndrome was observed, including motor scores and nonmotor scores, after i.t. injection of rIL-2 at doses of 2.0 × 10^4, 4.0 × 10^4 or 1.4 × 10^5 IU, 5% glucose in phosphate buffer (pH 7.0) as control. The total volume of injection was 30 µl. Values represent means ± SEM. Significance was defined as *p < 0.05 compared with control (one-way ANOVA followed by Fisher’s LSD test).
was widely distributed in lumbar spinal pia mater, DRG, sciatic nerve, spinal dorsal horn and CSF. The spinal cord plays an important role in the expression of autonomic and certain behavioral components of the opiate withdrawal syndrome [19], and the expression of the spinal-mediated withdrawal response was dependent on intact afferent stimuli [20]. In this study, the effect of IL-2 on the morphine withdrawal syndrome in mice and rats by i.t. injection was observed because i.t. injection is more practical in clinic than intracerebroventricular injection as shown by Woldbye et al. [14]. Our results showed that i.t. injection of IL-2 significantly suppressed the morphine withdrawal syndrome in mice. To observe more abstinence signs, further studies for the effect of i.t. IL-2 on the morphine withdrawal syndrome in rats were carried out. The results were consistent with experiments in mice.

Because the half-life time ($t_{1/2}$) of IL-2 in serum is very short [21], it may restrict the application of IL-2 for detoxification of addicts. To prolong this effect, IL-2 gene therapy technique was also carried out in this study. The effect of IL-2 gene on naloxone-precipitated morphine withdrawal syndrome at 24 h was observed after i.t. injection of liposome-mediated IL-2 gene. The effect of 8 μg pcDNA3-IL-2 was similar to that of the dose of 1 × 10^7 IU IL-2 protein on the number of jumps. The data of this study showed that IL-2 protein was expressed at high levels in CSF for 4–5 days. The detection of hIL-2 mRNA in situ hybridization in the spinal cord further indicated that the transfected hIL-2 gene could express at the spinal cord regions of functional modulation. This seemed to have more practical meaning for the use of IL-2 gene rather than IL-2 protein in the suppression of morphine withdrawal syndrome. IL-2 plays an important role in the regulation of the immune system and can be used in immunotherapy against glioma [22]. Addiction patients are usually deficient in the immune defense system [23] and IL-2 is a good immune augmentation agent. Using IL-2 for the above purpose may be another favorable point in the suppression of morphine withdrawal syndrome.

**CONCLUSION**

IL-2 and its gene can inhibit morphine withdrawal syndrome. This finding provides further evidence for the neuroregulatory function of an immunological molecule. IL-2 could be a cytokine useful in the relief of opioid addiction patients.

**REFERENCES**

9. Storkson RV, Kjorsvik A, Tjolsen A, Hole K. Lumbar catheterization of the lumen of 30 μl, IL-2 mRNA was detected in the section of lumbar spinal cord after intrathecal injection of pcDNA3 + lipofectamine (pcDNA3/lipo) or pcDNA3-IL-2/lipo (pcDNA3-IL-2/lipo). On the third day after i.t. injection of 25 μg of pcDNA3-IL-2 + lipofectamine (pcDNA3-IL-2/lipo) or 25 μg of pcDNA3-IL-2 + lipofectamine (pcDNA3-IL-2/lipo) in a total volume of 30 μl, IL-2 mRNA was detected in the section of lumbar spinal cord by in-situ hybridization. Photomicrographs showed transverse sections after i.t. injection of pcDNA3/lipo (a) or pcDNA3-IL-2/lipo (b).

**Fig. 2.** Interleukin (IL)-2 messenger RNA (mRNA) expression in the spinal cord after intrathecal (i.t.) delivery of hIL-2 gene. On the third day after i.t. injection of 25 μg of pcDNA3-IL-2 + lipofectamine (pcDNA3-IL-2/lipo) or 25 μg of pcDNA3-IL-2 + lipofectamine (pcDNA3-IL-2/lipo) in a total volume of 30 μl, IL-2 mRNA was detected in the section of lumbar spinal cord by in-situ hybridization. Photomicrographs showed transverse sections after i.t. injection of pcDNA3/lipo (a) or pcDNA3-IL-2/lipo (b).


Acknowledgements: We wish to thank Hua Xin High Biotechnology Inc. (China) for the gift of rIL-2 protein.