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Morphine suppresses proinflammatory cytokine production in human whole blood *in vitro*[1]

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ABSTRACT **AIM**: To study the effects of morphine on tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) production in human whole blood and the possible mechanism. **METHODS**: The seven human whole blood samples were collected, and each was aliquoted in six tubes. A total of 100 μl of whole blood was added with morphine sulfate (200 mg·L^{-1} and 2 mg·L^{-1}). Then lipopolysaccharide (LPS) (100 μg·L^{-1}) was added to the blood and incubated for 6 h at 37 ℃. Each whole blood was divided into six groups: drug-alone groups (group A_1, morphine 200 mg·L^{-1}; group A_2, morphine 2 mg·L^{-1}), activation groups (group B_1, morphine 200 mg·L^{-1} + LPS; group B_2, morphine 2 mg·L^{-1} + LPS), control group (group C_1), and LPS group (group C_2). The concentrations of TNF-α and IL-6 in plasma were measured by ELISA. **RESULTS**: The values of TNF-α production in group A_1, A_2 and C_1 were 240, 251, and 279 ng·L^{-1} ($P>0.05$), respectively; the values of IL-6 were 444, 490, and 561 ng·L^{-1} ($P>0.05$), respectively. The cytokine production in group B_1, B_2 and C_2 were 490, 534 and 1226 ng·L^{-1} (TNF-α), respectively; 1177, 1310 and 1563 ng·L^{-1} (IL-6), respectively. The levels in group B_1 and B_2 were less than that in group C_2 ($P<0.01$, or $P<0.05$), and the level in B_1 was less than that in B_2. **CONCLUSION**: Morphine alone has no effects on TNF-α and IL-6 production, but attenuates LPS-induced TNF-α and IL-6 production, and the high dose of morphine-induced effects on attenuation of TNF-α and IL-6 production increased are more than the low dose.

KEY WORDS pharmacodynamics; morphine; tumor necrosis factor-α; interleukin-6

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Cytokines are essential for hematopoiesis and immune responses, and they play a key role in the defense against infection. Lipopolysaccharide (LPS) is a potent inducer of the inflammation involver in the pathogenesis of septic shock. It has been demonstrated that proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-8, increase in patients with sepsis, trauma, and burns[1,2]. IL-6 mediates the acute-phase response. Morphine has advocated for anesthesia in septic or severely ill patients with cancers. It was reported that morphine affected LPS-induced TNF-α, IL-6 and IL-8 production in mice or rats. However, there are no reports on the effect of morphine on cytokine production in human whole blood. Therefore, in this study, we investigated the efficacy of morphine on LPS-induced TNF-α and IL-6 production in human whole blood.

1 MATERIALS AND METHODS

1.1 Reagents Phenol-extracted Escherichia Coli (serotype 0111: B_4); LPS and human TNF-α and IL-6 kits were purchased from Jing Mei Biotech. Co. Ltd.

1.2 Experimental methods After an internal review

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board approved the project, informed consent was obtained from 7 healthy volunteers. Five milliliters of venous blood were collected from each individual. The donors had no history of infection or allergy and never been subjected to immunosuppressive therapy. Whole blood was collected in edetic acid and aliquoted in tubes. A total of 100 μl of whole blood was added with morphine sulfate (200 and 2 mg·L^{-1}). Then LPS (100 μg·L^{-1}) was added to the blood and also incubated for 6 h at 37 ℃. Controls were incubated with phosphate-buffered saline (PBS). After incubation, the blood was centrifuged at 300 *g* for 10 min to remove blood cells. Supernatant samples were collected and stored at −70 ℃ until assayed. The plasma TNF-α and IL-6 concentrations were measured using commercially available enzyme-linked immunoassay (TNF-α and IL-6 ELISA kits). The intra- and interassay variations were both less than 10%. Detection limits for the ELISA kits were 10 and 15 ng·L^{-1}, respectively.

1.3 Statistical analysis All data were presented as $\bar{x} \pm s$. Analysis of variance (ANOVA) and *q*-test were used for statistical analysis. A significant difference was presumed at a probability value of $P < 0.05$.

2 RESULTS

2.1 Morphine alone had no effect on TNF-α and IL-6 production Two different concentrations of morphine were used to determine if there were effects on TNF-α and IL-6 production. The cytokine production in non-activation, such as drug-alone and control group was very low. The TNF-α production values in groups A_1, A_2 and C_1 were 240, 251 and 279 ng·L^{-1}, respectively; IL-6 values were 444, 490 and 561 ng·L^{-1}, respectively. None of the concentrations tested (200 and 2 mg·L^{-1}) had any effect on TNF-α and IL-6 production compared with control values ($P > 0.05$).

2.2 Morphine attenuated LPS-induced TNF-α and IL-6 production Whole blood was stimulated using LPS (100 μg·L^{-1}). LPS induced TNF-α and IL-6 production in human whole blood (Tab 1, $P < 0.01$). After adding different dose (200 and 2 mg·L^{-1}) of morphine, whole blood was stimulated with LPS. When the blood was incubated for 6 h, morphine significantly suppressed LPS-induced TNF-α and IL-6 production with their corresponding LPS group ($P < 0.01$, or $P < 0.05$), and the high dose of morphine-induced effects on attenuation of TNF-α and IL-6 production increased were more than the low dose.

Tab 1 Effect of morphine on LPS-induced TNF-α and IL-6 production in human whole blood ($\bar{x} \pm s$, $n = 7$)

Groups	TNF-α /ng·L^{-1}	IL-6 /ng·L^{-1}
Morphine		
200 mg·L^{-1}	240±79	444±230
2 mg·L^{-1}	251±76	490±199
200 mg·L^{-1} + LPS	490±143^{f}	1177±167^{e}
2 mg·L^{-1} + LPS	534±149^{f}	1310±384^{e}
Control	279±72	561±238
LPS	1226±177^{e}	1563±630^{e}

$^{e}P < 0.01$ *vs* their corresponding control group; $^{e}P < 0.05$, $^{f}P < 0.01$ *vs* their corresponding LPS group.

3 DISCUSSION

In the study, we demonstrated that morphine alone has no effect on TNF-α and IL-6 production, but it suppresses both LPS-induced TNF-α and IL-6 production. It was reported that TNF-α was the first cytokine expressed after LPS stimulation, after which it stimulated other cytokines including IL-6 secretion from macrophages, monocytes, neutrophils and endothelial cells[3]. The mechanism for the suppressive effect of morphine on cytokine production is not clear, it may be mediated by inhibiting NF-κB activity[4].

In previous studies, morphine affected LPS-induced TNF-α and IL-6 production in mice or rats. Subcutaneous injection of morphine decreased LPS-induced TNF-α production, and the effect was dose-dependent and reversible by naloxone[5]. In peritoneal mouse macrophages, nanomolar morphine concentrations were found to increase LPS-induced TNF-α and IL-6 synthesis. However, micromolar concentrations led to a reduction of TNF-α and IL-6 production[6]. The latter are contradictory to our study. A 100-fold higher LPS concentration was used, which may override low-dose morphine effects. Concentrations up to 20 mg·L^{-1} were determined after high-dose treatment with morphine[7].

In conclusion, we demonstrated that morphine inhibits the production of proinflammatory cytokines, such as TNF-α and IL-6, in human whole blood. We surmise that morphine is part of the antiinflammatory responses. Further study is required to elucidate the mechanism of the suppressive effect of morphine.

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吗啡抑制人外周血 TNF-α 和 IL-6 的表达的研究[1]

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摘要 目的: 研究吗啡对人外周血中肿瘤坏死因子-α (TNF-α) 和白细胞介素-6 (IL-6) 含量的影响, 探讨其对免疫炎症反应的可能机制。方法: 全血收集在试管内, 分装在 EP 管中, 每管取 100 μl 全血。实验分为吗啡 200 mg·L^{-1} 组(A_1 组)、吗啡 2 mg·L^{-1} 组(A_2 组)、吗啡 200 mg·L^{-1} + 脂多糖(LPS)(B_1 组)、吗啡 2 mg·L^{-1} 组 + LPS(B_2 组)、对照组(C_1 组)和 LPS 组(C_2 组)共 6 组(n = 7)。各组加入上述试剂, 用 PBS 补齐体积后, 在 37 ℃下孵育 6 h。用 ELISA 检测血清中 TNF-α 和 IL-6 含量。结果: 单独药物组(A_1 和 A_2 组) TNF-α 的浓度分别为 240 和 251 ng·L^{-1}, 与空白对照组(C_1 组, TNF-α 的浓度为 279 ng·L^{-1}) 比较, 无显著的统计学意义($P > 0.05$); IL-6 的浓度分别为 444、490 和 561 ng·L^{-1}, 亦无统计学意义($P > 0.05$)。激活组(B_1 和 B_2 组) 和 LPS 组(C_2 组) 分别为 490、534 和 1226 ng·L^{-1} (TNF-α); 1177、1310 和 1563 ng·L^{-1} (IL-6)。B_1 和 B_2 组明显低于 C_2 组($P < 0.01$ 或 $P < 0.05$), 且 B_1 组低于 B_2 组。结论: 吗啡对静息状态下 TNF-α 的表达无影响, 但可抑制 LPS 诱导的 TNF-α 表达, 且高剂量的吗啡对 LPS 诱导的 TNF-α 的抑制作用大于低剂量吗啡的作用。

关键词 药效学; 吗啡; 肿瘤坏死因子-α; 白细胞介素-6