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Effect of morphine on interleukin-1 and tumor necrosis factor α production from mouse peritoneal macrophages *in vitro*[1]

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AIM: To study the influence of morphine on lymphokine production from mouse peritoneal macrophages (PMØ). **METHODS**: The productions of interleukin-1 (IL-1) and tumor necrosis factor (TNF-α) from PMØ under various concentrations of morphine with or without naloxone were measured by testing thymocyte proliferation and L929 cell lysis *in vitro*. **RESULTS**: Both morphine (1×10^{-8}—1×10^{-3} mol L^{-1}) and naloxone (50 μmol L^{-1}) inhibited IL-1 production from mouse PMØ primed by sodium thioglycolate. Naloxone did not block the inhibitory effect of morphine. TNF-α production from PMØ was inhibited by only high concentration (1 mmol L^{-1}) of morphine which was not blocked by naloxone. **CONCLUSION**: Morphine had a direct inhibitory effect on PMØ, which was not mediated by opioid receptor of PMØ.

KEW WORDS morphine; naloxone; interleukin-1; tumor necrosis factor

Addiction to morphine is accompanied by an increased incidence of infections[1]. Reticuloendothelial system plays an important role in the host defense function. Morphine drastically reduces reticuloendothelial system activity[2] and then exacerbates infections. This paper is to study the direct effect of morphine on the function of peritoneal macrophages (PMØ) *in vitro*.

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MATERIALS AND METHODS

Reagents Morphine was produced by Qinhai Pharmaceutics and recrystallized by Dr YIN Dun-Xiang, Department of Synthetic Chemistry of our Institute. Naloxone, lipopolysaccharides (LPS), dactinomycin (Dac), and deoxyribonucleases (DNase) were from Sigma Co. Crystal violet (AR) was from Shanghai Chongming Chemical Reagent Factory. [^{3}H]TdR was from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. RPMI 1640 medium (Gibco Co) was supplemented with HEPES buffer 10 mmol L^{-1}, penicillin 100 kU L^{-1}, streptomycin 100 mg L^{-1}, *L*-glutamine 2 mmol L^{-1}, 2-mercaptoethanol 50 mol L^{-1}, and 10 % newborn bovine serum, pH 7.2.

Mice ICR mice, ♀, weighing 18 — 22 g; BALB/c mice, ♀, weighing 16—20 g, were purchased from Shanghai Animal Center, Chinese Academy of Sciences.

Cell line L929 cell line (tumorigenic murine fibroblast) was purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences. The cell line was subcultured with 10 % newborn bovine serum RPMI 1640 medium.

Production of interleukin-1 (IL-1) by PMØ[3] PMØ were obtained from mice ip injected with 10 % thioglycolate broth (Difco, 1 mL/mouse, 4 d later). The peritoneal cavities were lavaged with RPMI 1640 media. The cells were washed 3 times in RPMI 1640 media, placed in 24-well plates at 2×10^6 cells/well, and allowed to adhere at 37 ℃ in a 5 % CO_2 for 1 h. Nonadherent cells were washed out. IL-1 was induced with LPS (5 mg L^{-1}) from PMØ. Indometacin (10 mg L^{-1}) and morphine (1×10^{-6}—1×10^{-3} mol L^{-1}) with or without naloxone (50 μmol L^{-1}) were added, then the plates were incubated at 37 ℃ in 5 % CO_2 atmosphere for 24 h. After incubation, the supernatant was collected by centrifugation (600 × *g*, 10 min) and stored at −25 ℃ until assay.

IL-1 assay IL-1 activity was evaluated by thymocyte proliferation assay. Thymocytes (1.5×10^6 cells/well) isolated from BALB/c mice, were cocultured with PMØ supernatant in 96-well microtiter plates for 72 h in the presence of Con A (5 mg L^{-1}). The cultures were added with [^{3}H]TdR (24.5 kBq/well) for the final 6-h incubation and harvested onto glass fiber filter paper. IL-1 activity was evaluated by [^{3}H]TdR uptake.

Tumor necrosis factor α (TNF-α) production[4,5] The PMØ (1 mL, 2×10^6 cells/well) was cultured with LPS (10 mg L^{-1}). Morphine ($1 \times 10^{-8} - 1 \times 10^{-3}$ mol L^{-1}) with or without naloxone (50 μmol L^{-1}) was added in 24-well plate at 37 ℃ in a 5 % CO_2 incubator for 6 h, each sample duplicated. After incubation, all the supernatants containing TNF-α were collected by centrifugation ($600 \times g$, 10 min). The supernatants were stored at −25 ℃ until assay.

TNF-α assay The TNF-α activity was determined by the cytotoxocity assay against L929 cells. L929 cells (100 μL, 5×10^4 cells/well) were seeded into the 96-well plate and incubated at 37 ℃ in 5 % CO_2 atmosphere for 24 h. Culture medium was removed. TNF-α supernatant (100 μL) and RPMI 1640 medium containing Dac (final concentration 1 mg L^{-1}) were added. The plates were reincubated for 20 h. The cells were stained with 0.5 % crystal violet (containing 3.2 % formaldehyde isotonic solution) for 15 min. The plates were rinsed and dried. The residue was dissolved in 47.5 % ethanol (100 μL/well) which was then measured at 570 nm on an ELISA autoreader. TNF-α activity was calculated: Cytotoxicity (%) = (Control − Test)/Control × 100 %.

Effect of morphine on L929 cell activity As TNF-α assay, L929 cells (100 μL, 5×10^4 cells/well) were seeded into the 96-well plate. After 24-h incubation at 37 ℃ in 5 % CO_2 atmosphere, Dac (final concentration 1 mg L^{-1}) and morphine ($1 \times 10^{-8} - 1 \times 10^{-4}$ mol L^{-1}) were added. L929 cells activity was measured by ELISA autoreader after 20-h reincubation.

RESULTS

Morphine ($1 \times 10^{-8} - 1 \times 10^{-3}$ mol L^{-1}) inhibited IL-1 production from PMØ. Naloxone (50 μmol L^{-1}) also inhibited it and did not block the inhibitary effect of morphine (Tab 1).

Morphine had no influence on TNF-α production of PMØ at low concentration ($1 \times 10^{-8} - 1 \times 10^{-5}$ mol L^{-1}) while 1 mmol L^{-1} inhibited TNF-α production obviously and was not blocked by naloxone (50 μmol L^{-1}). Moreover, morphine $1 \times 10^{-8} - 1 \times 10^{-4}$ mol L^{-1} had no influence on L929 activity (Tab 1).

DISCUSSION

The present study demonstrated that *in vitro* morphine ($1 \times 10^{-8} - 1 \times 10^{-3}$ mol L^{-1}) inhibited IL-1 production, and morphine 1

Tab 1. Effects of morphine on L929 cell activity (n=4 wells/sample). LPS (6.25 mg L^{-1})-induced IL-1 activity (dpm/2×10^4 thymocytes) and TNF-α production (L929 cell specific lysis %) of peritoneal macrophages with or without naloxone (50 μmol L^{-1}). n=3 wells for 1 homogenate (pooled from 10 mice), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control group.

Morphine/ mol L^{-1}	L929 cell activity $A_{570\,nm}$	IL-1 activity/10^{-4} dpm −naloxone	IL-1 activity/10^{-4} dpm +naloxone	TNF-α activity/specific lysis % −naloxone	TNF-α activity/specific lysis % +naloxone
0	0.53±0.06	7.0±1.2	1.9±0.5	53.8±2.6	51.3±2.6
10^{-8}	0.53±0.10^a	3.6±0.8^b	1.8±0.5^a	53.8±2.6^a	48.7±2.6^a
10^{-7}	0.53±0.13^a	3.4±0.4^c	1.7±0.1^a	59.0±2.6^a	51.3±2.6^a
10^{-6}	0.54±0.04^a	3.1±1.2^b	2.1±0.5^a	56.4±2.6^a	46.1±7.7^a
10^{-5}	0.50±0.02^a	3.2±0.7^c	2.4±0.4^a	55.1±2.6^a	46.1±2.6^a
10^{-4}	0.55±0.06^a	—	—	—	—
10^{-3}	—	1.8±0.2^c	1.3±0.4^a	23.1±7.7^c	7.7±5.1^c

mmol L^{-1} also inhibited the TNF-α production from mouse PMØ induced by LPS. Since IL-1 and TNF-α are important cytokines produced by PMØ and play key roles in immune responses[5,7], our results suggested that the direct inhibitory effect of morphine on the function of macrophages might be one of the mechanisms for the increase of incidence of infection in morphine addicts.

Our previous work (to be published) had demonstrated that the effects of morphine on Con A-induced T-lymphocyte proliferation were blocked by naloxone (50 μmol L^{-1}), a μ opioid receptor antagonist. Now the inhibitory effects of morphine on the IL-1 and TNF-α production of PMØ were not antagonized by naloxone 50 μmol L^{-1}, which alone inhibited IL-1 production. This suggested that unlike the effect on T-lymphocyte, the inhibitory effect of morphine on PMØ was not mediated by opioid receptor.

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吗啡对腹腔巨噬细胞产生白细胞介素-1及肿瘤坏死因子α的体外影响

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目的：研究吗啡对腹腔巨噬细胞产生细胞因子的体外作用. **方法**：通过测定胸腺细胞的增殖及L929细胞的杀伤率观察存在或不存在纳洛酮的条件下不同浓度的吗啡对腹腔巨噬细胞产生IL-1及TNF-α的影响. **结果**：吗啡($1\times10^{-8}-1\times10^{-3}$ mol L^{-1})及纳洛酮(50 μmol L^{-1})都能抑制由巯基乙酸钠启动的腹腔巨噬细胞产生IL-1，但纳洛酮不能阻断吗啡的上述抑制作用. 对腹腔巨噬细胞TNF-α的产生，只有高浓度的吗啡(1 mmol L^{-1})具有抑制作用，也不能被纳洛酮阻断. **结论**：吗啡能直接抑制腹腔巨噬细胞的功能，而这种作用不是由腹腔巨噬细胞上阿片受体介导的.

关键词 吗啡；纳洛酮；白细胞介素-1；肿瘤坏死因子