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Morphine inhibited respiratory burst of neutrophils and scavenged oxygen free radicals

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AIM: To study the effects of morphine on active oxygen free radicals. **METHOD**: Chemiluminescence method was used to measure (a) active oxygen generation induced by respiratory burst of neutrophils from human blood stimulated with phorbol myristate acetate (PMA), (b) Superoxide anion (O_2^-) induced by xanthine-xanthine oxidase system, (c) hydroxyl radical (·OH) generated by ascorbic acid (AA)-Cu^{2+}-zymosan, and (d) the release of H_2O_2. **RESULTS**: The (a), (b), (c), and (d) were scavenged by morphine and their median inhibitory concentrations (IC_{50} and 95 % confidence limits) were 21.1 (13.0—34.0), 54.1 (50.0—58.5), 224.0 (128.2—390.8), and 66.9 (62.9—71.0), nmol L^{-1}, respectively. **CONCLUSION**: The immunosuppressant effect of morphine was related to its free radicals scavenging action.

KEY WORDS chemiluminescence; neutrophils; morphine; oxygen; free radicals

Morphine and its analogue abuse in humans and animals are associated with immunological changes, such as reduction of reticuloendothelial activity and phagocytic count, and induced exacerbation of infection in mice. Morphine suppressed natural killer cell activity, and caused thymic hypoplasia, and directly suppressed the generation of antibody-forming cells (AFC) *in vitro*[1-4]. Active oxygen free radicals might play a part in the functional activity of vairous immunocytes[5]. We raised the possibility that the immunosuppressant effect of morphine was related to its free radicals scavenging action. The present study was undertaken to examine by chemiluminescence method the effects of morphine on (a) the respiratory burst of neutrophils stimulated with phorbol myristate acetate (PMA), (b) superoxide generated by xanthine oxidase system, (c) hydroxyl radical produced by ascorbic acid (AA)-Cu^{2+} zymosan system, and (d) release of hydrogen peroxide.

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MATERIALS AND METHODS

Drugs Morphine hydrochloride (Mor, Shanghai Pharmceutical lot № 890212), fresh adult blood (Guangzhou Blood Bank); Luminol, PMA, xanthine, xanthine oxidase, zymosan, HEPES, glucan T500, and Ficoll-hypaqueq were purchased from Sigma Chemical Co, USA. Hydrogen peroxide 30 %, ascorbic acid (AA, Shanghai Chemical Reagent Co). Other reagents were of AR grade.

Apparatus FT662 Biochemistry luminometer (Beijing Nuclear Apparatus Factory), Z360K refrigerated centrifuge (Hermile, Germany).

Preparation of neutrophils[6] Human blood from healthy adult donors was anticoagulated with heparin (10 kU L^{-1}). The leukocytes were separated by mixing 10 mL of blood with 5 mL of glucan T500 4 % in saline. Red blood cells (RBC) were removed by lysis in NH_4Cl 0.9 %. Suspension containing approximately neutrophils 95 % was obtained from leukocytes suspension by standard with Hanks' balance saline solution (HBSS) and resuspended in HBSS buffer, pH 7.4, and heat-inactivated fetal calf serum 5 %. After a cell count, the neutrophils suspension (5×10^{10} L^{-1}) was kept on crushed ice.

Respiratory burst of neutrophils and chemilumi-

nescence[7]. The reaction mixtures included 0.5 mL HBSS supplemented with test drugs to which 0.9 mL of luminol (0.1 mmol L^{-1} in Me_2SO), 0.5 mL of neutrophils suspension (2×10^4 L^{-1}) were added. The reaction was initiated by the addition of 50 μL of PMA (1.25 mg L^{-1}). The resulting light output was recorded as mV and as counts per second calculus. The reaction temperature was maintained thermostatically at 37 ℃.

Determination of superoxide[8] The superoxide generation was induced by a mixture containing 0.8 mL of xanthine (0.02 mmol L^{-1}) and 50 μL of luminol (0.1 mmol L^{-1}). The reaction was initiated by the addition of 50 μL xanthine oxidase (7500 units L^{-1}) in a final volume of 1.0 mL PBS (pH 7.8) at 37 ℃. Maximal generation of superoxide was recorded by luminometer as mV and as counts per 6 s.

Generation of hydroxyl radicals[9] Reaction mixtures contained, in final volume of 3.2 mL, 0.2 mL of ascorbic acid 1.8 mmol L^{-1}, 0.4 mL of $CuSO_4$ 1.8 mmol L^{-1}, 0.2 mL of zymosan 7.5 g L^{-1}, 0.6 mL of PBS, pH 6.2. Following incubation at 25 ℃ for 10 min in a shaking water bath, the reaction was initiated by the addition of 0.6 mL of H_2O_2 33.3 mmol L^{-1}. The maximal generation of hydroxyl radicals was recorded by lumiometer as mV and as counts per 6 s.

Measurment of hydrogen peroxide[10] The release of H_2O_2 was determined, in reaction mixtures of 0.2 mL PBS (pH 8.0), 0.1 mL of H_2O_2 0.18 % solution, after incubating 3 min at 37 ℃, in addition of 0.1 mL of luminol (0.2 mmol L^{-1}), by luminometer as mV and as counts per 6 s.

Statistics Results were expressed as $\bar{x} \pm s$, and statistical significance of the differences between means was analyzed by two-tail t test. The IC_{50} values for drugs were determined by weighted probit analysis.

RESULTS

Inhibition of respiratory burst of neutrophils Mor inhibited the chemiluminescence of neutrophils stimulated with PMA was in a concentration-dependent manner (Tab 1). The IC_{50} value of Mor at peak (8 min) of chemiluminescence was 21.1 nmol L^{-1} (its 95 % confidence limits were 13.0—34.0 nmol L^{-1}).

Scavenging effect on superoxide The chemiluminescence produced by superoxide in xanthine-xanthine oxidase releasing system was inhibited in a concentration-dependent manner by Mor (Tab 2). Its IC_{50} was 54.1 (50.0—58.5) nmol L^{-1}.

Scavenging effects on hydroxyl radicals The chemiluminescence generation induced by AA-Cu^{2+}-zymosan system was inhibited by Mor in a concentration-dependent manner (Tab 2). The IC_{50} was 224.0 (128.2—390.8) nmol L^{-1}.

Tab 1. Inhibitory effects of morphine on respiratory burst of neutrophils. $n=6$, $\bar{x} \pm s$. [a] $P>0.05$, [b] $P<0.05$, [c] $P<0.01$ *vs* control.

	4 min	6 min	8 min	12 min	20 min
	Intensity of chemiluminescence/mV				
Control	84.2±13.2	221.0±8.9	364.5±20.4	136.0±13.1	34.5±4.9
Morphine (nmol L^{-1})					
33	50.5±4.7[c]	127.0±12.4[c]	186.2±24.3[c]	112.6±13.8[a]	21.2±4.6[b]
67	45.7±3.5[c]	97.4±5.0[c]	130.2±12.4[c]	65.2±8.2[b]	16.7±2.4[c]
133	22.7±6.4[c]	63.4±3.0[c]	106.5±11.3[c]	91.3±12.2[b]	12.3±1.8[c]
266	14.0±2.3[c]	32.6±6.2[c]	45.0±8.2[c]	30.0±6.1[c]	0
SOD (kU L^{-1})					
100	32.4±6.9[c]	93.7±12.6[c]	136.8±11.7[c]	96.3±7.5[b]	21.3±3.6[c]
200	18.2±1.8[c]	64.6±8.2[c]	117.2±11.7[c]	82.5±8.2[b]	17.5±2.7[c]

Tab 2. Scavenging effects of morphine on oxygen free radicals, $n=6$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

	Intensity of chemiluminesence (mV) (Rate of inhibition, %)		
	O_2^-	-OH	H_2O_2
Control	79.2±6.1	74.8±1.0	116.4±8.9
Morphine (nmol L^{-1})			
33	49.5±3.4^b(37.5)	66.7±3.4^a(10.8)	79.9±6.3^c(31.2)
67	34.2±2.9^b(56.8)	60.8±4.5^b(18.9)	67.9±5.1^c(41.7)
133	23.2±17.6^b(70.7)	50.2±2.4^c(32.9)	34.9±4.3^c(70.0)
266	12.3±0.9^c(84.5)	32.4±4.8^c(56.7)	25.5±2.7^c(78.1)
SOD (kU L^{-1})			
100	42.3±6.8^b(46.6)	61.0±1.9^b(18.3)	74.2±8.5^c(36.0)
200	28.4±7.2^b(64.4)	59.7±1.8^b(20)	36.7±6.2^c(68.4)

Effects on release of H_2O_2 Mor showed inhibitory effects on chemiluminescence induced by release of hydrogen peroxide (Tab 2). The IC_{50} was 66.9 (62.9 — 71.0) nmol L^{-1}.

DISCUSSION

Oxygen free radicals produced by the major immunocytes-neutrophils could be quickly detected by chemiluminescence opsonified with PMA. A high level of oxygen free radicals at local site selectively destroyed suppressor-effector T-lymphocytes, took part in local modulation of some bioactive substances, and was important related to process of immunoresponse[11]. The present study revealed that Mor had an inhibitory effect on respiratory burst of neutrophils. This probably involved in the mechanism of immunodepressive effect of Mor. Opiates receptors were reported to exist in normal blood T-lymphocytes and granulocytes and monocytes[12]. We wonder whether Mor binding with its membrane receptors of neutrophils directly triggered the inhibitory effect or Mor ought this effect to its scavenging action on oxygen free radicals generation induced by the activation of immunocytes. To test this assumption, we had further examined the effects of Mor on superoxide, hydrogen peroxide and hydroxyl radicals generation induced by various non cellular systems with chemiluminescence method. The results demonstrated that Mor significantly scavenged these oxygen free radicals in a dose-dependent manner. Because no experiment regarding direct interrelation between free radicals scavenging action of Mor and immune function was done and, moreover, the possible involvement of opiate receptor in the process remains to be elucidated, our present results just have provided some clues for exploring the mechanism of immunosuppressive action of Mor.

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445-448 16

吗啡抑制嗜中性白细胞呼吸暴发并清除氧自由基

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目的：研究吗啡对活性氧自由基的影响. **方法**：用化学发光法检测. (a)人血嗜中性白细胞受佛波醇刺激产生呼吸爆发的活性氧. (b) 黄嘌呤-黄嘌呤氧化酶体系产生的 $O_2^{\cdot}$；(c) AA-Cu^{2+}-酵母多糖产生的·OH；(d) 过氧化氢的释放反应. **结果**：吗啡清除 (a)，(b)，(c)，(d) 所产生的氧自由基，其 IC_{50}值(nmol L^{-1}) 分别为 21.1 (13.0—34.0)，54.1 (50.0—58.5)，224.0 (128.2—390.8)，和66.9 (62.9—71.0). **结论**：吗啡的氧自由基清除作用与吗啡的免疫抑制作用有关.

关键词 化学发光；嗜中性白细胞；吗啡类；氧；自由基