·论　著·

Low-dose pethidine inhibits acute cigarette smoking-induced leukocyte and nitric oxide elevation in lung in guinea pigs

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ABSTRACT: OBJECTIVE To investigate the delayed responses after acute smoking, we observed leukocyte and nitric oxide (NO) in BAL fluid in guinea pigs; and to further investigate the inhibitory effect of low-dose pethidine against the delayed response. **METHOD** Cell counts were determined in BAL fluid after inhalation six volumes of cigarette smoke (60mL, 75% concentration) successively via the smoking device; and the concentration of NO_2^- / NO_3^- was also measured to reflect the level of NO with biochemical method. The inhibitory effect against the above responses of pethidine was investigated in pethidine pre-treated group. **RESULTS** The total cell numbers, the percentage of neutrophils and the concentration of NO_2^- / NO_3^- in BAL fluid were increased significantly after smoke exposure. Pethidine 0.1 mg·kg^{-1} largely inhibited these responses. **CONCLUSION** A delayed neurogenic inflammation was observed in airways and parenchymal two hours after smoke exposure; and low-dose of pethidine significantly inhibit this delayed lung injury.

KEY WORDS: pethidine; smoking; airways; inflammation; nitric oxide; guinea pig

低剂量哌替啶抑制豚鼠吸烟所致肺内白细胞和 NO水平增加

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摘要:目的　以支气管肺泡灌洗液(BALF)中 NO水平和白细胞为指标,观察豚鼠定量吸烟后肺、气道的迟发性反应;以及很低剂量的哌替啶对这些反应的抑制作用。方法　经吸烟装置,豚鼠自主吸入 60mL香烟烟雾(75%浓度)/次,共 6次,每次间隔 20min。2h后检测 BALF中白细胞数及其分类,以及 BALF中 NO_2^- / NO_3^-的浓度,以反映气道内 NO水平。同时在药物组中观察很低剂量哌替啶对上述反应的抑制作用。结果　豚鼠定量吸烟 2h后,BALF中细胞数、中性粒细胞百分比和 NO_2^- / NO_3^-水平显著增高;哌替啶 0.1mg·kg^{-1}能明显抑制上述反应。结论　多次吸烟 2h后,豚鼠肺、气道出现神经源性的迟发性炎症反应,很低剂量的哌替啶有效地抑制豚鼠吸烟产生的迟发性炎症反应。

关键词:哌替啶;吸烟;气道;炎症;一氧化氮;豚鼠
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Cigarette smoking has been implicated in many disorders. The impairment will be more prominent in airways because the lung is the primary target organ responding to cigarette smoke. However, till now, few have been done about the effect of smoking on airways and the related diseases preventions. Investigators have focused on the effect of smoking on chronic lung injury in past years. However, the acute effect of cigarette smoking on airways has attracted less attention.

Investigators have suggested that cigarette smoke exposure stimulates the afferent sensory nerve in airways to trigger the release of tachykinins directly from the fiber endings and further to increase the release of acetycholine (Ach) from the cholinergic nerves, leading to evoke the initial neurogenic response in airways[1,2]. We had also observed this initial responses to acute smoking, i.e. smoke-induced neurogenic reactions such as bronchoconstriction and plasma exudation in airways. In addition to

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the initial neurogenic response, smoke exposure also provokes subsequent lung injury both in airways and parenchymal. And the initial neurogenic response may contribute to the development of the subsequent lung injury, i.e. delayed lung injury[3].

Inflammatory cell infiltration and abnormity of NO release is believed to contribute to inflammatory processes in airways. However, there is still controversy about the effect of smoking on NO release. The association between acute smoking and the synthesis and release of NO has not been reported.

The opioid-receptor agonists (such as morphine, pethidine) have been considered to elevate airway tone and forbidden to be used in patients suffering pulmonary diseases such as asthma. However, recent studies found that very low-dose opioid-receptor agonists wouldn't elevate airway tone, actually they exhibit to reduce airway tone and inhibit airway neurogenic inflammation. The inhibitory effect of low-dose opioid-receptor agonists is via activation of prejunctional opioids receptors existed on airway sensory nerve endings, thereby inhibit sensory neuropeptide to release[4~7]. Consistent with these reports, in a previous study, we also demonstrated that pethidine 0.01 ~ 1 mg·kg^{-1} significantly reduced bronchoconstriction and plasma exudation, the initial airway responses after smoke exposure (data not shown).

Thus, based on these observations, the aim of this study is to: ① clarify the process of smoke-induced delayed response several hours after smoke exposure and to better understand the pathophysiologic processes of smoke-induced neurogenic reactions in airways through observing the leukocyte and NO in BAL fluid in guinea pigs in vivo; ② further investigate whether low-dose of pethidine can reduce the cigarette smoke-induced delayed lung injury.

1 Materials

1.1 Animals

Hartley guinea pigs of either sex (body weight (359 ±35) g) were purchased from Laboratory Animal Center of Medical School of Zhejiang University.

1.2 Drugs and Reagents

Pethidine (meperidine, Shenyang First Pharmaceutical Factory); nitric oxide reagent (Nanjing Jiancheng Bioengineering Institute); urethane (Shanghai Chemical Reagent Company); Wright-Giemsa dye (Nanjing Jiancheng Bioengineering Institute).

1.3 Cigarettes

The cigarettes we used herein are the series 4R1 research cigarettes, 8.4cm length, containing 2.45mg of nicotine and 35.3mg of tar per cigarette, produced by the Tobacco Research Institute, University of Kentucky, USA.

1.4 Apparatus

Smoking device (made by ourselves)[4]; Eppendorf Centrifuge 5804R (Germany); Olympus BX51 (Japan); BIO-TEK (ELX800, USA); SHZ-88(Jiangsu Laboratory Apparatus Factory).

2 Methods

2.1 Exposure to cigarette smoke

Animals were randomly divided into three groups: unexposed control, smoke-exposed group and pethidine (0.1mg·kg^{-1}) pretreated group. Methods of generating and delivering cigarette smoke were the same as those described in a previous report[4]. Guinea pigs were anesthetized with intraperitoneal injection of urethane (2g·kg^{-1}) and laid supine. Then the animals were intubated through a tracheostomy and a catheter was introduced into the jugular vein to administer drugs. Fifteen min later the animals were exposed to 60mL of cigarette smoke (75% concentration) for 3min under normal atmospheric pressure, followed by 20min of air exposure. This procedure was repeated for six times. Thus, the animals were successively exposed to a total of 360mL of cigarette smoke during 2hours. The unexposed control animals were exposed to room air through the smoking device for the same period of cigarette smoke exposure. Based on the previous results, pethidine (0.1mg·kg^{-1}) was administered intravenously and 10min later the animals were exposed to cigarette smoke the same as the smoke-exposed group.

2.2 Bronchoalveolar Lavage

Immediately after total smoke exposure, the animals were killed. A total of 5mL of cold sterile saline was instilled into the right lung through the right bronchus in three times and aspirated by gentle syringe suction. A total of 4.5mL of injected saline was recovered. Total cell counts were determined under light microscopy. Then the lavage was pooled and centrifuged at 2000r/min for 15min at 4℃. The cell-free supernatant was collected and stored at -28℃ until used. Differential profiles were determined by Wright-Giemsa staining after the cytospin preparation.

2.3 Measurement of nitric oxide

The NO is short life and rapidly convert to NO_2^- or NO_3^- in tissue. Thus we measure the concentration of NO_2^- / NO_3^- to reflect the level of NO. We measured the samples with a nitric oxide reagent according to the manufacture's specifications.

2.4 Statistics

The data were analyzed statistically using the One way ANOVA and unpaired *t*-test with sigma stat software; *p* values less than 0.05 were considered as statistically significant; data are expressed as mean ±SD.

3 Results

3.1 Cell counts in BAL fluid

There was a significant increase in total cell numbers recovered in BAL fluid after cigarette smoke exposure. The smoke-exposed group contained approximately twice as many cells com-

pared to the unexposed control (Tab 1).

Differential cell counts was determined in this study. The cells in BAL fluid were mostly macrophages. Cigarette smoke exposure induced a significant increase in the percentage of neutrophils. The percentage was two times higher than that of the unexposed control. The percentage of macrophages and lymphocytes showed no significant differences among these groups.

A significant increase in leukocyte was noted due to acute effect of cigarette smoke exposure (mainly alveolar macrophages, Tab 1).

3.2 NO_2^- / NO_3^- concentration in BAL fluid

We measured the concentration of NO_2^- / NO_3^- in BAL fluid to reflect the level of NO. The concentration of NO_2^- / NO_3^- was elevated significantly from (8.33 ±1.56) μmol• L^{-1} to (13.29 ±3.01) μmol• L^{-1} two hours after smoke inhalation (Fig 1).

3.3 Effect of low-dose pethidine

In animals pre-treated with pethidine 0.1 mg• kg^{-1} intravenously contained significantly less cells recovered in BAL fluid as compared to the smoke-exposed but untreated animals. The percentage of neutrophils was also decreased with pethidine pretreatment. The infiltration of inflammatory cells in airways was significantly alleviated (Tab 1).

Pethidine also significantly attenuated the elevation of NO_2^- / NO_3^- from (13.29 ±3.01) μmol• L^{-1} to (8.57 ±2.11) μmol• L^{-1} in BALF induced by acute effect of cigarette smoke exposure (Fig 1).

Tab 1 The acute effect of cigarette smoke exposure on cellular profile in BAL fluid and the inhibitory effect of low-dose pethidine (0.1 mg• kg^{-1})

	Total cell numbers and differential cell counts		
	control (n=7)	cs (n=8)	pethidine + cs (n=8)
Total cell numbers (10^7 /L)	97.96 ±9.57	180.88 ±14.63[2)]	113.48 ±5.82[2,4)]
Macrophages (10^7 /L)	75.38 ±5.93	143.35 ±12.85[2)]	94.08 ±6.82[2,4)]
(%)	78.81 ±6.63	78.25 ±4.97	82.05 ±4.11
Lymphocytes (10^7 /L)	15.99 ±6.25	22.44 ±7.69	15.92 ±1.66
(%)	16.56 ±5.75	12.10 ±3.81	13.75 ±1.54
Neutrophils (10^7 /L)	1.85 ±0.38	9.41 ±3.51[2)]	2.62 ±1.29[4)]
(%)	1.94 ±0.43	4.70 ±1.08[2)]	2.30 ±1.19[3)]

Note: [1)] $P<0.05$, [2)] $P<0.01$ vs control; [3)] $P<0.05$, [4)] $P<0.01$ vs cs group; cs = cigarette smoke exposure

4 Discussion

In the present study, a marked increase in leukocyte (mainly alveolar macrophages) in BAL fluid was observed, and percentage of neutrophils and the concentration of NO was also increased significantly in response to the acute effect of cigarette smoke exposure. Pre-treatment with pethidine 0.1 mg• kg^{-1} before smoke challenge largely reduced the above injurious responses. Studies have reported that the inhibition on smoke-induced neurogenic responses by opioid-receptor agonists was via activation of opioid receptors existed on airway sensory nerve endings, thereby inhibit neurotransmission, for the opioid-receptor antagonist naloxone abolished this inhibition[5]. Thus, from these observations we can deduce that the early-released mediators such as tachykinins and Ach play an important role in producing the smoke-induced delayed lung injury. These mediators may increase the influx of inflammatory cells, and as proinflammatory mediators to activate the inflammatory cells to release a lot of mediators and oxygen radicals, thereby induce further cellular reactions and enhance the ongoing lung injury.

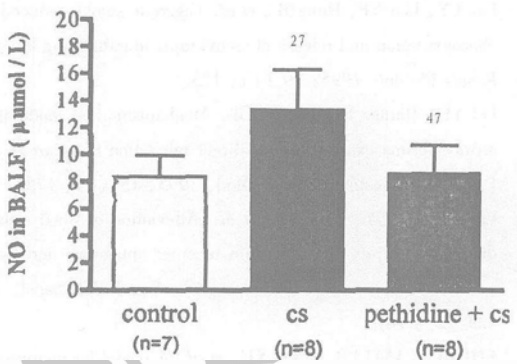

Fig 1 The acute effect of cigarette smoke exposure on the concentration of NO_2^- / NO_3^- in BAL fluid and the inhibitory effect of low-dose pethidine (0.1 mg• kg^{-1})

[1)] $P<0.05$, [2)] $P<0.01$ vs control; [3)] $P<0.05$, [4)] $P<0.01$ vs cs group; cs = cigarette smoke exposure

Pethidine is a μ-opioid-receptor agonist. In this study, the dose of pethidine used herein has previously been shown to inhibit initial neurogenic inflammation in vivo in guinea pigs. We found that pethidine 0.1 mg• kg^{-1} largely reduced the accumulation of inflammatory cells and elevation of NO in BAL fluid after smoke exposure. These findings confirmed the inhibitory effect of pethidine against the neurogenic responses induced by smoke exposure and broaden the recognition of the effect of pethidine on airways. We found that pethidine 0.1 mg• kg^{-1} could not completely abolish the acute lung injury induced by smoke exposure. In addition to sensory irritation, we believed that there existed other chemical processes in airways to inhaled smoke, such as oxidative reaction. The opioids could only inhibit the sensory irritation and have no effect against other chemical reactions. Otherwise, pethidine 10 mg• kg^{-1} failed to reduce the initial smoke-induced neurogenic responses (data not shown).

NO is known to play a prominent role in several pathophysiological conditions, especially in airway inflammations[8,9]. A marked elevation of NO in BAL fluid after smoking was observed in our study and this result suggested that the initial smoke-induced neurogenic response provokes the subsequent, NO-participated inflammatory reaction. The partial inhibition of the release of NO may alleviate this response. The deleterious effect of NO

appears to be caused by its ability to form peroxynitrite and nitrate tyrosine residues in airways[10].

Taken together, our study ① demonstrated a delayed inflammatory response evidenced by increased infiltration of inflammatory cells (mainly alveolar macrophages) and elevation of NO in airways after acute smoking; ② Low-dose pethidine could actually inhibit the delayed lung injury due to sensory irritation after acute smoking.

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