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# Effect of melatonin on reactive oxygen species in rats with asthma

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**Abstract: Objective** Asthma is recognized as a chronic airway inflammatory disease. Reactive oxygen species can induce airway inflammation. The aim of this study was to explore the effect of melatonin (MT) on the content of reactive oxygen species and airway inflammation in rats with bronchial asthma. **Methods** Twenty2four Sprague2Dawley (SD) rats were randomly assigned into 3 experimental groups (8 in each): 1) Asthma group: the rats were immunized on day 1 by intraperitoneal injection of 100 mg ovalbumin (OVA) in 1 ml saline with 100 mg of aluminum hydroxide. After 14 days, the rats were challenged with aerosolized 1% OVA for 20 mins per day for 7 consecutive days; 2) MT group: OVA2sensitized rats were given intraperitoneal injection of 10 mg/kg MT 30 mins before each OVA challenge; and 3) Control group: OVA was replaced with normal saline. Airway responsiveness to aerosolized acetylcholine was detected 6 hrs after the last challenge. Then the rats were lavaged and total and differentiated leukocytes counts in bronchoalveolar lavage fluid (BALF) were performed after Wright2Giemsa staining. At the same time, the content of reactive oxygen species (ROS) in the lung tissues was assessed with chemical colorimetry. **Results** After OVA challenge, there was a significant decrease in airway responsiveness and the number of lymphocytes and eosinophils in the BALF of the MT group compared with the Asthma group ($P < 0.05$). The MT group also showed a significantly lower ROS level in the lung tissues compared with Asthma group ($P < 0.05$). **Conclusions** MT can decrease airway inflammation and the content of ROS in asthmatic rats, which may be the underlying protective mechanisms of MT against asthma.

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**Key words:** Melatonin; Asthma; Reactive oxygen species; Rat

## 褪黑素对哮喘大鼠肺组织活性氧产生的影响

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[**摘 要**] **目的** 支气管哮喘是慢性气道炎症性疾病,肺组织活性氧产生可以导致气道炎症,本研究目的探讨褪黑素(MT)对哮喘模型大鼠肺组织活性氧(ROS)生成以及气道炎症的影响。**方法** 将 24 只大鼠随机分为 3 组:哮喘组($n = 8$):用 10%鸡卵白蛋白(OVA)1 ml、氢氧化铝凝胶 100 mg 无菌腹腔注入,2 周后用 1% OVA 超声雾化吸入 20 min,连续激发 1 周致其哮喘发作;MT 组($n = 8$):模型制作同哮喘组,在每次激发前 30 min 腹腔注入 MT 10 mg/kg;对照组($n = 8$):以生理盐水代替 OVA 吸入。每组分别于最后一次激发后 6 h 测定气道反应性;取支气管肺泡灌洗液(BALF)进行白细胞计数、分类;取肺组织进行活性氧产量测定。**结果** MT 组大鼠气道反应性及 BALF 中炎性细胞数明显低于哮喘组,差异有显著性($P < 0.05$)。肺组织活性氧产量在哮喘组、MT 组及对照组分别为(114.8 ±11.3) U/mgprot、(95.2 ±5.9) U/mgprot 及(87.5 ±7.4) U/mgprot,哮喘组高于其它两组,差异均有显著性($P < 0.05$)。**结论** 哮喘组大鼠肺组织活性氧产量增加。MT 干预可以降低肺组织活性氧产生,降低气道炎症和气道高反应性,这可能是其治疗哮喘的保护机制。 [**中国当代儿科杂志,2004, 6(6): 453 - 455**]

[**关 键 词**] 褪黑激素;哮喘;活性氧;大鼠

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Bronchial asthma (BA) is a chronic airway inflammatory disease with unknown causes. The inflammatory cells, such as eosinophil, lymphocyte, neutrophil, and the produced cytokines and inflammatory mediators, are involved in the pathogenesis of asthma. Reactive oxygen species (ROS) are multifunctional bioactive substances produced by airway inflammatory cells, immunocyte and structural cells. Excessive production of ROS can cause the airway inflammation and high responsiveness[1]. Melatonin (MT), mainly secreted by the pineal gland, is a neuroendocrinal hormone with multibiological functions such as immune regulation and antioxidation[2]. MT can protect the lungs from oxidative damage caused by various oxidants[3]. This study aims to investigate whether MT has protective effect against oxidative damage in rats with asthma.

## Materials and methods

**Establishment of animal model and grouping[4]**

Twenty-six healthy Sprague-Dawley (SD) rats, weighing 120 - 170 g, with the age of 8 - 12 weeks, were randomly assigned into 3 groups ($n$ = 8 each): an Asthma group, a MT group and a Normal control group. The rats in the Asthma group were given intraperitoneal injection of 100 mg ovalbumin (OVA, sigma Company) in 1 ml saline and 100 mg aluminum hydroxide gel. After 14 days, 1 % VOA was administered by ultrasonic aerosol inhalation (type 405 medical fog generator) daily for 7 days, for 20 minutes each time, to induce with asthma attack. The rats in the MT group were induced asthma as the Asthmatic group, but they were intraperitoneally injected with 10 mg/kg MT (Sigma, USA) 30 minutes before each challenge. The control rats were treated as the MT group, except that OVA and MT were replaced by normal saline (NS).

**Determination of airway responsiveness[5]**

Rats of each group were intraperitoneally injected with 7.5 mg/kg diazepam 6 hours after the last challenge, and were then administered with aerosol inhalation of 0.01, 0.1 and 1 μmol/L acetylchloline (ACH) in turn with a 30 minutes interval. Their respiratory rates were recorded. The airway responsiveness was defined as the negative logarithm of the ACH concentration which makes the respiratory rate increase by 30 %.

**Count and classification of inflammatory cells in bronchoalveolar lavage fluid (BALF)**

After an airway responsiveness evaluation, the rats were anesthetized with pentobarbital intraperitoneally. Immediately after endotracheal intubations and decapitation, 5 ml Hanks solution was injected into bronchia 3 times. The retrieved fluid was centrifugated at 1 500 rpm for 10 minutes and the supernatant fluid was discarded. The cells were suspended in 1 ml Hanks solution. The number of cells in 0.1 ml solution were counted under hemocytometer. In order to classify various cells, 0.2 ml solution was smeared and stained with Wright-Giemsa.

**Determination of the content of reactive oxygen species in pulmonary tissue**

After the bronchi had been lavaged, the upper lobe of left lung was quickly removed and washed with iced NS. Then 0.3 g the lobe (wet weight) was taken and homogenized in cooled NS by an electric homogenizer. The supernatant fluid was extracted after centrifuge at 6 000 rpm for 5 minutes at 4 ℃[6]. The content of ROS was measured according to the manufacture's instructions.

**Statistical analysis**

All data were analyzed by SPSS. Data were presented as $x \pm s$. Differences among multiple groups were analyzed by the one-way ANOVA. A $q$ test was performed to analyze the differences between two groups. A $P$ of less than 0.05 was considered significant.

## Results

**Airway responsiveness of various groups**

The airway responsiveness of the Asthma group (7.8 ±0.5) was significantly higher than that of the Control group (6.3 ±0.5) and the MT group (6.6 ± 0.7, both $P < 0.05$). There is no significant difference between the MT and the Control groups.

**Count and classification of inflammatory cells in BALF**

The amount of inflammatory cells in the Asthma

group was significantly higher than that of the Control and the MT groups ( $P < 0.05$) (Table 1).

**The content of ROS in the pulmonary tissues**

The content of ROS in pulmonary tissues of the Asthma group (114.8 ±11.3 U/mg prot) was significantly higher than that of the Control group (87.5 ±7.4 U/mg prot) and the MT group (95.2 ± 5.9 U/mg prot, both $P < 0.05$).

**Table 1** Inflammation cells in the BALF in various groups ( $n = 8$, $x \pm s$, $\times 10^7$/L)

| Group | Amount of total cells | Counting of classified cells | | | |
|---|---|---|---|---|---|
| | | Macrophage | Neutrophil | Lymphocyte | Eosinophil |
| Control | 44.3 ±7.2 | 38.9 ±4.5 | 1.6 ±1.1 | 1.9 ±1.1 | 1.0 ±0.8 |
| Asthma | 78.3 ±12.9[a] | 48.1 ±8.1[a] | 10.4 ±4.1[a] | 7.5 ±2.7[a] | 7.3 ±1.5[a] |
| MT | 64.0 ±10.6[b] | 41.0 ±5.8 | 7.8 ±2.4[a] | 4.4 ±2.2[a,b] | 4.3 ±2.0[a,b] |
| $F$ | 21.27 | 4.75 | 20.25 | 14.39 | 36.48 |
| $P$ | < 0.05 | < 0.05 | < 0.05 | < 0.05 | < 0.05 |

Note: a Compared with the Control group, $P < 0.05$; b Compared with the Asthma group, $P < 0.05$

## Discussion

In this study, the ROS in the pulmonary tissue of the asthmatic rats significantly increased as compared with the control rats, which indicates ROS may be involved in the pathogenesis of bronchial asthma.

The rhythm disorder of MT secretion is related with the pathogenesis of asthma[7]. Recently the antioxidation of MT has drawn much attention. There are several explanations for this effect. One is that MT can catch free radicals or provide or get one electron to relieve the oxidations of free radicals. Another is that MT has high fat solubility, so it can enter the cells, activate GSH-PX, inhibit the activity of NOS, and prohibit the expressions of NF-κB and AP-1[8] to exert an anti-oxidation effect. In this experiment, MT protected the asthmatic rats from the oxidated damages and decreased the airway inflammation and the airway responsiveness. Nevertheless, the underlying mechanism requires further study.

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