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Relationship Between Substance P and Asthma

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Abstract: Objective To study the changes of substance P (SP) content in plasma of asthmatic children and in the blood, bronchoalveolar lavage fluid (BALF) and lung tissues of asthmatic guinea pigs and SP mRNA expression, and to investigate the relationship between SP and asthma of children. **Methods** We determined the contents of plasma SP by the method of immunoassay at the stage of acute attack and clinical remission in 35 children with asthma. On the basis of asthma model of guinea pigs, we examined the contents of SP in the blood, BALF and lungs. The expression of SP mRNA in lungs was examined by RT-PCR. **Results** ①At the stage of acute attack, the level of SP was higher than that of the stage of clinical remission and normal controls ($P < 0.01$). At the stage of clinical remission, the difference of SP contents was not obvious compared with that of controls ($P > 0.05$). ② The SP content of severe asthmatic children was higher than that of mild asthmatic children ($P < 0.05$). ③In asthmatic guinea pig groups, SP contents in the blood, BALF and lungs were higher than those of normal ones ($P < 0.01$), and the expressions of SP mRNA in lungs were higher ($P < 0.01$). In repeatedly attack group, the SP content of guinea pigs was higher than that of asthma group ($P < 0.05$), and the expression of SP mRNA in lungs was also higher ($P < 0.01$). **Conclusions** SP levels increased in asthmatic children and asthmatic guinea pigs, and the expression of SP mRNA was up-regulated in the lungs of asthmatic guinea pigs. They were positive related to the severity of asthma. So SP has close relationship with asthma and may play a role in the mechanism of asthma. **[Chin J Contemp Pediatr, 2003, 5(3): 185-188]**

Key words: Substance P; Asthma; Children; Guinea pig; mRNA

P 物质与哮喘的关系

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[**摘 要**] **目的** 动态研究哮喘患儿血浆及哮喘豚鼠血液、支气管肺泡灌洗液(BALF)和肺组织中 P 物质(SP)含量及 SP mRNA 表达变化,探讨 SP 与小儿哮喘发病机制的关系。**方法** 用放射免疫方法,动态测定 35 例不同严重程度哮喘患儿急性发作期及其临床症状缓解期的血浆 SP 含量变化;并在豚鼠哮喘模型上同期进行了不同严重程度哮喘豚鼠血液、BALF 和肺组织中 SP 含量的对比研究,利用 RT-PCR 方法研究肺组织 SP mRNA 表达变化。**结果** ①哮喘患儿急性发作期血浆 SP 含量较其自身症状缓解期及正常对照组均明显增高,差异有显著性($P < 0.01$);症状航馄谐 ⊃ 19P 含量较正常对照组差异无显著性($P > 0.05$);②重症哮喘急性发作期血浆 SP 含量,较轻、中度哮喘急性发作期的含量显著增高,差异有显著性($P < 0.05$);③哮喘豚鼠血液、BALF 和肺组织中 SP 含量较正常豚鼠均显著增高($P < 0.01$),反复诱喘豚鼠各组织中 SP 含量均显著高于诱喘一次豚鼠($P < 0.05$);④哮喘豚鼠肺组织内 SP mRNA 表达较正常组显著增高,反复诱喘豚鼠 SP mRNA 表达显著高于诱喘一次的豚鼠($P < 0.01$)。**结论** 哮喘患儿及哮喘豚鼠 SP 含量增加,哮喘豚鼠肺组织中 SP mRNA 的表达明显上调,均与病情呈显著正相关。SP 与小儿哮喘关系密切,可能参与了小儿哮喘的发病机制。

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[**关 键 词**] P 物质;哮喘,小儿;豚鼠;mRNA

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Bronchial asthma is a chronic inflammation of airway caused by many cells and cellular elements.

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Recently, neurogenic inflammation in airway which mediated by neuropeptide secreted by sensory nerve is being got attention[1]. Substance P(SP) is an important sensory neuropeptide leading to neurogenic inflammation in airway. There is now convincing evidence that SP is an important neurotransmitters of the non-cholinergic excitatory nerves and has a close relationship with asthma[1,2]. The aim of this study was to explore the relationship between SP and asthmatic children based on clinic and animal model.

Materials and methods

Clinical materials

Thirty-five asthmatic children (18 cases of severe asthma and 17 cases of mild asthma) were enrolled in this study. Their age ranged from 7 months to 12 years old. Among them, 16 cases were less than 3 years old, and the rest were more than 3 years old. All the patients had not received glucocorticoid by vein or oral route 3 days prior to administration. The control subjects (n = 15) were healthy children with no history of asthma, bronchiolitis and family history of asthma.

Animals

The guinea pigs were provided by China Medical University. They were in the same genus, the same age (1.5 - 2.0 months), the same body weight (250 - 275 g) and the same breeding conditions.

Collection and disposal of clinical sample

In asthma group, 4.0 ml blood was collected at the stage of acute attack and clinical remission. Then it was placed into a pre-colded tube containing 40 μl 10 % EDTA and 1 000 kIU Aprotinin. After centrifugation (4 000 rpm) at 4 ℃ for 15 min, the plasma was stored at - 70 ℃. The blood of control group was collected and disposed in the same way.

Detection of plasma SP

The plasma was acidified with 1 % Trifluoroacetic acid(TFA), and centrifugated at 4 ℃ for 20 min. The supernatant was purified by the pre-treated SEP-pak C_{18}. Then it was washed with 1 % TFA (3 ml) twice and 3 ml 60 % acetonitrile (in 1 % TFA) once. Eluent was collected and dried to powder in a lyophilizer. The powder was stored at - 70 ℃ to detect SP by radioimmunoassay.

Animal model

The model was established according to the method discribed by Hutson[3]. The guinea pigs were sensitized by exposure to aerosolized ovalbumin (1 % wt/vol in 0.9 % sterile sodium chloride) for 3 min on the day 1, 4, 7; then the guinea pigs were exposed to aerosolized 2 % ovalbumin for 5 min on the day 21 to provoke asthma. 45 guinea pigs of the same conditions were divided into 3 groups (n = 15 each): ①asthma grioup, the animals were sensitized with the above-mentioned ways; ② repeatedly attack of asthma, in this group the animals were exposed to aerosolized 2 % ovalburmin every other day for 1 week after the first induction of asthma on the day 21; ③control group, the aerosolized ovalbumin was replaced by NS.

Collection of bronchoalveolar lavage fluid(BALF)

A sterilized tube was inserted into trachea 20 - 24 hours after the last exposure to ovalbumin or NS in three groups, then 6 ml NS was perfused slowly for 3 times. After the BALF was drawn out, 1.0 ml BALF was placed into a pre-colded tube containing 1 000 kIU Aprotinin to wait for detection of SP. The blood of guinea pigs was collected from carotid artery and disposed as the clinic sample (see above).

Lung tissues collection

We opened the thoracic cavity of the guinea pigs, removed the lung tissue into a tube without Rnase and stored the tissues in liguid nitrogen.

Detection of SP in lung tissues

The lung tissues was cut into 1 ×1 mm sections and put into 0.5 mol/L glacial acetic acid. They were heated for 10 min in a water-bath box of 95 ℃, then they were homogenized and neutralized with NaOH. After centrifugation at 4 ℃ for 20 min, the supernatant was stored at - 70 ℃ to detect SP. The protein quantity of the lung tissues was detected by the methods of Coomassie Light Blue.

Detection of SP mRNA

Using the method of RT-PCR to detect SP mRNA of lung tissues in all groups. Total RNAs were extracted using TRIZOL reagent. 3 μl total RNA was taken to synthesize the first chain of cDNA. The amplified fragment of SP was 260 bp. The upstream of the primmer (primer A) was 5'-ACCAACACTTCAGAACCCAACC-3', the down-stream

primer B was 5'-AACAGACCGTAGTACCACTCA-3'. The reaction mixture included cDNA 4 μl, dd H_2O 11.8 μl, 5 ×buffer 5 μl, dNTPS 2 μl, TaqE 0.2 μl, primer A and B each 1 μl. The PCR was conducted as follows: 94 ℃3 min →94 ℃40 s →56 ℃1 min →72 ℃1 min →72 ℃5 min. After 32 cycles, it was extended at 72 ℃for 5 min. The TRIZOL was provided by Promega company. TakaRa reverse transcription kit was provided by TakaRa company. The agarose gel electrophoresis was used to detect the amplified SP products. The density of bands was assessed and the amount of SP mRNA was determined according to the ratio to β-actin.

Statistical analysis

Data were expressed as $\bar{x} \pm s$. Statistical analysis was performed with paired samples *t* test or independent samples *t* test by SPSS 10.0 software.

Results

The contents of plasma SP at the stage of attack and clinical remission

The content of plasma SP at the stage of attack was significantly higher than that of clinical remission and control group [(160.41 ±54.04) pg/ml vs (77.57 ±31.16) pg/ml, (160.41 ±54.05) pg/ml vs (78.12 ±35.38) pg/ml] ($P < 0.01$). No significant difference in plasma SP between the clinical remission stage and the control groups was found ($P > 0.05$).

Comparisons of plasma SP in asthma children of different degrees and different stages

The content of plasma SP at the stage of acute attack in severe asthma was significantly higher than that of the mild asthmatic children ($P < 0.05$). At the stage of clinical remission, no significant difference between plasma SP in severe and mild asthmatic children was found. ($P > 0.05$). See Table 1.

Table 1 Comparisons of plasma SP contents in asthmatic children of different degrees and different stages ($\bar{x} \pm s$, pg/ml)

Groups	n	the stage of acute attack	the stage of clinical remission
Mild asthma	17	137.08 ±30.18	68.94 ±26.56
Severe asthma	18	178.05 ±61.97	73.05 ±31.07
t		2.462	0.42
P		< 0.05	> 0.05

The contents of SP in the blood, BALF and lung tissues of different groups

The contents of SP in the blood, BALF and lung tissues in groups of asthma and repeadly attack of asthma were significantly higher than those in control group ($P < 0.01$). In repeatedly attack group, they were significantly higher than those in asthma group ($P < 0.05$). See Table 2.

Table 2 Contents of SP in the blood, BALF and lung tissues of different groups ($\bar{x} \pm s$)

Groups	n	plasma SP (pg/ml)	BALF SP (pg/ml)	lung tissues SP (ng/g)
Control	15	84.12 ±33.57	31.75 ±21.15	42.30 ±11.50
Asthma	14	132.21 ±46.2[a]	89.66 ±39.52[a]	78.3 ±15.60[a]
Repeatedly attack of asthma	13	245.18 ±139.2[a,b]	122.66 ±26.45[a,b]	148.10 ±101.0[a,b]

Note: a vs control group $P < 0.01$; b vs asthma group $P < 0.05$

SP mRNA expression in the lung tissue of guinea pigs

SP mRNA expression in the lung tissue of asthma and repeatedly attack of asthma groups were significantly higher than that in control group (0.99 ± 0.02 vs 0.19 ±0.05, 1.02 ±0.01 vs 0.19 ±0.05), ($P < 0.01$). In repeatedly attack group, it was significantly higher than that in asthma group (1.02 ± 0.01 vs 0.99 ±0.02), ($P < 0.01$). SP mRNA expression in the lung tissue of control group was weak.

Discussion

Bronchial asthma is a kind of chronic inflammation of airways, whose mechanism is complex. Neurogenic inflammation of airways mediated by neuropeptides may have a close relationship with asthma.

SP (11 amino acid) is one of important mediators of neurogenic inflammation in airways, which localizes in unmyelinated sensory nerves (C-fibres) and is known as sensory neuropeptide. It is also termed as tachykinins because of its rapid spasmogenic effect on smooth muscle of airways[1,2]. SP immunoreactive nerves in the airway have been found within the airway epithelium, submucus glands, airway smooth muscle and surrounded blood vessels. There are much evidences that SP has a potent effect on airway neurogenic inflammation, including smooth muscle contraction, vasodilatation and oedema, mucus secretion, inflammatory cell activation[1,2]. SP can increase the expression of adhesion molecules on vascular endothelium and stimulate human T and B lymphocytes, monocytes, eosinophils and fibroblasts. It can induce TNF-α mRNA expression and TNF-α secretion and facilitate histamine release from bronchoalveolar lavage cells in both nonasthma coughers and cough variant asthmatics[4,5,6]. More studies have found that SP in the lung may take an important role in bronchial hyperresponsiveness[7,8].

Our study showed that the content of plasma SP at the stage of acute attack was significantly higher than those at the stage of clinical remission and control group. At the stage of attack, plasma SP of severe asthma children was significantly higher than that of the mild asthmatic children. Our studies showed that the contents of SP in the blood, BALF and lung tissue of asthmatic guinea pigs were significantly higher than those of normal control, and the SP in the group of repeatedly attack was the higest. All these results were accordant with other overseas studies of adult's asthma. Tomaki's study showed that SP content significantly increased in induced sputum from patients with asthma. Where asthma was more severe, SP was more higher. There was a negative relationship between SP content and FEV1/ FVC[9].

Our further studies by the method of RT-PCR showed that the expressions of SP mRNA in the lung tissue of asthmatic guinea pigs were significantly higher than that of control group, and the SP in repeatedly attack group was the highest. These suggested that the SP mRNA expression was up-regulated in asthma, the elevated SP content may be related to the up-regulated SP mRNA.

All these confirmed that SP might have a close relationship with asthma in children and play an important role in the pathogenesis of asthma.

Barnes believed that neurogenic inflammation in airways was involved in the mechanisms and symptoms of asthma, furthermore it may contribute and amplify the inflammation in asthmatic airways[10]. Spina considered that the chronic release of neuropeptides in the airway wall might play an important role in the activation of inflammatory and immune cells which led to reconstruction of airway wall[11]. Thus, further study of relationship between SP and asthma may contribute to develop new drugs that inhibit neurogenic inflammation of airways.

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