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Effect of 1,25-dihydroxyvitamin D3 on mast cells tryptase in asthmatic guinea pigs

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ABSTRACT

Objective: To explore the effect of 1,25-dihydroxyvitamin D3 on the mast cell tryptase (MCT) in asthmatic guinea pigs.

Methods: A total of 60 male or female healthy guinea pigs were randomly divided into control group (group A), asthmatic group (group B), and 1,25-dihydroxyvitamin D3 group (group C), with 20 cases in each group. To establish asthmatic guinea pig models, 1 mL peanut oil was filled into stomach in the morning in group A and group B, and 1 mL peanut oil with 1,25-dihydroxyvitamin D3 was filled into stomach in group C. Airway resistance (Re) of asthmatic guinea pigs was detected, and the bronchoalveolar lavage fluid (BALF) cells were counted. Lung tissue with HE and MCT immunohistochemical staining were used to observe the pathological changes in lung tissue and the distribution of MCT.

Results: After injection of different concentration of acetylcholine chloride, the Re in group B and group C were increased significantly compared with group A ($P < 0.05$); compared with group B, the Re in group C were decreased significantly ($t = -5.385, -5.761, -6.184, -13.574$, $P < 0.05$); the total number of BALF cells and eosinophils were increased significantly in group B and C ($t = 19.618, 9.598, 10.854, 5.388$, $P < 0.05$); compared with group B, the total number of BALF cells and eosinophils in group C was decreased significantly ($t = -5.555, -5.392$, $P < 0.05$); the number of tryptase positive cells in group B was increased significantly than that in group A ($t = 21.312$, $P < 0.05$), and in addition to the alveolar septum and submucosa, the cells were also distributed around blood vessels and outside the cells; the number of tryptase positive cells in group C was decreased significantly compared with group B, and the difference was statistically significant ($t = 5.043$, $P < 0.05$).

Conclusions: After the asthmatic guinea pigs are treated with 1,25-dihydroxyvitamin D3, their BALF, Re, infiltration degree of inflammatory cells in the trachea and lung tissue and airway inflammatory reaction are reduced significantly. 1,25-dihydroxyvitamin D3 has a certain inhibiting effect on the activation of mast cells and the release of MCT granules.

1. Introduction

1,25-dihydroxyvitamin D3 [1,25-$(OH)_2D_3$] is the main active form of vitamin D, which belongs to the steroid hormone. In addition to promoting the absorption of calcium and phosphorus in the mucosa of small intestine and regulating the deposition and release of bone calcium, it can also participate in the differentiation and regulation of the immune system to play its biological activity. Related reports indicate that the immune mechanism of bronchial asthma is regulated by many factors, and the 1,25-$(OH)_2D_3$ can improve the symptoms of chronic airway inflammation in asthma, with a significant negative correlation between them [1–4]. There is close relationship between the type I allergy and the incidence of asthma. The main mechanism is that after antigen stimulates body, specific IgE class antibody is produced and combined with mast cells, making the body sensitized. And as a specific marker of mast cells, the mast cell tryptase (MCT) is the marker of disease

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activation and mediation [5,6]. In the study, we mainly analyzed the pathological changes, airway responsiveness changes and MCT distribution in lung tissue of asthmatic guinea pigs, so as to explore the effect of 1,25-$(OH)_2D_3$ on asthmatic reaction.

2. Materials and methods

2.1. Experimental materials and reagents

60 healthy guinea pigs were randomly divided into control group (group A), asthmatic group (group B), and 1,25-$(OH)_2D_3$ group (group C), with 20 cases in each group. There were 10 males and 10 females in group A, weighing 269 g–358 g with a mean of (304.31 ± 20.12) g; there were 11 males and 9 females in group B, weighing 257 g–360 g with a mean of (305.29 ± 20.64) g; there were 9 males and 11 females in group C, weighing 260 g–354 g with a mean of (308.19 ± 21.03) g. The difference in gender and weight among each group was not statistically significant ($P > 0.05$).

Ovalbumin (OVA) and 1,25-$(OH)_2D_3$ were purchased from American SIGMA Company, acetycholine chloride (Ach) was purchased from Beijing Zhongsheng Ruitai Science and Technology Co. Ltd., and Sumianxin was provided by Third Military Medical University; 402 type ultrasonic atomizer came from Xi'an Yongxing Medical Instrument Co. Ltd.; anti tryptase monoclonal antibody AA1 was purchased from Shanghai Bio-Sun Science and Technology Co. Ltd.; SP immuohistochemistry kit was purchased from Shenzhen Neobioscience Technology Company; 8L sealed perspex box (20 cm × 20 cm × 20 cm) was self-made.

2.2. Methods

2.2.1. Medication

One mL physiological saline was injected into the abdominal cavity in guinea pigs of group A. One mL 10% OVA solution was intraperitoneally injected in 1–15 d and 1% OVA was taken by aerosol inhalation for 30–60 min in 16–30 d. The reaction of guinea pigs in each group was observed. The same process was implemented in guinea pigs of group A, while the drug was replaced by physiological saline. The animal models were successfully established when the typical symptoms of asthma attack appeared in group B and group C, such as quickened and deepened breath, quietness and stillness, and arching back. One mL peanut oil was filled into stomach in the morning in group A and group B, and 1 mL peanut oil with 1,25-$(OH)_2D_3$ was filled into stomach in group C (2.5 $\mu g \cdot kg^{-1} \cdot d^{-1}$), both continuing for 15 d. The materials were drawn 30 days later.

2.2.2. Determination of airway responsiveness

The guinea pigs were anesthetized and fixed on the operation table. After stripping tissue, the internal jugular vein and trachea intubation was respectively conducted. The lung function of guinea pigs was measured using whole body plethysmography, with animal breathing machines assisting ventilation (tidal volume 6 mL/kg, frequency 70/min). The guinea pigs were intravenously injected with physiological saline and observed. The animal basic expiratory airway resistance (Re) was determined as the basic control value. Then Ach was intravenously injected by several times (concentration increasing by times was 40, 80, 160, 320 μg/kg, and next dose was injected after Re reduced to normal). Re changes before and after each drug injection were recorded. Increasing rate of Re = measured value/basic value of Re × 100%.

2.2.3. Classification and counting of bronchoalveolar lavage fluid (BALF) cells

The chest was opened quickly to perform a ligation on the right main bronchus of guinea pigs. A silicone tube was inserted into the bronchus, and the lavage was carried on using a syringe (a total of 2 times, 4–6 mL/time, the recovery rate must be more than 80%). The recovered BALF was centrifuged (10 min, 1 500 r/min). After a dilution of cell residue, the BALF was dropped in the blood cell counting plate for counting. Hematoxylin-eosin staining (HE staining) was used for cell classification.

2.2.4. HE and MCT immunohistochemical staining of lung tissue

Liver puncture was performed according to standard procedure. The tissue in superior lobe of right lung was removed to make sections (4 μm) and then performed with HE and MCT immunohistochemical staining. The pathological changes in lung tissue and the distribution of MCT granules in guinea pigs were recorded.

2.3. Statistical analysis

The statistical data were analyzed with SPSS17.0 software. Measure data were shown as mean ± sd. Multiple sample means were analyzed by single factor analysis of variance, while comparison between two groups by t test. P value <0.05 was considered statistically significantly different.

3. Results

3.1. Contrast of general situation

In group A, no death of guinea pigs appeared in the experimental process, and no abnormality was shown after the aerosol inhalation of physiological saline. In group B, 1 guinea pig was dead because of hypersensitivity. After the aerosol inhalation of OVA, most showed accelerated respiratory rhythm and abdominal respiration. Some could hear wheezing rale. Those with severe symptoms had a fall and convulsive behavior. In group C, 1 guinea pig was dead because of improper intragastric administration. The early symptoms were similar to group B after the aerosol inhalation of OVA, along with asthma reaction. The spirit and diet condition was improved in the later stage after the treatment of 1,25-$(OH)_2D_3$ intragastric administration.

Table 1
Comparison of airway responsiveness among each group of guinea pigs after injection of Ach.

Drugs	Group A (n = 20)	Group B (n = 19)	Group C (n = 19)
Physiological saline	4.97 ± 0.16	5.14 ± 0.21	5.01 ± 0.17
Ach 40 μg/mL	7.09 ± 0.30	9.45 ± 0.76*	8.14 ± 0.68*,△
Ach 80 μg/mL	7.88 ± 0.66	10.70 ± 0.89*	9.12 ± 0.77*,△
Ach 160 μg/mL	8.49 ± 0.80	19.11 ± 1.51*	16.29 ± 1.22*,△
Ach 320 μg/mL	8.69 ± 0.93	32.06 ± 3.38*	20.74 ± 1.75*,△

*$P < 0.05$ compared to group A; △$P < 0.05$ compared to group B.

Table 2
Comparison of classification and counting of BALF cells among each group of guinea pigs.

Group	*n*	Total cell number (× 10^8/L)	Lymphocyte	Monocyte	Neutrophil	Eosinophil
Group A	20	9.11 ± 1.53	0.214 ± 0.027	0.678 ± 0.121	0.053 ± 0.038	0.033 ± 0.025
Group B	19	21.87 ± 2.45*	0.225 ± 0.043	0.626 ± 0.059	0.048 ± 0.024	0.121 ± 0.032*
Group C	19	17.49 ± 2.41*,△	0.258 ± 0.042	0.652 ± 0.066	0.056 ± 0.021	0.070 ± 0.026*,△

*$P < 0.05$ compared to group A; △$P < 0.05$ compared to group B.

3.2. Comparison of airway responsiveness

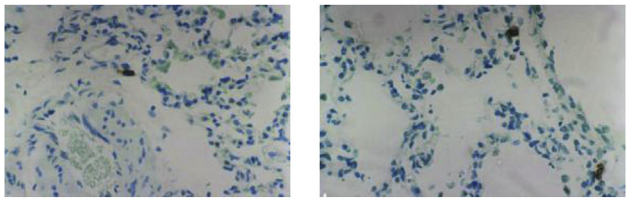
Compared with group A, the Re after injection of each gradient of Ach in group B and group C increased significantly ($P < 0.05$); compared with group B, the Re in group C decreased significantly, and the difference was statistically significant ($t = -5.385, -5.761, -6.184, -13.574$, $P < 0.05$) (Table 1).

3.3. Classification and counting of BALF cells

Compared with group A, the total number of BALF cells and eosinophils raised significantly in group B and group C ($t = 19.618, 9.598, 10.854, 5.388$, $P < 0.05$); compared with group B, the total number of BALF cells and eosinophils in group C decreased significantly, and the difference was statistically significant ($t = -5.555, -5.392$, $P < 0.05$) (Table 2).

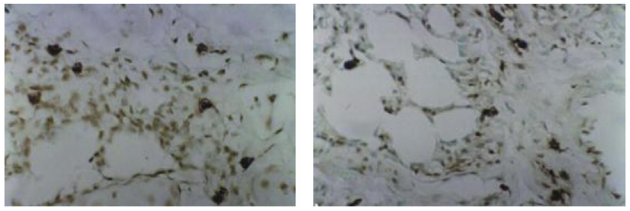
3.4. HE staining of lung tissue

In group B, the bronchial lumen narrowed. A large amount of mucus gathered to form mucus embolus. Some showed epithelial tissues shedding. Lots of inflammatory cells infiltrated in the mucosal tissue, which were mainly eosinophils. The tracheal smooth muscles became hypertrophic, and hyperplastic and hypertrophic muscle cells were observed under a high power microscope. In group C, the nonspecific inflammation of airway mucosa reduced significantly when compared with group B. In group A, the pathological changes above were not observed.



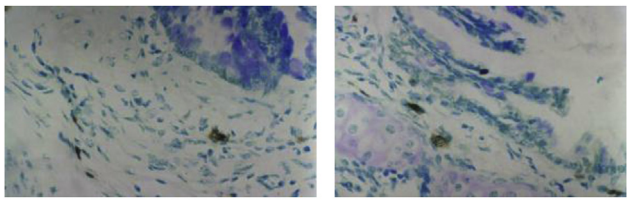
A B

Figure 1. Expression of tryptase positive cells in guinea pigs of group A (×400). A: Alveolar septum; B: Airway submucosa.



A B

Figure 2. Expression of tryptase positive cells in group B (×400). A: Alveolar septum; B: Airway submucosa.



A B

Figure 3. Expression of tryptase positive cells in group C (×400). A: Alveolar septum; B: Airway submucosa.

3.5. MCT immunohistochemical staining of lung tissue

Observed through a high power microscope, the expression of tryptase positive cells in group B and group C was more than that in group A. The cells were mostly distributed in the alveolar septum and under the mucosa in group A (Figure 1). While in group B and group C, in addition to the alveolar septum and submucosa, the cells also distributed around blood vessels and outside the cells (Figures 2 and 3). The number of tryptase positive cells in group B (17.28 ± 2.17) increased significantly than that in group A (3.21 ± 1.89) ($t = 21.312$, $P < 0.05$); the number of tryptase positive cells in group C (13.92 ± 1.93) decreased significantly compared with group B, and the difference was statistically significant ($t = 5.043$, $P < 0.05$).

4. Discussion

Asthma is a chronic airway inflammatory reaction involving a variety of cells and their components, including mast cells and eosinophils. It can easily cause recurrent wheezing, shortness of breath, sense of suppression in the chest and other symptoms. It has a close relationship with airway hyper responsiveness, in which Re mostly increases. In this experiment, the guinea pigs in group B and group C were sensitized by OVA injection and then induced by aerosol inhalation to establish asthma animal models. Asthma reaction and increase of Re appeared in both early stages. And in the later stage of group B, the MCT immunohistochemical staining of lung tissue in guinea pigs showed that the bronchial lumen narrowed, the epithelial tissues swelled and shedded, lots of inflammatory cells like eosinophils infiltrated in the mucosal tissue, and the muscle cells showed hyperplasia and hypertrophy. No pathological changes above were shown in group A, indicating the reliable establishment of asthma models in the experiment.

Clinical researches in recent years show that as an immunomodulator, vitamin D may participate in the etiology of bronchial asthma through a variety of ways [7,8]. Chinellato *et al.* [9] analyzed the cause of children's asthma in Italy through cross sectional study. The results of study shows that the level of 1,25-$(OH)_2D_3$ has a significant negative correlation with the incidence degree of asthma. Goleva E and others [10] found

that in patients with asthma, the less the 1,25-$(OH)_2D_3$ is, the lower the glucocorticoid receptor sensitivity will be, resulting in higher airway responsiveness and thus worse lung function. The study of Jolliffe DA [11] shows that in patients with asthma, after treatment of 1,25-$(OH)_2D_3$, airway inflammation reaction and other pathological changes are improved, and the number of acute attacks and symptoms are both relieved. At the same time, 1,25-$(OH)_2D_3$ can promote the down regulation of Th1 and the up regulation of Th2 in T helper lymphocyte (Th) [12]. It is shown in this experiment that, after the establishment of asthmatic models in group C and treatment of 1,25-$(OH)_2D_3$, the BALF, Re, infiltration degree of inflammatory cells in the trachea and lung tissue of guinea pigs are reduced significantly, thus leading to relief of airway inflammatory reaction in asthmatic guinea pigs.

Tryptase is a neutral serine protease tetramer with structure of active center and conformation of substrate recognition that are similar to trypsin. It shows highly selective expression in mast cells. When the body is sensitized, a large number of β-tryptases are released in the blood as the main products of activated mast cells. It is shown in the study that compared with group A, the number of mast cells and MCT granules increases with statistical significance, which is consistent with foreign related reports [13–15]. And after the treatment of 1,25-$(OH)_2D_3$ in group C, the number of mast cells and MCT granules decreases, which indicates that vitamin D may have a certain inhibiting effect on the activation of mast cells and the release of MCT granules.

In summary, the activation of mast cells is the key to the incidence of asthmatic allergy. After the asthmatic guinea pigs were treated with 1,25-$(OH)_2D_3$, their BALF and airway responsiveness are reduced significantly and the airway inflammatory reaction is alleviated, which has a certain inhibiting effect on the activation of mast cells and the release of MCT granules.

Conflict of interest statement

We declare that we have no conflict of interest.

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