

# The Effect of Adenosine A<sub>2A</sub> and A<sub>2B</sub> Antagonists on Tracheal Responsiveness, Serum Levels of Cytokines and Lung Inflammation in Guinea Pig Model of Asthma

Laleh Pejman<sup>1</sup>, Hasan Omrani<sup>1</sup>, Zahra Mirzamohammadi<sup>1</sup>, Amir Ali Shahbazfar<sup>2</sup>, Majid Khalili<sup>3</sup>, Rana Keyhanmanesh<sup>3\*</sup>

<sup>1</sup> Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

<sup>2</sup> Department of Pathology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran.

<sup>3</sup> Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

## ARTICLE INFO

### Article Type:

Research Article

### Article History:

Received: 23 July 2013

Revised: 14 August 2013

Accepted: 1 September 2013

ePublished: 24 December 2013

### Keywords:

ZM241385

MRS1706

Adenosine A<sub>2A</sub> and A<sub>2B</sub> receptor

Asthma

Guinea pig

## ABSTRACT

**Purpose:** Nowadays adenosine is specified as an important factor in the pathophysiology of asthma. For determining the effect of different A<sub>2</sub> receptors, in this investigation the effect of single dose of selective adenosine A<sub>2A</sub> and A<sub>2B</sub> antagonists (ZM241385 and MRS1706) on different inflammatory parameters; tracheal responsiveness to methacholine and ovalbumin, total and differential cell count in bronchoalveolar lavage (BAL), blood levels of IL-4 and IFN- $\gamma$  and lung pathology of guinea pig model of asthma were assessed.

**Methods:** All mentioned parameters were evaluated in two sensitized groups of guinea pigs pretreated with A<sub>2A</sub> and A<sub>2B</sub> antagonists (S+Anta A<sub>2A</sub>, S+Anta A<sub>2B</sub>) compared with sensitized (S) and control (C) groups.

**Results:** The tracheal responsiveness to methacholine and OA, total cell and eosinophil and basophil count in BAL, blood IL-4 level and pathological changes in pre-treated group with MRS1706 (S+Anta A<sub>2B</sub>) was significantly lower than those of sensitized group ( $p < 0.01$  to  $p < 0.05$ ). In pretreated group with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>), all the above changes were reversed.

**Conclusion:** These results showed a preventive effect of A<sub>2B</sub> antagonist (MRS1706) on tracheal responsiveness to methacholine and OA, total and differential cell count in bronchoalveolar lavage, blood cytokines and pathological changes. Administration of ZM241385, selective A<sub>2A</sub> antagonist, deteriorated the induction effect of ovalbumin.

## Introduction

Asthma is a chronic disease characterized by a variety of features including reversible airways obstruction, airway inflammation and an increased airway responsiveness.<sup>1</sup> Evidence has increasingly implicated adenosine in the pathophysiology of asthma.<sup>2</sup> Adenosine is the breakdown product of ATP via endogenous ecto-ATPases and is also present at cell surface in cultured airway epithelial cells.<sup>3</sup> Adenosine in a signaling nucleoside is eliciting many physiological responses. Elevated levels of adenosine have been found in bronchoalveolar lavage, blood and exhaled breath condensate of patients with asthma. In addition, inhaled adenosine-5'-monophosphate induces bronchoconstriction in asthmatics but not in normal subjects. Studies on animals and humans have shown that bronchoconstriction is most likely due to the release of inflammatory mediators from mast cells. However a number of evidences suggest that adenosine modulates

the function of many other cells involved in airway inflammation such as neutrophils, eosinophils, lymphocytes and macrophages.<sup>4</sup>

It has become clear that biological functions of adenosine are mediated by four distinct subtypes of receptors (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>) and that biological responses are determined by the different pattern of receptors distribution in specific cells. Adenosine receptors are ubiquitously expressed throughout the body, with virtually all cells expressing one or more adenosine receptor subtype. With respect to the lung, little is known about the relative expression of adenosine receptor subtypes; however, binding studies in healthy peripheral lung tissue have suggested that A<sub>2</sub> receptor subtypes are much more abundant than the A<sub>1</sub> and A<sub>3</sub> receptor subtypes.<sup>5</sup>

For determining the effect of different A<sub>2</sub> receptors in pathophysiology of asthma, in this investigation the

\*Corresponding author: Rana Keyhanmanesh, Tuberculosis and Lung Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Tel: +98(411)3364664, Email: [keyhanmaneshr@tbzmed.ac.ir](mailto:keyhanmaneshr@tbzmed.ac.ir), [rkeyhanmanesh@gmail.com](mailto:rkeyhanmanesh@gmail.com)

effect of selective adenosine A<sub>2A</sub> and A<sub>2B</sub> antagonists (ZM241385 and MRS1706) on tracheal responsiveness to methacholine and ovalbumin (OA), total and differential cell count in bronchoalveolar lavage, blood levels of IL-4 and IFN- $\gamma$  and lung pathology of guinea pig model of asthma were assessed.

## Materials and Methods

### *Animal sensitization and animal groups*

Forty male adult Dunkin-Hartley guinea pigs (400–700 g) were used throughout the study. They were allowed to acclimatize to the new situation for ten days. The animals were group-housed in individual cages in climate-controlled animal quarters and were given water and food ad libitum, while a 12-h on/12-h off light cycle was maintained.

Animals randomly divided to four groups; Control group (C), Sensitized group with ovalbumin (OA, S), sensitized groups pretreated with selective A<sub>2A</sub> antagonist (ZM241385) and selective A<sub>2B</sub> antagonist (MRS1706) (S+Anta A<sub>2A</sub> and S+Anta A<sub>2B</sub> groups). Each of these antagonists (Tocris bioscience, UK) with 3 mg/kg dose was injected i.p. on day 10 of induction protocol.<sup>6,7</sup>

Sensitization of animals to OA (Grade II Sigma Chemical Ltd., UK) was performed according to our previous study.<sup>8</sup> Briefly, on the first day, 100 mg of OA, dissolved in saline, injected intraperitoneally and other 100 mg of OA subcutaneously. A week later, subsequent 10 mg of OA was injected intraperitoneally. Then from day 14, sensitized animals were exposed to an aerosol of 4% OA for 18  $\pm$  1 days, 4 min daily. The aerosol was administered in a closed chamber with dimensions 30  $\times$  20  $\times$  20 cm. Control animals were treated similarly but saline was used instead of OA solution. The study was approved by the ethical committee of the Tabriz University of Medical Sciences.

### *Tissue preparation*

Guinea pigs were killed by a blow on the neck and the trachea was removed. In each animal, after separation of the trachea from adjacent tissues, one tracheal chain was prepared as follows: The trachea was cut into 10 rings (each containing 2–3 cartilaginous rings) and sutured together to form a tracheal chain. Then all the rings (except terminal rings) were cut open opposite the trachealis muscle to clarify the muscular response. Finally tissue was suspended in a 20-mL organ bath (Schuler organ bath type 809, Germany) containing Krebs-Henseliet solution of the following composition (mM): NaCl; 120, NaHCO<sub>3</sub>; 25, MgSO<sub>4</sub>; 0.5, KH<sub>2</sub>PO<sub>4</sub>; 1.2, KCl; 4.72, CaCl<sub>2</sub>; 2.5 and dextrose 11. The Krebs solution was maintained at 37 °C and gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Tissue was suspended under isotonic tension of 1 g and allowed to equilibration for at least 1 hour while it was washed with Krebs solution every 15 min.<sup>9</sup>

Responses were measured using an isometric transducer (ADInstruments, Spain) with a sensitivity range of 0–25 g. These responses after amplifying with ML/118 quadbridge amplifier (March-Hugstetten, Germany) were recorded on a powerlab (ML-750, 4 channel recorder; March-Hugstetten, Germany).

### *Assessment of tracheal response to Methacholine*

In each experiment, a concentration-response curve of the tracheal chain was obtained. Consecutive concentrations of methacholine hydrochloride (Sigma Chemical Ltd., UK); including 10<sup>-7</sup> to 10<sup>-2</sup> M, dissolved in saline; were added every 3 minutes. The contraction due to each concentration was recorded at the end of 3 minutes and the effect reached a plateau in all experiments. Then the percentage of the tracheal smooth muscle contraction due to each concentration of methacholine in proportion to the maximal contraction obtained by its final concentration was plotted against log concentration of methacholine. A concentration-response curve of methacholine was performed in the tracheal chain of each studied animal. The effective concentration of methacholine causing 50% of maximum response (EC50) was measured from the methacholine response curve in each experiment using 50% of the maximum response in the Y axis and measuring the dose of methacholine causing this response in the X axis. The contractility response to 10  $\mu$ M methacholine as the magnitude of contraction was also measured.

### *Measurement of tracheal response to Ovalbumin (OA)*

The tracheal response of all animals to a 0.1% solution of OA was measured in each studied animal as follows: 0.5 mL of 4% OA solution (dissolved in saline) was added to the 20-mL organ bath and the degree of tracheal chain contraction was recorded after 10 min and was expressed as a proportion (in percentage) to the contraction obtained with 10  $\mu$ M methacholine.

The measurements of tracheal response to methacholine and OA were performed in random order.

### *Lung lavage and its white blood cell count*

Coincident with preparing the tracheal chain, a cannula was located into the remaining trachea and the lungs were lavaged with 5 mL of saline 4 times (total: 20 mL). One mL of lung lavage fluid (LLF) was stained with Turk solution and counted in duplicate in a hemocytometer (in a Burkner chamber). The Turk solution consisted of 1 mL of glacial acetic acid, 1 mL of gentian violet solution 1% and 100 mL of distilled water.

The remaining LLF was centrifuged at 2500  $\times$  g at 4 °C for 10 min. The supernatant was removed. The smear was prepared from the cells and stained with Wright-Giemsa. According to staining and morphological criteria, differential cell analysis was carried out under a light microscope by counting 100 cells twice and the percentage of each cell type calculated.<sup>10</sup>

### Measurement of blood interleukin 4 (IL-4) and interferon- $\gamma$ (IFN- $\gamma$ ) levels

Ten milliliters of peripheral blood was obtained immediately after sacrificing the animals and placed at room temperature for 1 hour. The samples were then centrifuged at 3500g 4 °C for 10 min. The supernatant was collected and immediately stored in deep freezer at -70°C until analyzed. Finally, blood IL-4 and IFN- $\gamma$  levels were measured using the sandwich ELISA method.<sup>11</sup>

### Pathological evaluation

Guinea pigs were sacrificed by a cervical dislocation, and their lungs and trachea were removed and placed into 10% buffered formalin (37%, Merck, Germany). Seven days later, the tissues were dried using an Autotechnicon apparatus by passage of the tissues through 70% ethanol and xylol to clear the tissues and then paraffin block the tissues. The specimens were cut into 4- $\mu$ m slices and stained with hematoxylin and eosin (H&E stain). The tissues were then evaluated under a light microscope. For each specimen, at least 10 airways and vessels were evaluated.<sup>8</sup>

The following pathological changes in the lungs of the sensitized groups were observed: vascular and airway membrane hyperplasia, mucosal plug, local epithelial denudation, eosinophil and lymphocyte infiltration and emphysema. These changes were scored as follows: no pathologic changes = 0, patchy changes =1, local changes =2, scattered changes =3 and severe changes =4.

### Statistical analysis

The data of tracheal response to methacholine (EC<sub>50</sub>), tracheal contractility response, tracheal response to OA, total WBC numbers and differential WBC counts are quoted as mean  $\pm$  SEM. The data of three sensitized groups were compared with controls using one-way analysis of variance (ANOVA) with Tukey-Kramer post-test. Moreover, the data of the sensitized group were compared with control and treated guinea pigs using one-way analysis of variance (ANOVA) with Tukey-Kramer post-test. The data between groups of animals treated with antagonists using the unpaired t-test. Significance was accepted at  $p < 0.05$ .

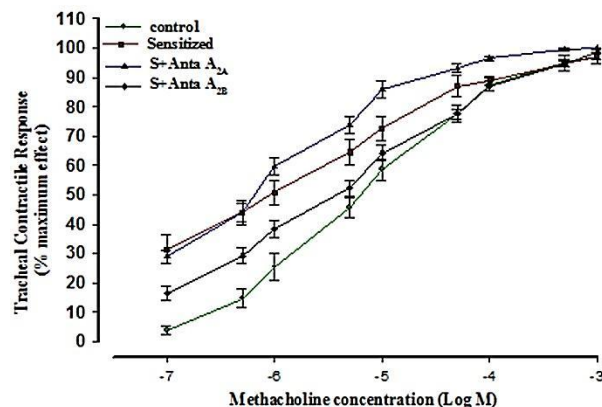
## Results

### Tracheal response to methacholine

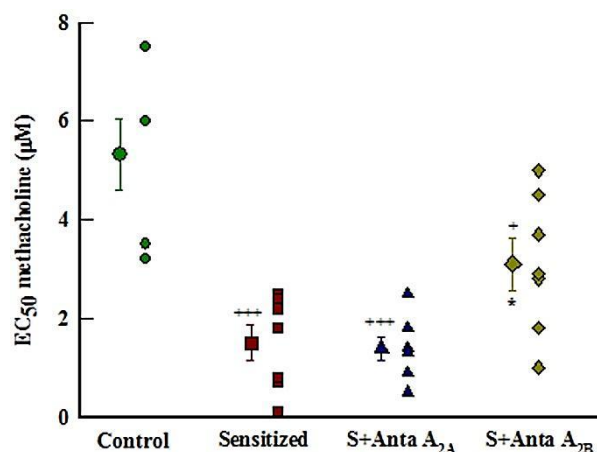
Concentration response curves to methacholine in non incubated tissues showed left ward shift of the curve in group S compared to group C. However, the curve of S+Anta A<sub>2B</sub> group was shifted to right compared to group S. Pretreatment with Anta A<sub>2A</sub> caused left ward shift compared to S group (Figure 1).

The mean value of EC<sub>50</sub> in tracheal chains of group S (1.50 $\pm$ 0.36  $\mu$ M) was significantly lower than in group C (5.31 $\pm$ 0.71  $\mu$ M,  $p < 0.001$ , Figure 2). The mean value of EC<sub>50</sub> in tracheal chains in pre-treated group with Anta A<sub>2A</sub> (1.38 $\pm$ 0.24 $\mu$ M) was non significantly lower than sensitized group; but administration of Anta A<sub>2B</sub>

(3.10 $\pm$ 0.54  $\mu$ M,  $P < 0.01$ ) caused significant improvement compared to the group S (Figure 2). However, the mean value of EC<sub>50</sub> in tracheal chains of S+Anta A<sub>2B</sub> group was still significantly lower than in group C ( $P < 0.05$ , Figure 2).



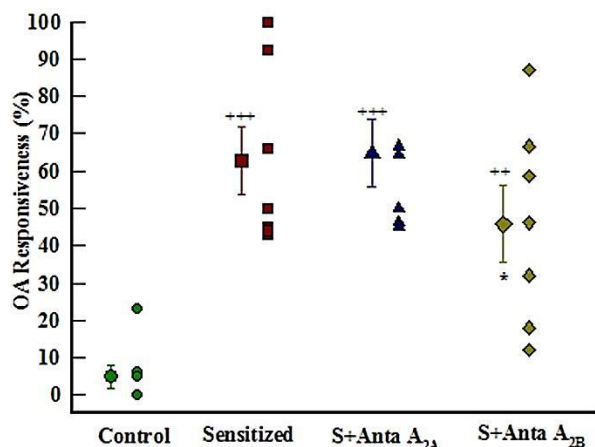
**Figure 1.** Cumulative log concentration-response curves of methacholine induced contraction of isolated trachea in control (C), sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs in the organ bath (for each group, n=7).



**Figure 2.** Individual values and mean $\pm$ SEM (big symbols with bars) of tracheal response to methacholine (EC<sub>50</sub>) in control (C), sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n=6). Statistical differences between control and different groups: +;  $p < 0.05$ , ++;  $p < 0.01$ , +++;  $p < 0.001$ . Statistical differences between sensitized and treated groups: \*;  $p < 0.05$ , \*\*;  $p < 0.01$ , \*\*\*;  $p < 0.001$ .

### Tracheal response to ovalbumin

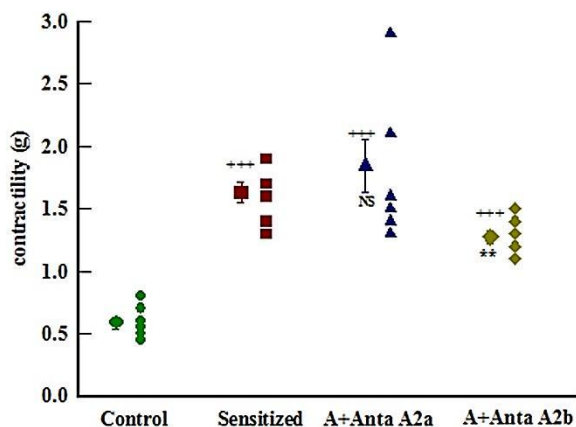
Tracheal response to OA in tracheal chains of group S (62.889.12%, range 42.80-100%) was significantly higher than in group C (4.853.18%, range 0-23%,  $p < 0.001$ , Figure 3). Tracheal response to OA in group S+Anta A<sub>2A</sub> (64.729.01%, range 45-114.45%) was non significantly higher than that of S group but pre-treatment with Anta A<sub>2B</sub> (45.7810.24%, range 12-78%) was significantly improved compared to sensitized group ( $p < 0.05$ , Figure 3). However, tracheal response to OA in S+Anta A<sub>2B</sub> group was still significantly higher than in group C ( $P < 0.01$ , Figure 3).



**Figure 3.** Individual values and mean $\pm$ SEM (big symbols with bars) of tracheal response to ovalbumin in control (C), sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n=6). Statistical differences between control and different groups: +; p<0.05, ++; p<0.01, +++; p<0.001. Statistical differences between sensitized and treated groups: \*: p<0.05, \*\*: p<0.01, \*\*\*: p<0.001.

### Contractility

The contractility response of tracheal chains to methacholine of group S ( $1.63\pm 0.08$  g) was significantly higher than that of group C ( $0.58\pm 0.04$  g, p<0.05). The contractility response in treated group with Anta A<sub>2B</sub> ( $1.27\pm 0.05$  g) caused significant decrease compared with S group (p<0.01) although this response was still significantly higher than controls (p<0.001). There was no significant difference in the contractility response of S+Anta A<sub>2A</sub> group ( $1.84\pm 0.21$  g) with group S (Figure 4).

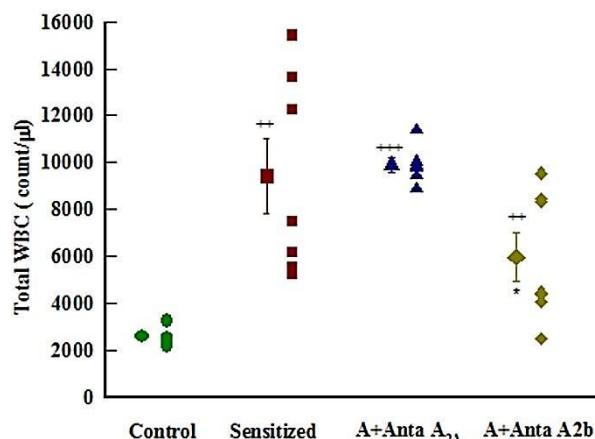


**Figure 4.** Individual values and mean $\pm$ SEM (big symbols with bars) of tracheal contractility response to 10  $\mu$ M methacholine in control (C), sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n=6). Statistical differences between control and different groups: +; p<0.05, ++; p<0.01, +++; p<0.001. Statistical differences between sensitized and treated groups: \*: p<0.05, \*\*: p<0.01, \*\*\*: p<0.001.

### Total white blood cell count

The mean value of total white blood cell (WBC) in LLF of group S ( $9421.43\pm 169.1$ ) was significantly higher than that of group C ( $2580\pm 180.02$ , P<0.01)

(Figure 5). The WBC in S+Anta A<sub>2A</sub> group ( $9896.43\pm 288.80$ ) was non significantly higher than sensitized animals. The WBC in S+Anta A<sub>2B</sub> group ( $5950\pm 1035.7$ ) showed significant improvement compared to that of S group (p<0.05, Figure 5). However, the mean value of WBC in this group was still significantly higher than in group C (p<0.01, Figure 5).



**Figure 5.** Individual values and mean $\pm$ SEM (big symbols with bars) of total LLF WBC number in control (C), sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n=6). Statistical differences between control and different groups: +; p<0.05, ++; p<0.01, +++; p<0.001. Statistical differences between sensitized and treated groups: \*: p<0.05, \*\*: p<0.01, \*\*\*: p<0.001.

### Differential count of WBC in Lung Lavage fluid

There was a significant decrease in neutrophil, lymphocyte and monocyte but significant increase of eosinophil and non-significant increase of basophil in LLF of group S compared to those of group C (p<0.001 for all cases, Figure 6a-e). Administration of Anta A<sub>2A</sub> caused significant decline in lymphocyte (p<0.01) and significant increment in eosinophil count (p<0.05) but the neutrophil, monocyte and basophil counts were not significantly different from S group. However treatment of sensitized animals with Anta A<sub>2B</sub> caused significant improvement in all LLF different cell counts (p<0.001 for all cases) but there were still significant differences in eosinophil and lymphocyte count of this group in comparison with those of group C (p<0.05 to P<0.001, Figure 6a-e).

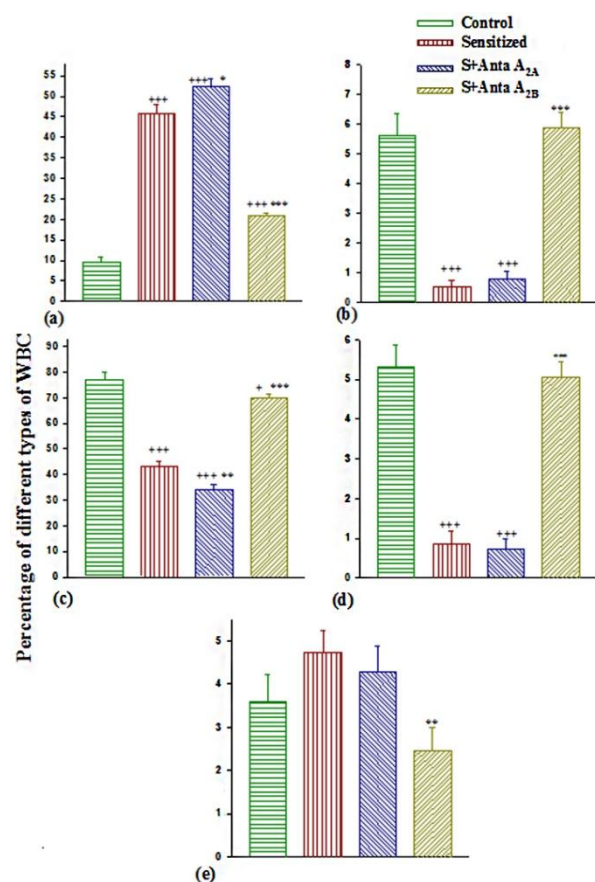
### Blood IL-4 and IFN- $\gamma$ levels

The mean value of the blood IL-4 levels in groups S ( $47.41\pm 1.98$ ), S + Anta A<sub>2A</sub> ( $49.48\pm 2.74$ ) and S + Anta A<sub>2B</sub> ( $45.24\pm 2.15$ ) were significantly higher than that of group C ( $39.78\pm 2.10$ , p < 0.05, Figure 7a). However, the mean values of the IL-4 in these pretreated groups were not significantly lower than those of group S (Figure 7a).

The mean value of the blood IFN- $\gamma$  levels of group S ( $117.37\pm 2.7$ ) and S + Anta A<sub>2B</sub> ( $120.02\pm 2.95$ ) was significantly higher than that of group C ( $104.97\pm 2.70$ ,



$p < 0.05$ , Figure 7b). The mean value of IFN- $\gamma$  in S+Anta A<sub>2A</sub> group ( $101.14 \pm 3.24$ ) was significantly lower than in group S ( $p < 0.01$ , Figure 7b).



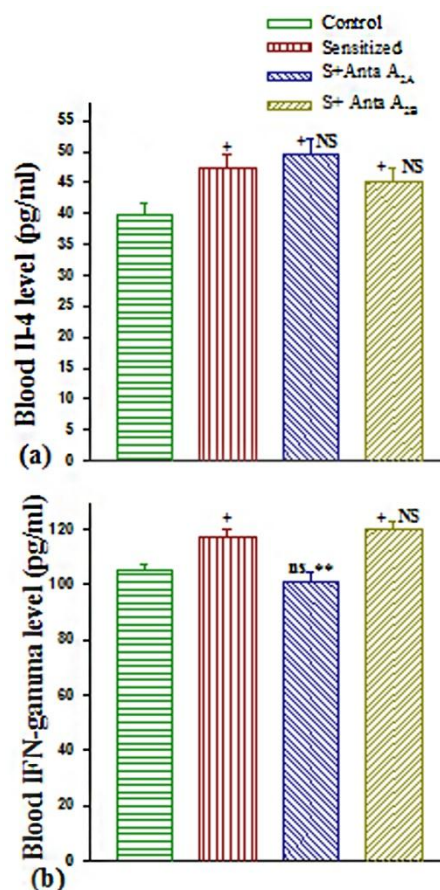
**Figure 6.** The percentages of eosinophil (a), neutrophil (b), lymphocyte (c), monocyte (d) and basophil (e) of lung lavage fluid in control, sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n=15). Statistical differences between control and different groups: +;  $p < 0.05$ , ++;  $p < 0.01$ , +++;  $p < 0.001$ . Statistical differences between sensitized and treated groups: \*:  $p < 0.05$ , \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ .

### Pathological results

All pathological changes in the S and S+A<sub>2A</sub> groups, including vascular membrane hyperplasia ( $3.0 \pm 0.3$  and  $2.90 \pm 0.28$  respectively), airway membrane hyperplasia ( $2.54 \pm 0.28$  and  $2.54 \pm 0.31$  respectively), mucosal plug ( $2.72 \pm 0.14$  and  $2.27 \pm 0.23$  respectively), local epithelial denudation ( $2.18 \pm 0.23$  and  $2.45 \pm 0.21$  respectively), eosinophil and lymphocyte infiltration ( $1.55 \pm 0.20$  and  $2.18 \pm 0.22$  respectively) and emphysema ( $2.0 \pm 0.27$  and  $2.18 \pm 0.23$  respectively) were significantly higher than control group ( $0.33 \pm 0.21$ ,  $0.17 \pm 0.16$ ,  $0 \pm 0$ ,  $0.17 \pm 0.16$ ,  $0 \pm 0$  and  $0.17 \pm 0.16$  for vascular and airway membrane hyperplasia, mucosal plug, local epithelial denudation, infiltration and emphysema respectively,  $p < 0.05$  for all, Figure 8, 9 a-f).

In S+A<sub>2B</sub> group, some of pathological changes; the mucosal plug ( $1.14 \pm 0.34$ ), local epithelial denudation ( $0.86 \pm 0.26$ ) and emphysema ( $1.42 \pm 0.30$ ) were

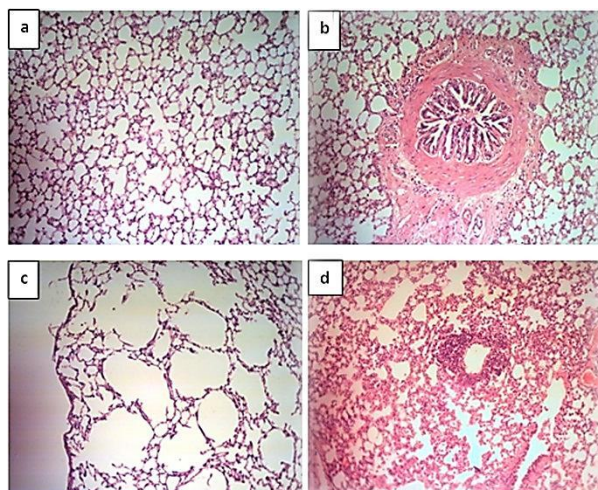
significantly higher than controls ( $p < 0.05$ ). however, the pathological changes such as vascular membrane hyperplasia ( $1.42 \pm 0.48$ ) and airway membrane hyperplasia ( $0.86 \pm 0.34$ ), the mucosal plug, local epithelial denudation in group S+A<sub>2B</sub> were significantly lower than S group ( $p < 0.05$ , Figure 8, 9 a-f).



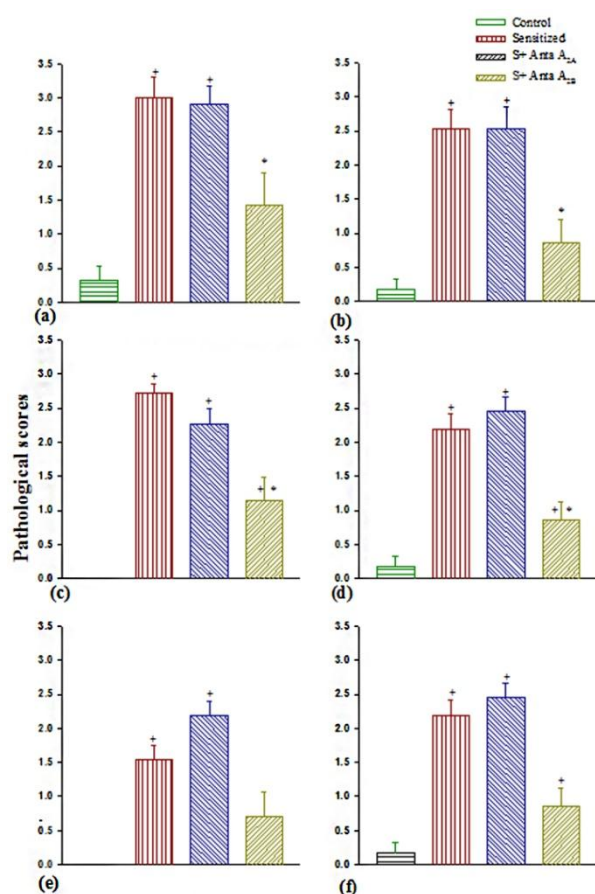
**Figure 7.** The blood IL-4 (a) and IFN- $\gamma$  (b) levels (pg/ml) in control, sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n = 8). Statistical differences between the control and the different groups: ns; no significant difference, +;  $p < 0.05$ . Statistical differences between sensitized and treated groups: NS; no significant difference, \*\*  $p < 0.01$ .

### Discussion

In the present study, the effect of ZM241385 (selective adenosine A<sub>2A</sub> antagonist) and MRS1706 (selective adenosine A<sub>2B</sub> antagonists) on tracheal responsiveness to methacholine and OA, total and differential cell count in bronchoalveolar lavage, blood levels of IL-4 and IFN- $\gamma$  and lung pathology of guinea pig model of asthma were examined. The results showed increased tracheal responsiveness to methacholine and OA, increased contractility response, increment of LLF total WBC count, eosinophil and basophil number (non-significantly), elevated blood IL-4 and IFN- $\gamma$  and numerous lung tissue pathological changes but decreased neutrophil, lymphocyte and monocyte in sensitized compared to control animal which was similar to the results of our previous studies.<sup>11,12</sup>



**Figure 8.** Photographs of a lung specimen in guinea pigs: a- control normal lung tissues (C); b- sensitized (S) with airway membrane hyperplasia and mucosal plug; c- S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) with severe emphysema and d- S treated with Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) with local epithelial denudation (magnification for each group; 10×20).



**Figure 9.** The vascular (a) and airway membrane hyperplasia (b), mucosal plug (c), local epithelial denudation (d), eosinophil and lymphocyte infiltration (e) and emphysema (f) of lungs in control, sensitized (S), S treated with Anta A<sub>2A</sub> (S+Anta A<sub>2A</sub>) and Anta A<sub>2B</sub> (S+Anta A<sub>2B</sub>) guinea pigs (for each group, n = 8). Statistical differences between the control and the different groups: ns; no significant difference, +; p < 0.05. Statistical differences between sensitized and treated groups: NS; no significant difference, \* p < 0.05.

Exogenous and endogenous adenosine; a ubiquitous purine nucleoside, has essential role in the pathogenesis of asthma and other lung inflammatory disorders. This concept is based on the fact that adenosine receptors are present in many cell types involved in airway inflammation.<sup>13</sup> It is now clear that the main mechanism responsible for exogenous adenosine inhalation-induced bronchoconstriction is mediators release from mast cells although there are some evidences for neural pathways activation.<sup>14</sup> In addition to this effect, the increased level of adenosine found in biological fluids, such as bronchoalveolar lavage and exhaled breath condensate of patients with asthma.<sup>15</sup> Although the precise source of adenosine release (mast cells, smooth muscle, epithelial cells) remains uncertain it is likely that adenosine may contribute to the bronchoconstriction induced by other stimuli such as allergens, hypoxia, lung injury and chronic inflammation.<sup>16</sup> However the bio-availability of adenosine is an important determinant of its biological functions, the pattern of expression and distribution of its different receptors (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, A<sub>3</sub>) in the anatomical and structural sites of the respiratory system and in immune or inflammatory cells, are responsible for this matter that adenosine may exert either deleterious or protective roles in the lung. However the inflammatory cytokines can regulate the expression of adenosine receptors, adenosine had a role in the inflammatory environment.<sup>17</sup>

The data strongly suggest that activation of adenosine A<sub>2A</sub> receptors, which are present in most of the inflammatory cells (such as neutrophils, mast cells, macrophages, eosinophils, platelets and T cells) inhibit inflammatory responses via affecting multiple aspects of the inflammatory process, modulating neutrophils activation and degranulation, oxidative species production, adhesion molecules expression, cytokines release and mast cells degranulation.<sup>5,18-20</sup>

In this study, single dose administration of ZM241385, selective adenosine A<sub>2A</sub> antagonist, caused increased tracheal responsiveness (decreased EC<sub>50</sub> and incremental contractility), tracheal response to OA and total WBC count and eosinophil and basophil number in LLF and pathological changes and decrease in neutrophil, monocyte and lymphocyte count in comparison to controls. These changes were more than those of sensitized group. It showed that this A<sub>2A</sub> receptor antagonist deteriorated the effect of ovalbumin in inducing asthma in guinea pigs. It has been predicted by exerting inhibitory effects of activation of these receptors on multiple inflammatory cell types mentioned before.

In addition, administration of selective adenosine A<sub>2A</sub> antagonist, ZM241385, caused increased IL-4 level and decreased IFN- $\gamma$  in blood. In asthma, the inflammation is regulated by two subsets of CD4<sup>+</sup> helper T cells; Th1 and Th2 balance. IFN- $\gamma$  secretes mostly by Th1 cells whereas IL-4 produces mostly by Th2 cells. In fact, asthma is associated with a shift in immune

responses away from a Th1 (IFN- $\gamma$ ) pattern and toward a Th2 (IL-4, IL-5 and IL-13) profile.<sup>21</sup> So one of proposed mechanisms of selective adenosine A<sub>2A</sub> antagonist could be its effect on regulation of T helper cells subtypes. Also the decreased lymphocyte count in this study can support this suggested mechanism.

Expression of adenosine A<sub>2B</sub> receptors has been found in bronchial epithelium, cultured human airway smooth muscle, human mast cells, monocytes and fibroblasts.<sup>4</sup> Increasing evidences suggest that in rodents and man activation of adenosine A<sub>2B</sub> receptors modulates mast cells function. Adenosine signaling through the A<sub>2B</sub> receptors can stimulate the production of IL-8, IL-4, IL-13 and VEGF from mast cells. This receptors signaling is an important factor of aberrant dendritic cell differentiation and generation of tolerogenic, angiogenic, and pro-inflammatory cells that produce VEGF, IL-8, IL-6, IL-10, COX-2 and TGF- $\beta$ . In addition, A<sub>2B</sub> receptors engagement can promote the production of IL-6 and osteopontin from macrophages; IL-6 and MCP-1 from bronchial smooth muscles; increases in IL-6 release from fibroblasts; induces myofibroblasts differentiation; and induces the expression of fibronectin in type II lung epithelial cells. A<sub>2B</sub> receptor signaling also contributes to the maintenance of airway surface liquid height in airway epithelial cells and vascular barrier function in endothelial cells.<sup>22</sup> It suggests that adenosine, via A<sub>2B</sub> receptors participates in the remodelling process occurring in chronic inflammatory lung diseases.<sup>23</sup> Taken together these evidences suggest that adenosine A<sub>2B</sub> receptor are deeply involved in the mechanisms underlying mediators release by mast cells, the major mechanism by which adenosine induces bronchoconstriction and airway inflammation in asthma. Therefore it has been suggested that targeting adenosine receptors might be a possible approach for the development of anti-inflammatory treatments in diseases characterized by chronic airway inflammation such as asthma and COPD. Currently there is an agreement that development of selective adenosine A<sub>2B</sub> receptor antagonists might be the most appealing approach.<sup>14,24</sup>

Some authors have speculated that the pro- and anti-inflammatory property of adenosine may be dictated by its level in the lung.<sup>14,16</sup> Lung inflammation determines a hypoxic environment in which adenosine is generated. In the initial stage, low levels of adenosine activate high affinity receptors, such as adenosine A<sub>2A</sub> receptors, and this triggers a protective pathway. However higher levels of adenosine are released when lung inflammation is severe, and these, activating the low affinity adenosine A<sub>2B</sub> receptors, may trigger deleterious signaling pathways that further exacerbate inflammation.<sup>16</sup>

Single dose administration of MRS1706, selective adenosine A<sub>2B</sub> antagonist, in this study, improved the changes in tracheal responsiveness, total WBC count and lung pathological changes. This drug ameliorated

the variations in differential WBC compared to asthmatic guinea pigs. However it could not prevent completely in comparison to controls. Moreover, this drug caused increase in IFN- $\gamma$  and decrease in IL-4 level in comparison to sensitized animals although it could not reach those of controls. The lymphocyte count was also higher than sensitized group. These results were in line with previous studies. Mustafa and his colleagues in 2007 showed that other antagonist of adenosine A<sub>2B</sub> receptors, CVT-6883, inhibited the airway inflammation.<sup>25</sup> These studies confirmed the role of A<sub>2B</sub> receptors in the pathophysiology of asthma.

### Conclusion

In Conclusion, the results showed that administration of single dose of selective adenosine A<sub>2A</sub> (ZM241385) deteriorated the inflammatory changes of ovalbumin induced asthma and single dose prescription of A<sub>2B</sub> antagonists (MRS1706) could prevent these changes.

### Acknowledgements

This investigation was supported by Tuberculosis and lung research center of Tabriz University of medical sciences as the part of the thesis of Msc student, Miss Pejman.

### Conflict of Interest

The authors declare that they have no conflict of interest.

### References

1. Keir S, Page C. The rabbit as a model to study asthma and other lung diseases. *Pulm Pharmacol Ther* 2008;21(5):721-30.
2. Rorke S, Holgate ST. Targeting adenosine receptors: novel therapeutic targets in asthma and chronic obstructive pulmonary disease. *Am J Respir Med* 2002;1(2):99-105.
3. Kornerup KN, Page CP, Moffatt JD. Pharmacological characterisation of the adenosine receptor mediating increased ion transport in the mouse isolated trachea and the effect of allergen challenge. *Br J Pharmacol* 2005;144(7):1011-6.
4. Spicuzza L, Di Maria G, Polosa R. Adenosine in the airways: implications and applications. *Eur J Pharmacol* 2006;533(1-3):77-88.
5. Brown RA, Spina D, Page CP. Adenosine receptors and asthma. *Br J Pharmacol* 2008;153 Suppl 1:S446-56.
6. Fozard JR, Mccarthy C. Adenosine receptor ligands as potential therapeutics in asthma. *Curr Opin Investig Drugs* 2002;3(1):69-77.
7. Smith N, Broadley KJ. Adenosine receptor subtypes in the airways responses to 5'-adenosine monophosphate inhalation of sensitized guinea-pigs. *Clin Exp Allergy* 2008;38(9):1536-47.
8. Keyhanmanesh R, Boskabady MH, Khamneh S, Doostar Y. Effect of thymoquinone on the lung pathology and cytokine levels of ovalbumin-



- sensitized guinea pigs. *Pharmacol Rep* 2010;62(5):910-6.
9. Keyhanmanesh R, Boskabady MH. Relaxant effects of different fractions from *Tymus vulgaris* on guinea-pig tracheal chains. *Biol Res* 2012;45(1):67-73.
  10. Keyhanmanesh R, Boskabady MH, Eslamizadeh MJ, Khamneh S, Ebrahimi MA. The effect of thymoquinone, the main constituent of *Nigella sativa* on tracheal responsiveness and white blood cell count in lung lavage of sensitized guinea pigs. *Planta medica* 2010;76(3):218-22.
  11. Boskabady MH, Keyhanmanesh R, Khameneh S, Doostdar Y, Khakzad MR. Potential immunomodulation effect of the extract of *Nigella sativa* on ovalbumin sensitized guinea pigs. *J Zhejiang Univ Sci B* 2011;12(3):201-9.
  12. Boskabady MH, Keyhanmanesh R, Khamneh S, Ebrahimi MA. The effect of *Nigella sativa* extract on tracheal responsiveness and lung inflammation in ovalbumin-sensitized guinea pigs. *Clinics (Sao Paulo)* 2011;66(5):879-87.
  13. Spicuzza L, Bonfiglio C, Polosa R. Research applications and implications of adenosine in diseased airways. *Trends Pharmacol Sci* 2003;24(8):409-13.
  14. Polosa R, Rorke S, Holgate ST. Evolving concepts on the value of adenosine hyperresponsiveness in asthma and chronic obstructive pulmonary disease. *Thorax* 2002;57(7):649-54.
  15. Huszar E, Vass G, Vizi E, Csoma Z, Barat E, Molnar Vilagos G, et al. Adenosine in exhaled breath condensate in healthy volunteers and in patients with asthma. *Eur Respir J* 2002;20(6):1393-8.
  16. Blackburn MR. Too much of a good thing: adenosine overload in adenosine-deaminase-deficient mice. *Trends Pharmacol Sci* 2003;24(2):66-70.
  17. Khoa ND, Montesinos MC, Reiss AB, Delano D, Awadallah N, Cronstein BN. Inflammatory cytokines regulate function and expression of adenosine A<sub>2A</sub> receptors in human monocytic THP-1 cells. *J Immunol* 2001;167(7):4026-32.
  18. Lappas CM, Sullivan GW, Linden J. Adenosine A<sub>2A</sub> agonists in development for the treatment of inflammation. *Expert Opin Investig Drugs* 2005;14(7):797-806.
  19. Thiel M, Chouker A, Ohta A, Jackson E, Caldwell C, Smith P, et al. Oxygenation inhibits the physiological tissue-protecting mechanism and thereby exacerbates acute inflammatory lung injury. *PLoS Biol* 2005;3(6):e174.
  20. Constance N, Wilson S, Mustafa J, Abbracchio MP, Heidelberg SD. Adenosine receptors in health and disease. Dordrecht: Springer; 2009.
  21. Schmidt-Weber CB, Blaser K. The role of the FOXP3 transcription factor in the immune regulation of allergic asthma. *Curr Allergy Asthma Rep* 2005;5(5):356-61.
  22. Zhoua Y, Schneidera DJ, Blackburna MR. Adenosine signaling and the regulation of chronic lung disease. *Pharmacol Ther* 2009;123(1):105-16.
  23. Zhong H, Belardinelli L, Maa T, Zeng D. Synergy between A<sub>2B</sub> adenosine receptors and hypoxia in activating human lung fibroblasts. *Am J Respir Cell Mol Biol* 2005;32(1):2-8.
  24. Holgate ST. The Quintiles Prize Lecture 2004. The identification of the adenosine A<sub>2B</sub> receptor as a novel therapeutic target in asthma. *Br J Pharmacol* 2005;145(8):1009-15.
  25. Mustafa SJ, Nadeem A, Fan M, Zhong H, Belardinelli L, Zeng D. Effect of a specific and selective A<sub>2B</sub> adenosine receptor antagonist on adenosine agonist amp and allergen-induced airway responsiveness and cellular influx in a mouse model of asthma. *J Pharmacol Exp Ther* 2007;320(3):1246-51.