



Antiasthmatic Effect of Saxagliptin on Ovalbumin-Induced Allergic Asthma in Mice

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ABSTRACT

In the current study, the protective activity of saxagliptin (SAX) on ovalbumin (OVA)-induced allergic asthma in mice was evaluated.

Methods: Mice with OVA-induced allergic asthma were orally administered SAX at a dose of 10 mg/kg/day for 6 days. We determined the count of total cells, differential inflammatory cells and interleukin (IL)-13 concentration in bronchoalveolar lavage fluid (BAL). The concentration of lipid peroxidation marker, malondialdehyde (MDA), was also examined in lung homogenate. Histopathological alterations in lung tissues were also detected by Hematoxylin and Eosin staining.

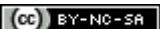
Results: SAX inhibited OVA-induced airway inflammation and oxidative stress in the lung. OVA-induced allergic asthma was suppressed by SAX as evidenced by decreased lung body weight ratio and leukocyte infiltration in the lungs, and also suppressed OVA-induced elevation of level of IL-13 in BAL. In addition, SAX decreased the MDA concentration in lung homogenate.

Conclusion: Our study demonstrated that SAX attenuated OVA-induced allergic asthma and it may represent a promising agent for the control of allergic asthma.

Keywords: Saxagliptin, interleukin, ovalbumin, asthma, inflammation

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INTRODUCTION

Allergic asthma is one of the most prevalent chronic diseases and affects peoples in all ages. Moreover, the prevalence of asthma has augmented in the last 20 years [1]. Allergic asthma is identified by airway occlusion, increased mucus secretion, and bronchial infiltration by eosinophils, neutrophils, mast cells, and T lymphocytes. T lymphocytes play major roles in airway inflammation and remodeling through cytokines. T helper 2 (Th2) cytokines, including IL-13, prompt allergen-specific immunoglobulin (Ig) E production and inflammatory mediator release from mast cells.

Dipeptidyl peptidase-4 (DPP-4) inhibitors are broadly used therapeutic approach for type 2 diabetes. They inhibit the enzyme DPP-4, there for extending the physiological actions of the incretin hormones, with a clinically relevant attenuation in serum glucose level[2]. However, DPP-4 are universal enzymes that are produced in different types of cells and tissues [3]. Hence, DPP-4 inhibitors may valuably attenuate chronic inflammation and oxidative stress in different diseases. Among DPP-4 inhibitors, SAX has anti-inflammatory properties, antioxidant and immunomodulatory properties [2, 4, 5]. The therapeutic approaches for asthma are composed of several bronchodilators and anti-inflammatory drugs. Because lung and systemic oxidative stress augment inflammatory processes related to asthma[6], the antioxidant and anti-inflammatory activities may propose SAX as a treatment strategy to attenuate allergic asthma manifestation.

As long as, this study was performed to test whether SAX attenuates airway alterations in *OVA*-induced allergic asthma as well as to elucidate that the protective effect of SAX in this study is related to the anti-inflammatory and antioxidant activity.

MATERIALS AND METHODS

Animals: Adult male Swiss albino mice (7 weeks old, n=30) were used for this experiment (obtained from VACSERA, Giza, Egypt). The animals were fed with a balanced chow diet with free access to water *ad libitum*, housed in cages under pathogen-free conditions, and conserved on a 12/12-h light/dark cycle. All experimental procedures used in this study were designed in compliance with guiding principles for the care and use of laboratory animals approved by “The research Ethics Committee”, Faculty of Pharmacy, Mansoura University, Egypt.

Chemicals and drugs: Ovalbumin (*OVA*) was purchased from Loba Chemie PVT. Ltd. (Bombay,

India). **Saxagliptin monohydrate (SAX), Onglyza 5mg tablets**, manufactured by *Bristol Myers Squibb* (Pennington,NJ,USA), was purchased from the market. It was prepared as 0.25% suspension in 0.5% CMC immediately before use. Other chemical reagents used in the experiment were analytical grade.

***OVA*-induced allergic asthma protocol and treatment:** In order to evaluate the preventive potential of SAX on *OVA*-induced allergic asthma in mice, mice were randomly allocated into three equal groups (n =10/group) as follows: Control (*CTL*) group, allergic asthma group (*OVA* group), and treatment group(SAX group) [7].

Briefly, mice were sensitized with an IP injection of 20 µg *OVA* and 1 mg aluminum hydroxide [AL(OH)₃] on days 0 & 7. Then they were challenged, on days 14, 15 and 16 with nebulization of 1% (w/v) *OVA* in normal saline, 1 hr after administration of SAX for 30 minutes[8].

Mice in the *CTL* group were sensitized and challenged with saline. On days 11–16, mice in the SAX group were administered SAX orally at dose of 10mg/kg/day[9, 10]; mice in *CTL* group and *OVA* group were given saline. On Day 17, mice were sacrificed 24 hrs after the last *OVA* challenge and bronchoalveolar lavage fluid (BAL) in each group was collected, and the lung tissues were also isolated and washed with ice cold 1.15% potassium chloride (KCL) (pH 7.45) then weighed for calculation of lung per body weight ratio[11].

Collection of BAL and counting of total and differential leukocytes: For preparation of BAL, 0.5 ml of cold saline was infused through the lung and withdrawn for 3 times via tracheal cannula. The BALF was then centrifuged at 1000 rpm at 4°C for 10 minutes. The supernatants were stored at -80°C for biochemical measures and cytokines detections. Pellets containing BAL cells were resuspended in cold saline for total and differential leukocytes count. The total number of inflammatory cells was counted, then the samples were centrifuged onto glass slides and the numbers of eosinophils, neutrophils, macrophages and lymphocytes in BALF were detected by Wright–Giemsa staining[12].

Determination of IL-13 level in BAL: The level of IL-13 in BAL was quantified using ELISA kits according to the manufactory instructions.

Preparation of lung homogenate and determination of lipid peroxidation marker in the lung tissues: Left lung specimen was homogenized as previously described[13].Briefly, left lung sections were weighed and homogenized

in 1.15% KCL solution to prepare 10% w/v lung homogenate. Lung homogenate was centrifuged and the supernatant was collected after centrifugation. The level of malondialdehyde (MDA), lipid peroxidation indicator, was measured in lung tissues following the procedure of Gerard-Monnier *et al.*, (1998) [14].

Lung histology examination: The right lung lobe from each animal was removed and fixed in 10% (v/v) neutral buffered formalin. The tissue was dehydrated, embedded in paraffin, sectioned at 5 mm thickness. Then the sections were stained with hematoxylin and eosin (H&E) stains. The histopathological alterations of the lung tissues were observed by treatment-blinded pathologist.

Statistical analysis: All results were expressed as means \pm standard error of mean (SEM). Statistical analysis was performed by using Graph Pad Prism V. Statistical significance ($P < 0.05$) was assessed by one-way analysis of variance (ANOVA) followed by Tukey test.

RESULTS

Effect of SAX on lung/body weight ratio in OVA-induced allergic asthmatic mice: The lung/body weight ratio of OVA group was significantly increased by 31% compared to the CTL group. In SAX group, the lung/body weight ratio significantly lowered by 22% when compared to that of the OVA group as shown in Figure (1).

Effect of SAX on total and differential cell count in BAL of OVA-induced allergic asthmatic mice: To assess whatever SAX could attenuate infiltration of inflammatory cells, total and differential cell count in BAL was determined. Mice in OVA group demonstrated significant elevation in the infiltration and the accumulation of total cells, macrophages, neutrophils, eosinophils, and lymphocytes compared to CTL group. However, SAX treatment significantly decreased the accumulation of these cells in comparison with OVA group (table 1).

Effect of SAX on IL-13 level in BAL of OVA-induced allergic asthmatic mice: As presented in figure 2, mice sensitized and challenged with OVA showed significant increase in the IL-13 concentration in BAL when compared to mice in CTL group. This OVA-exacerbation of IL-13 was significantly decreased by treatment with SAX compared to OVA-sensitized and challenged mice.

Effect of SAX on lipid peroxidation marker in the lung tissues of OVA-induced allergic asthmatic mice: Mice in OVA group showed

marked increase in MDA concentration in lung tissues. The increased MDA levels in the lung of mice were significantly attenuated upon SAX administration in comparison with OVA-treated mice (Figure 3).

Effect of SAX on histopathological alterations in the lung tissues of OVA-induced allergic asthmatic mice: To explore the effect of SAX on OVA-induced allergic asthma, the histopathological changes in lung tissues from mice in each group were observed using H&E staining. As shown in Figure 4, mice sensitized and challenged by OVA demonstrated extensive infiltration of inflammatory cells surrounding the bronchi, thickened walls of bronchial epithelium and congested blood vessels, indicating airway inflammation of the OVA-treated mice. SAX treatment attenuates bronchial airway inflammation and accumulation of inflammatory cells.

DISCUSSION

In the current study, we explored the antiasthmatic effect of SAX on OVA-induced allergic asthma. We demonstrated for the first time that oral administration of SAX attenuated asthma-associated lung injury by decreasing the infiltration of inflammatory cells around bronchial airway and vessels, interfering with IL-13 production in BAL and suppressing lipid peroxidation occurred in the lung tissues following sensitization and challenge with OVA.

In recent years, several studies have demonstrated that DPP4 could become a therapeutic approach for management of allergic asthma [15]. Moreover, SAX, a DPP-4 inhibitor, was reported to have antioxidant, antifibrotic and anti-inflammatory activities which may be beneficial in treatment of asthma and other inflammatory diseases [7, 16, 17]. In agreement with these studies, in the current work, we provide novel evidence that SAX has antiasthmatic activity and could mitigate allergic airway inflammation.

Th-2 immune response is considered as the hallmark of OVA-induced allergic asthma through infiltration of inflammatory cells and expression of Th2 cytokines, such as IL-4, IL-5 and IL-13 [18, 19]. IL-4, and IL-5 control the maturation, survival and differentiation of eosinophils [20]. IL-13, another key cytokine in asthma, promotes B-cell differentiation and plays a dominant role in airway inflammation and hyper responsiveness and mucus secretion [21]. Recent studies provided evidence that inhibition of Th2 immune response could mitigate allergic asthma [12].

Results of our study showed that notable increase in the total and differential inflammatory cell counts in BALF of OVA- challenged mice was abolished by SAX. In addition, SAX also decreased the level of Th2-associated IL-13 and the number of eosinophils and basophils, suggesting that antiasthmatic effect of SAX may be related to interference with Th2 immune response. Furthermore, histological analysis showed that SAX mitigated OVA-induced lung tissue injury; providing evidence for the protective effect of SAX against asthma.

Oxidative stress has been participated in the development of OVA-induced allergic asthma. In patients with allergic inflammatory airway, recruited inflammatory cells release excess reactive oxygen species (ROS) with a shift toward massive level of oxidative stress [22, 23].MDA, one of

several by-products of lipid peroxidation process, was measured in lung homogenate to reflect the redox imbalance in asthmatic airways. In the present study, mice in SAX group had decreased level of MDA in lung homogenate as compared to CTL mice. This apparent SAX antioxidant activity is consistent with some previous findings reporting antioxidant activity of SAX in various tissues [4, 5, 24].

CONCLUSION

We demonstrated that SAX mitigated the symptoms of asthma in OVA-induced allergic asthmatic mice. Further, this study showed that SAX inhibited leukocyte infiltration, cytokine production and oxidative stress in the allergic asthmatic lung, which may support the anti-asthmatic effect of SAX.

Table 1: Table 1: Effect of oral administration of SAX (10 mg/kg/day) on total and differential inflammatory cells counts in bronchoalveolar lavage fluid (BAL)of OVA-induced allergic asthmatic mice.

Group	Total leucocyte count X 10 ⁴	Neutrophil count X 10 ⁴	Lymphocyte count X 10 ⁴	Monocyte count X 10 ⁴	Eosinophil count x 10 ⁴
CTL group	7.35 ± 0.2	6.07 ± 0.3	0.7 ± 0.02	0.23 ± 0.01	0.21 ± 0.01
OVA group	32.4 ± 0.8*	27.02 ± 1.3 *	4.07 ± 0.2 *	0.65 ± 0.01 *	0.65 ± 0.01*
SAX group	10.28 ± 0.6 *#	8.3 ± 0.7#	1.46 ± 0.24 #	0.32 ± 0.02 *#	0.20 ± 0.02#

OVA challenge was performed for 30 min after OVA sensitization on day 0 & 7 and SAX was administered once daily 1 hr before OVA challenge from days 11 to 16. CTL (control group), OVA (Ovalbumin group) and SAX (Saxagliptin group). Values represent the mean ± SEM, n=10. Data were statistically evaluated by means of *one way analysis of variance* followed by *Tukey-Kramer* multiple comparisons test.

* $P < 0.05$, compared with CTL group

$P < 0.05$, compared with OVA group

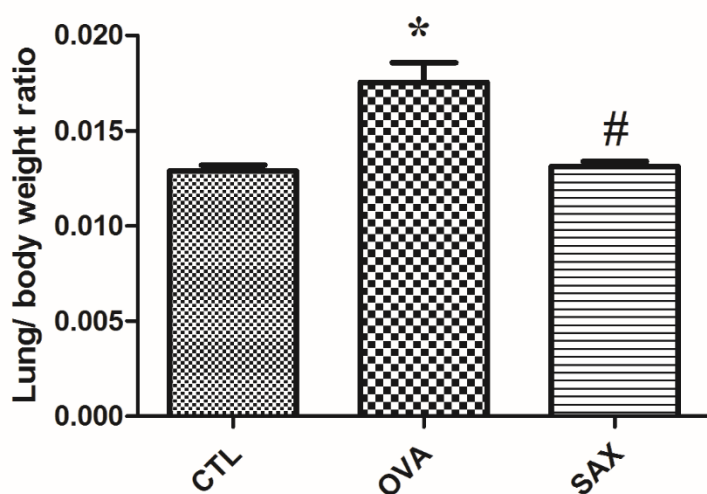


Figure 1: Effect of oral administration of SAX (10 mg/kg/day) on lung /body weight ratio in OVA-induced allergic asthmatic mice.

OVA challenge was performed for 30 min after OVA sensitization on day 0 & 7 and SAX was administered once daily 1 hr before OVA challenge from days 11 to 16. CTL (control group), OVA (Ovalbumin group) and SAX (Saxagliptin group). Values represent the mean ± SEM, n=10. Data were statistically evaluated by means of *one way analysis of variance* followed by *Tukey-Kramer* multiple comparisons test.

* $P < 0.05$, compared with CTL group; # $P < 0.05$, compared with OVA group

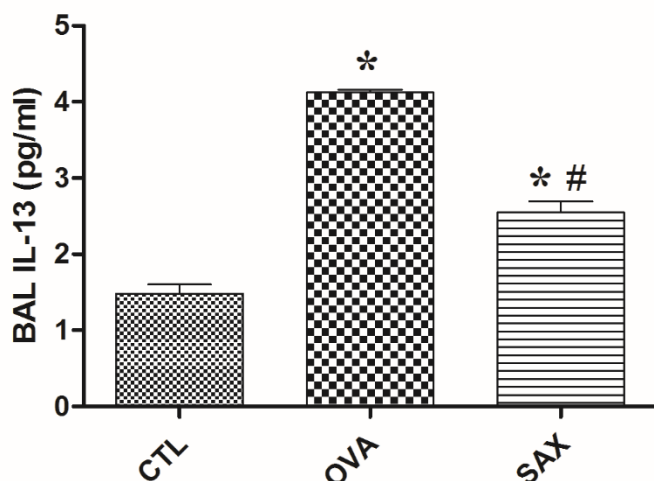


Figure 2: Effect of oral administration of SAX (10 mg/kg/day) on IL-13 level in BAL of OVA-induced allergic asthmatic mice

OVA challenge was performed for 30 min after OVA sensitization on day 0 & 7 and SAX was administered once daily 1 hr before OVA challenge from days 11 to 16. CTL (control group), OVA (Ovalbumin group) and SAX (Saxagliptin group). Values represent the mean \pm SEM, n=10. Data were statistically evaluated by means of *one way analysis of variance* followed by *Tukey-Kramer* multiple comparisons test.

* $P < 0.05$, compared with CTL group; # $P < 0.05$, compared with OVA group

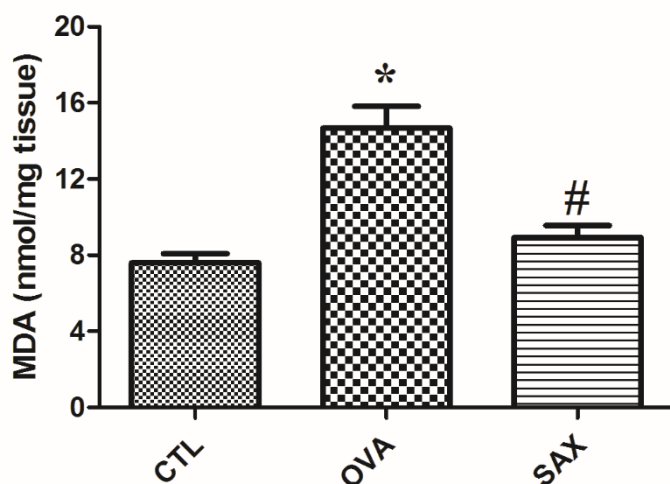


Figure (3): Effect of oral administration of SAX (10 mg/kg/day) on MDA, lipid peroxidation marker, in the lung tissues of OVA-induced allergic asthmatic mice.

OVA challenge was performed for 30 min after OVA sensitization on day 0 & 7 and SAX was administered once daily 1 hr before OVA challenge from days 11 to 16.

CTL (control group), OVA (Ovalbumin group), SAX (Saxagliptin group) and MDA (malondialdehyde).

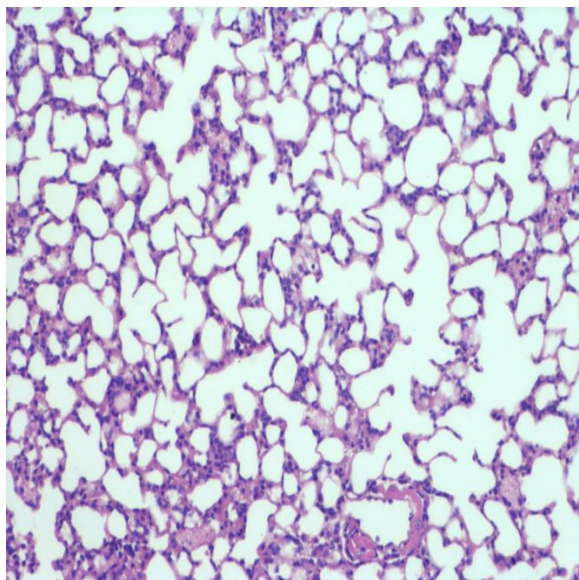
Values represent the mean \pm SEM, n=10.

Data were statistically evaluated by means of *one way analysis of variance* followed by *Tukey-Kramer* multiple comparisons test.

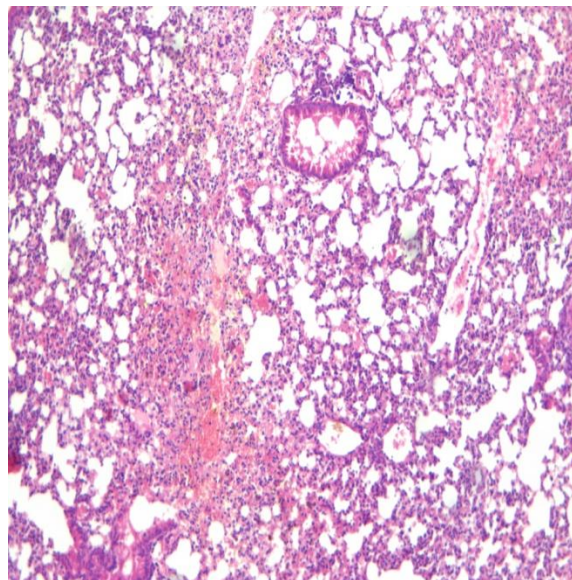
* $P < 0.05$, compared with CTL group

$P < 0.05$, compared with OVA group

CTL



OVA



SAX

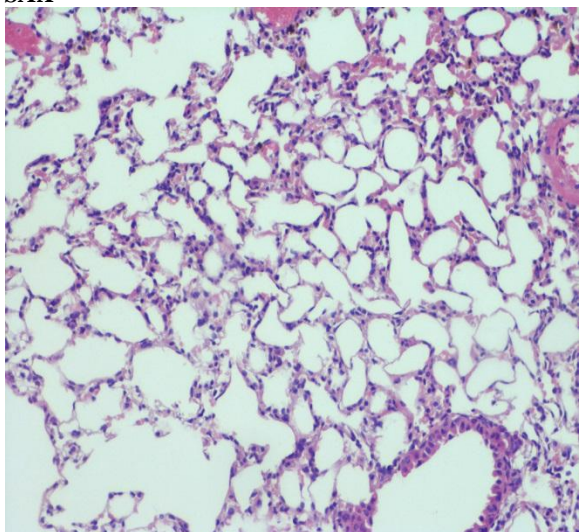


Figure 4: Effect of oral administration of SAX (10 mg/kg/day) on histopathological alterations (hematoxylin-eosin staining, 20x) in the lung tissues of OVA-induced allergic asthmatic mice.

(CTL) control group showed normal bronchial airway. (OVA) OVA group showed extensive infiltration of inflammatory cells surrounding the bronchi, thickened walls of bronchial epithelium and congested blood vessels (SAX) Saxagliptin group showed attenuation of bronchial airway inflammation and infiltration of inflammatory cells.

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