Potential effect of one-alpha-hydroxy vitamin D3 (alfacalcidol) on experimentally induced allergic asthma

Aliaa A. Abd-Elsattar, Mona M. Amin, Nadia H. Elbarody, Ragaa H. Abaza

Background: Alfacalcidol is a vitamin D analog mainly used in the management of osteoporosis. Apart from the well-known role of vitamin D in bone and calcium metabolism, an immune-modulator role of vitamin D in allergic diseases was suggested.Aim: To evaluate the potential effect of alfacalcidol either alone or in combination with salbutamol or/and prednisolone and its role in the protection and management of experimentally induced allergic asthma. **Materials and methods:** Male guinea pigs were divided into two groups: group A constituted normal guinea pigs (received saline) and group B constituted ovalbumin-sensitized guinea pigs, which were divided into eight subgroups: group B1 (control): groups from B2 to B4 was treated with salbutamol, prednisolone, alfacalcidol, respectively, for 1 week, while group B5 was treated with salbutamol+prednisolone; group B6 was treated with salbutamol+alfacalcidol; group B7 was treated with prednisolone+alfacalcidol; and group B8 was treated with salbutamol, prednisolone, and alfacalcidol for 1 week. Twenty-four hours after the last dose, the animals were subjected to both: (a)pharmacological studies, (b) histopathological studies (group A, group B1, B3, and B4). **Results:** (a) Pharmacological studies: sensitization of guinea pigs caused a significant increase in amplitude of histamine-induced contractions of isolated tracheal smooth muscles in comparison to that of the normal one. Treatment of sensitized guinea pigs with salbutamol, prednisolone, or alfacalcidol produced a significant reduction in the amplitude of histamine-induced contractions in comparison to sensitized nontreated guinea pigs. Addition of alfacalcidol to salbutamol or prednisolone in the treatment of sensitized guinea pigs caused more decrease in the amplitude of histamine-induced contractions. However, the highest reduction in the amplitude of contractions of isolated tracheal strips was produced by the treatment of guinea pigs with a combination of salbutamol, prednisolone, and alfacalcidol. (b) Histopathological examinations: treatment of sensitized guinea pigs with alfacalcidol resulted in an anti-remodeling effect, while prednisolone showed better anti-inflammatory effect, when it was compared with that of the control group. **Conclusion:** Alfacalcidol was shown to possess anti-inflammatory and anti-remodeling effects in allergic asthma. It was superior to prednisolone regarding the anti-remodeling effect. So, alfacalcidol could be beneficial in the management of allergic asthma.

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Introduction

Allergic asthma is a chronic lung disease characterized by airway inflammation, airflow limitation, airway hyperresponsiveness, episodic wheeze, and cough [1]. When the inflammation is severe or prolonged, it produces structural changes in the airway wall leading to airway remodeling [2]. These structural changes can include thickening of the basement membrane, subepithelial fibrosis, airway smooth muscle (ASM) hypertrophy and hyperplasia, blood vessel proliferation and dilatation, and mucous gland hyperplasia and hypersecretion [3]. Alfacalcidol is a synthetic vitamin D3 analog that is frequently used to treat osteoporosis [4]. Current therapeutic regimen for asthma includes mainly β2-adrenergic agonists such as bronchodilators and corticosteroids to target airway inflammation and airway hyperresponsiveness [5]. Corticosteroids have been successful in controlling asthma symptoms, but are unable to reverse airway remodeling and the symptoms persist with prolonged use of these steroids [6]. Recent studies have suggested a possible role of vitamin D as a pivotal immune-modulator in allergic asthma [7].

So, the aim of the present study is to evaluate the potential effect of alfacalcidol, either alone or in combination with salbutamol or/and prednisolone in the protection and management of experimentally induced allergic asthma.

Materials and methods

Animals

Male guinea pigs weighing 300–500 g were housed at the Animal House of the Faculty of Medicine (Girls), Al-Azhar University. The protocol of the study was approved by the ethics committee of the Faculty of Medicine (Girls), Al-Azhar University.

Drugs

1α-hydroxycholecalciferol (alfacalcidol): was supplied as a glass ampoule containing 1 μg/0.5 ml and it was given intraperitoneally.

Salbutamol sulfate: was supplied as syrup for oral administration contains 2 mg of salbutamol/5 ml.

Prednisolone sodium phosphate: it was supplied as an oral solution. Each 5 ml contains 5 mg prednisolone.

Chemicals

(1) Ovalbumin: was supplied as a fine yellow powder. It was freshly dissolved in distilled water and was given first by intraperitoneally injection and then by the inhalation route using a nebulizer at a concentration of 4%.
(2) Histamine acid phosphate: was supplied as a white powder which was freshly dissolved in distilled water.
(3) Formaldehyde 10%: was supplied as a bottle containing 500 ml aqueous solution.
(4) Kreb's solution: The Staff of Department of Pharmacology; Edinburg (1970) [8].

Design of the experiment

Guinea pigs were divided into two groups:

Group A: normal guinea pigs (received saline) and group B: sensitized guinea pigs.

Then the sensitized animals were divided into eight subgroups: group B1 (control) received saline. Group B2 received salbutamol (465 μg/kg orally twice daily), group B3 received prednisolone (2.325 mg/kg orally once daily), and group 4 received alfacalcidol (0.23 μg/kg intraperitoneally once daily) for 1 week. While group B5 was treated with (salbutamol+prednisolone), group B6 was treated with (salbutamol+alfacalcidol), group B7 was treated with prednisolone+alfacalcidol), and group B8 was treated with salbutamol, prednisolone, and alfacalcidol for 1 week. At 24 h after the last dose the animals were subjected to both:

(a) Pharmacological studies (all groups), (b) histopathological studies (group A and group B1, B3, and B4): by light microscope.

Methods

Sensitization was done according to the allergic asthma model given by Boskabady *et al.* [9].

Doses of salbutamol, prednisolone, and alfacalcidol correspond to the human therapeutic doses were calculated according to the method by Paget and Barnes [10].

In the pharmacological study, the effect of the above-mentioned drugs on contractions of tracheal strips induced by increasing doses of histamine (5–80 μg/ml) was recorded.

The chest was opened and pieces from both lungs representing both large and small airways as well as lung tissues were excised and fixed with 10% formaldehyde. The specimens were sent to the Histopathology Department, Faculty of Medicine (Girls) Al-Azhar University to be stained for light microscopic examination.

Statistical analysis

Analysis of data was conducted through SPSS program version 24

Data were expressed as mean±SEM.

Comparison between groups was done using:

(1) Student's *t* test for quantitative data of two independent samples.
(2) One-way analysis of variance was used for comparing between more than two groups followed by Turkey's post-hoc test.

Statistical significance was acceptable to a level of *P* value less than 0.05.

Tables and graphs were performed using Prism software program (GraphPad Software, incorporated version). Graphs were sketched using GraphPad Prism (ISI, USA) software (version 5) computer program.

Results

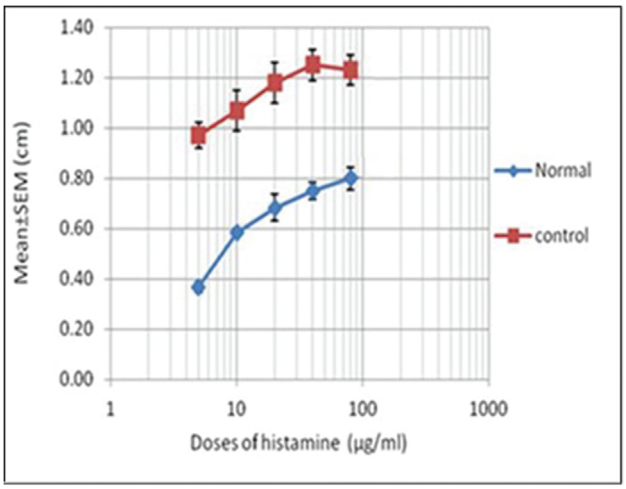
Pharmacological studies

Sensitization of guinea pigs by ovalbumin, caused a significant increase in the amplitude of histamine-induced contractions (Fig. 1).

Treatment of sensitized guinea pigs with salbutamol, prednisolone, or alfacalcidol resulted in a significant reduction in the amplitude of histamine-induced contractions when it was compared with that of sensitized nontreated guinea pigs (Fig. 2 and Table 1).

When alfacalcidol was added to salbutamol or prednisolone in the treatment of sensitized guinea pigs, it caused more decrease in the amplitude of histamine-induced contractions and the difference in the decrease was found to be statistically significant as compared with control, sensitized group (Fig. 3 and Table 2).

Figure 1

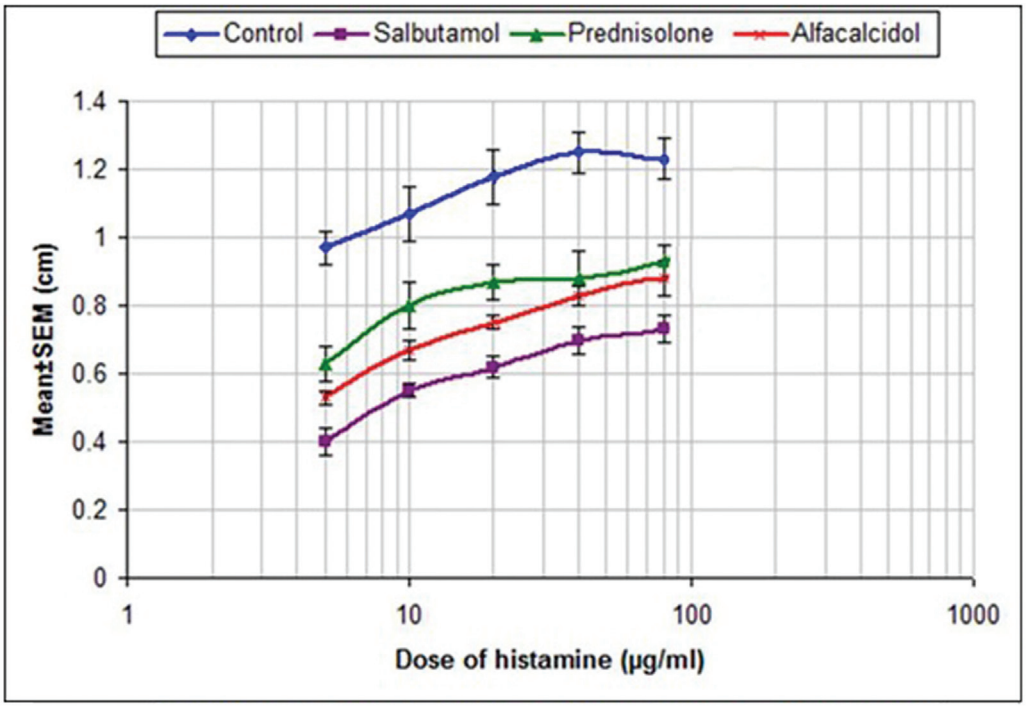


Mean±SEM amplitude of histamine (5–80 µg/ml)-induced contractions (cm) of tracheal spiral strips of normal group and control groups.

The highest reduction in the amplitude of contractions of isolated tracheal smooth muscle was produced by the treatment of guinea pigs with a combination of the three drugs: salbutamol, prednisolone, and alfacalcidol. This reduction was more than that produced in either salbutamol-treated or prednisolone-treated groups (Fig. 4 and Table 1.

The highest reduction in the amplitude of tracheal contractions was recorded for the group treated with salbutamol, prednisolone, and alfacalcidol) followed, respectively, by the group treated with salbutamol and prednisolone, salbutamol and alfacalcidol, salbutamol, prednisolone and alfacalcidol, alfacalcidol and then the group treated with prednisolone (Fig. 5).

Figure 2



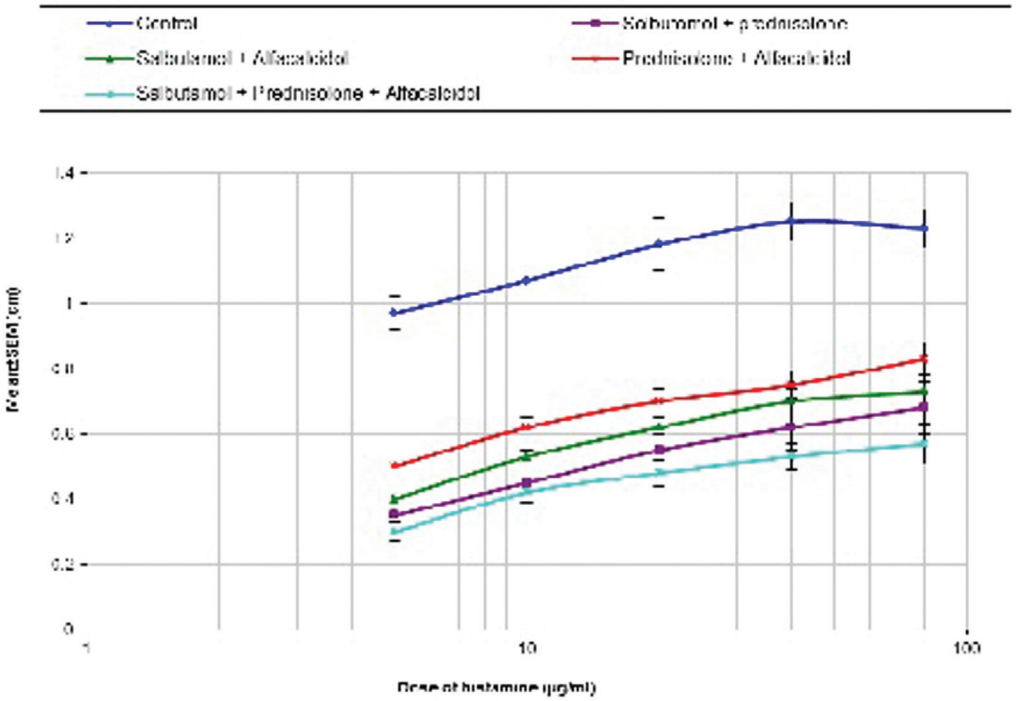
Effect of salbutamol, prednisolone, or alfacalcidol treatment on the amplitude of histamine-induced contractions (mean±SEM) of tracheal strips isolated from sensitized-treated groups in comparison to the control group.

Table 1 Effect of treatment of different groups on histamine-induced contractions (in cm) of tracheal strips isolated from sensitized-treated groups in comparison to the control group

Doses of histamine (µ/ml)	Control (sensitized)	Sensitized then treated with						
		Salbutamol	Prednisolone	Alfacalcidol	Salbutamol +prednisolone	Salbutamol +alfacalcidol	Prednisolone +alfacalcidol	Salbutamol +prednisolone +alfacalcidol
5	0.97±0.05	0.40±0.04*	0.63±0.05*	0.53±0.05*	0.35±0.02*	0.40±0.03*	0.50±0.00*	0.30±0.03*
10	1.07±0.08	0.55±0.02*	0.80±0.07*	0.67±0.03*	0.45±0.05*	0.53±0.02*	0.62±0.03*	0.42±0.03*
20	1.18±0.08	0.62±0.03*	0.87±0.05*	0.75±0.02*	0.55±0.05*	0.62±0.03*	0.70±0.04*	0.48±0.04*
40	1.25±0.06	0.70±0.04*	0.88±0.08*	0.83±0.03*	0.62±0.07*	0.70±0.04*	0.75±0.04*	0.53±0.04*
80	1.23±0.06	0.73±0.04*	0.93±0.05*	0.88±0.05*	0.68±0.08*	0.73±0.06*	0.83±0.05*	0.75±0.06*

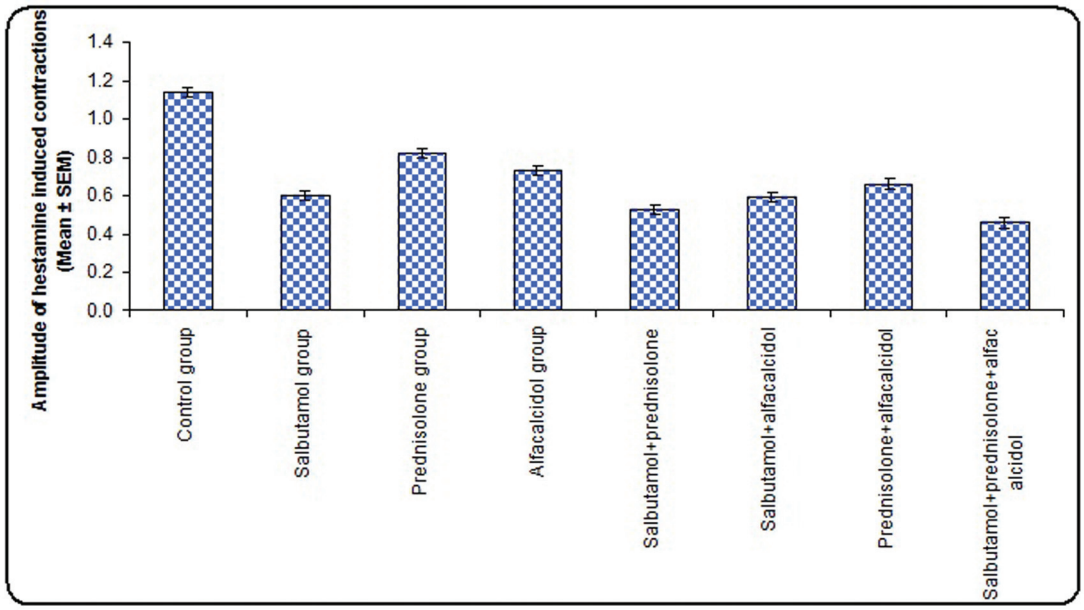
*Significant in comparison to the control group.

Figure 3



Effect of salbutamol+prednisolone, salbutamol+alfacalcidol, prednisolone+alfacalcidol and salbutamol+prednisolone+alfacalcidol treatment on the amplitude of histamine-induced contractions (mean±SEM) of tracheal strips isolated from sensitized-treated groups in comparison to the control group.

Figure 4



Comparison between the amplitude of histamine-induced contractions (cm) of tracheal spiral strips isolated from sensitized nontreated group and treated groups either alone or in combinations (one-way analysis of variance test).

Histopathological examination

The sensitized group showed manifestation of airway inflammation and remodeling associated with intrabronchial inflammatory exudates and hyperplasia of bronchial epithelium (Fig. 6a). Increased number of goblet cells and mast cells was also present (Fig. 6b, c, respectively).

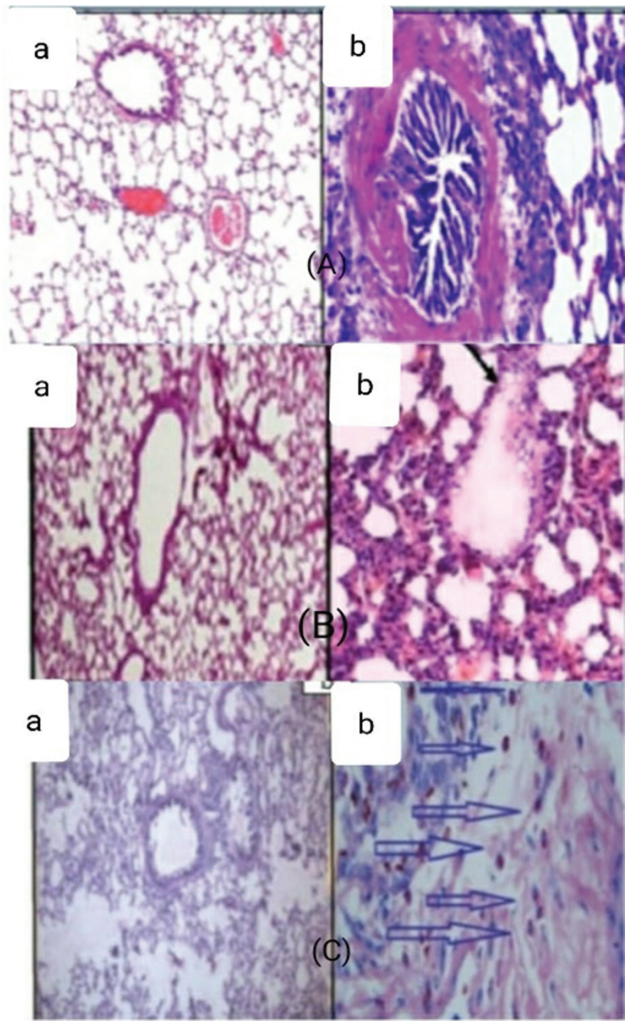
Histopathological examination of lung sections from prednisolone-treated and alfacalcidol-treated groups

Table 2 Comparison between the amplitude of histamine-induced contractions (cm) of tracheal spiral strips isolated from sensitized nontreated group and treated groups either alone or in combinations (one-way analysis of variance test)

Groups	Histamine	
	Mean±SEM	Range
Control group	1.14±0.03	0.8–1.5
Salbutamol group	0.60±0.03[a,b]	0.3–0.9
Prednisolone group	0.82±0.03[a]	0.5–1.2
Alfacalcidol group	0.73±0.03[a]	0.5–1
Salbutamol+prednisolone	0.53±0.03[a,c,d]	0.3–0.9
Salbutamol+alfacalcidol	0.60±0.03[a,c]	0.3–0.9
Prednisolone+alfacalcidol	0.66±0.03[a,c]	0.4–1.2
Salbutamol+prednisolone+alfacalcidol	0.46±0.02[a,b,c,e]	0.2–0.8

Data are mean±SEM and analysis was done by one-way analysis of variance followed by Turkey's post-hoc test. a, compared with the control group; b, compared with prednisolone or alfacalcidol; c, compared with alfacalcidol; d, compared with (salbutamol+alfacalcidol) or (prednisolone+alfacalcidol); e, compared with all the previous treated groups. *P* value less than 0.05.

Figure 5



(A) Photomicrographs for lung sections isolated from normal and sensitized guinea pigs stained with H&E: (a) normal and (b) sensitized, showed manifestation of airway inflammation and remodeling. (B) Photomicrographs for lung sections isolated from normal and sensitized guinea pigs stained with PAS stain to show goblet cells: (a) normal. (b) Sensitized: showed increased number of goblet cells. (C) Photomicrographs for lung sections isolated from normal and sensitized guinea pigs stained with toluidine blue to show mast cells: (a) normal, (b) sensitized, showed an increased number of mast cells. H&E, hematoxylin and eosin.

showed that the prednisolone-treated group showed dilated bronchial lumen decrease in the inflammatory reaction with absence of mast cells and decreased goblet cells (Fig. 7b), while the alfacalcidol-treated group showed mild-to-moderate alveolar and peribronchial inflammatory cell infiltrate (Fig. 7c). As compared with the prednisolone-treated group, alfacalcidol-treated group showed decreased goblet cell number and a decrease in the number of mast cells (Fig. 8a, b).

Discussion

Epidemiologic evidence links poor vitamin D status to osteoporosis, several cancers, diabetes mellitus, multiple sclerosis, tuberculosis, and autoimmune diseases [6].

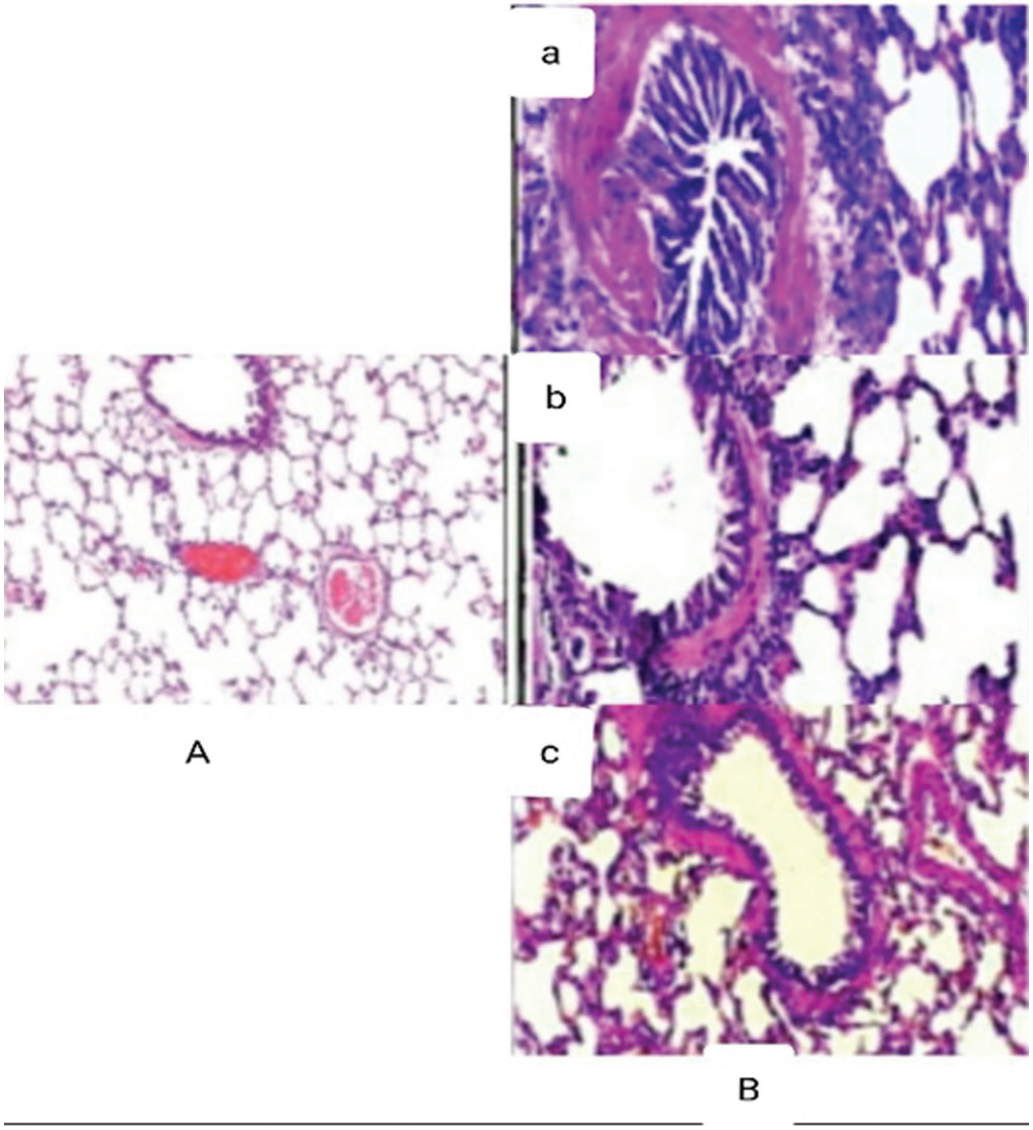
Unfortunately, the effective therapeutic doses that were required to treat these disorders can produce substantial hypercalcemia. This limitation of calcitriol therapy has spurred the development of vitamin D analogs that retain the therapeutically important properties of 1,25(OH)2D3, but with reduced calcemic activity [11].

Increased responsiveness to histamine in sensitized guinea pigs, which was demonstrated in this study, reflects ASM hyperreactivity, which could be linked to chronic airway inflammation [12].

Salbutamol is β_2-adrenoreceptor agonist which can induce the relaxation of ASM by coupling with β_2-adrenoreceptors, which is the major anti-asthmatic mechanism of these drugs [13].

The presumed mechanism of prednisolone is due to its inhibitory action on antigen-induced release of ASM spasmogens and inhibition of the release of a range of

Figure 6



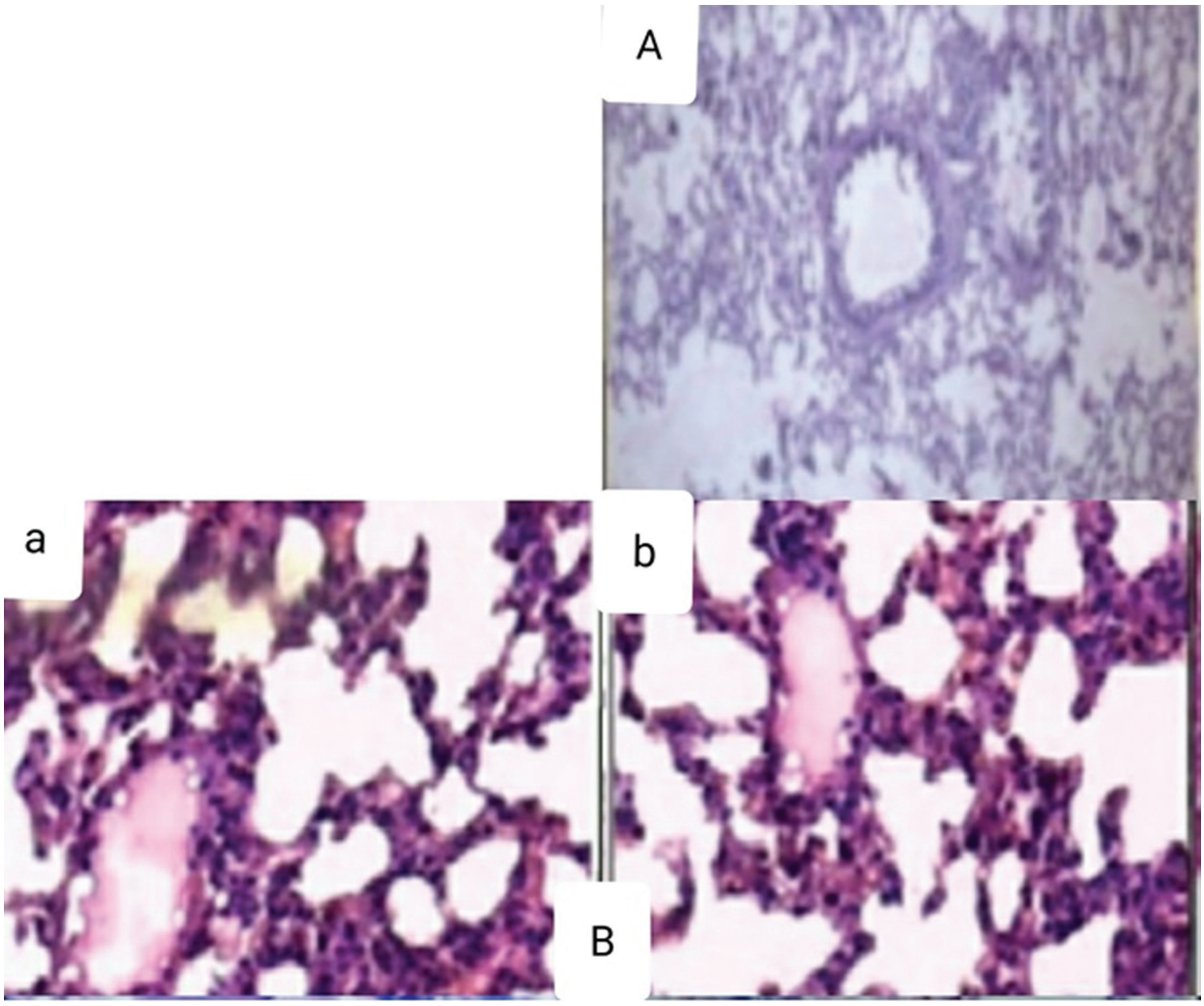
(B) Photomicrographs for lung sections stained with H&E, isolated from sensitized (nontreated) and treated guinea pigs: (a) sensitized (nontreated), (b) sensitized-treated with prednisolone: showed dilated bronchial lumen, decreased vascularity of the surrounding lung tissue and decrease in the inflammatory reaction. (c) sensitized-treated with alfacalcidol: showed mild-to-moderate alveolar and peribronchial inflammatory cell infiltrate and decreased smooth muscle hyperplasia. (A) All in comparison to normal. H&E, hematoxylin and eosin.

cyclooxygenase-derived spasmogens [14]. Prednisolone has very potent anti-inflammatory effect caused by the inhibition of transcription of various proinflammatory proteins such as lipocortin-1 [15]. This leads to a decrease in the release of arachidonic acid as well as the release of prostaglandins and leukotrienes from inflammatory cells of the lung [16]. It also acts by inhibiting the induction of cyclooxygenase-2 enzyme [17].

The beneficial role of alfacalcidol in this study may be mediated through its broncho-relaxant, anti-inflammatory, and anti-remodeling effects. The broncho-relaxant effect of alfacalcidol could be attributed to its potent modulator effect on ASM cells. Vitamin D has been shown to inhibit the progression of the cell cycle, thus decreasing ASM proliferation. It also decreases the production of potent inflammatory mediators such as tumor necrosis factor-α, interleukin-8, and regulated upon activation, normal T cell expressed, and secreted (RANTES), which results in reduced chemotaxis of inflammatory cells to the airways. Vitamin D has also been shown to modulate the expression of mediators involved in collagen deposition and airway remodeling. The overall effect of this modulation is decreased inflammation, airway hyperresponsiveness, and airway remodeling associated with asthma [5].

In-vitro challenge with ovalbumin evokes mast cell degranulation and release of mediators that induce ASM contractions [18]. So, the reduction in

Figure 7



(A) (a) normal, (b) photomicrographs for lung sections stained with PAS, isolated from prednisolone-treated and alfacalcidol-treated guinea pigs: (a) prednisolone-treated group, (b) alfacalcidol-treated group, (c) photomicrographs for lung sections stained with Toluidine blue isolated from prednisolone-treated and alfacalcidol-treated groups.

contraction of tracheal strip with vitamin D could be potentially attributed to its effect on mast cells [19].

Concerning the anti-inflammatory and anti-remodeling effects of alfacalcidol, Bosse *et al.* [20] have provided the first evidence that VDR is present and functional in bronchial smooth muscle cells. They also demonstrated that the expression of many genes including those implicated in asthma predisposition and pathogenesis, in addition to genes that may play an important role in airway remodeling.

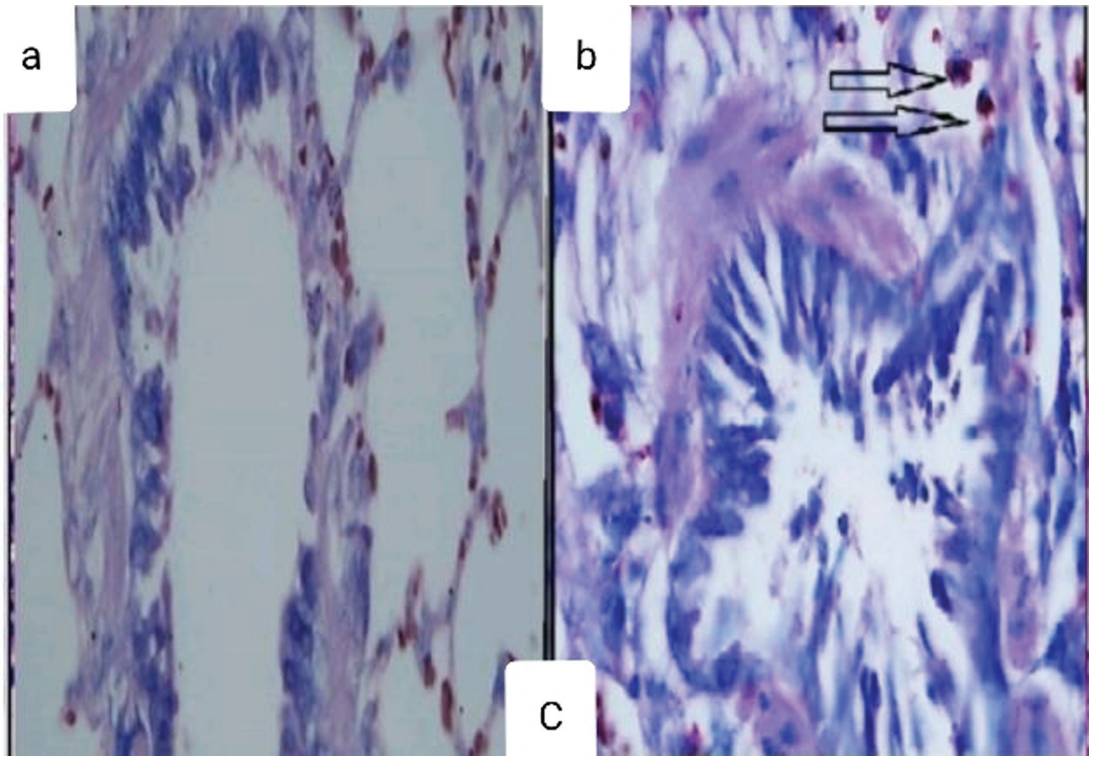
Other studies have highlighted the therapeutic role of vitamin D supplementation in the airways of animal models. Lai *et al.* [21] reported that intraperitoneal administration of 1,25(OH)2D3 at the time of allergen challenge was associated with attenuation of established structural changes in the airways, such as goblet cell hyperplasia, increased ASM mass, and reduced chronic inflammation in the lung tissue.

Alfacalcidol caused a decrease in the number of mast cells, which play an important role in allergic asthma. This result is in accordance with other studies by Baroni *et al.* [22], who reported a dose-dependent inhibition of mast cell differentiation by 1,25(OH)2 D3 at various stages of mast cell development. Moreover, Sandhu and Casale [23] mentioned that mast cells have been shown to be increased in the airways of asthmatic patients compared with healthy controls.

When alfacalcidol was added to salbutamol in the treatment of sensitized guinea pigs, it caused more bronchial relaxation, which may be attributed to its anti-inflammatory and anti-remodeling effects.

When alfacalcidol was added to prednisolone, it caused more bronchial relaxation which may be caused by its anti-inflammatory effect. This effect also proved that

Figure 8



(a) Prednisolone-treated group and (b) alfacalcidol-treated group.

alfacalcidol can enhance steroid responsiveness. The mechanism by which alfacalcidol enhances steroid responsiveness was studied by Xystrakis *et al.* [24], where they showed that adding vitamin D to cell cultures increases glucocorticoid-induced secretion of interleukin-10, which inhibit cytokine secretion by allergen-specific Th2 cells. Moreover, Sutherland *et al.* [25] reported that a low vitamin D level is associated with impaired lung function and increased steroid use or decreased steroid response.A recent clinical investigation also showed that high vitamin D levels are associated with better lung function, less airway hyperresponsiveness, and improved glucocorticoid response [26].

Conclusion

Alfacalcidol can be used as an adjuvant in the management of asthma in addition to β_2-adrenoceptor agonists and corticosteroids. Its use is attributed to its broncho-relaxant, anti-inflammatory, and anti-remodeling effects.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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