

Assessment of the Mechanistic Role of Cinnarizine in Modulating Experimentally-Induced Bronchial Asthma in Rats

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Key Words

Cinnarizine · Dexamethasone · Bronchial asthma · Inflammatory infiltration · Oxidative stress · Rat

Abstract

Background/Aims: Calcium influx, inflammatory infiltration, cytokine production, immunoglobulin E activation and oxidative stress play coordinated roles in bronchial asthma pathogenesis. We aim to assess the protective effect of cinnarizine against experimentally induced bronchial asthma.

Methods: Bronchial asthma was induced by ovalbumin sensitization and challenge. Rats were allocated into a normal control, an asthma control, a dexamethasone (standard) treatment, and 2 cinnarizine treatment groups. The respiratory functions tidal volume (TV) and peak expiratory flow rate (PEFR), the inflammatory cytokines tumor necrosis factor- α (TNF- α) and interleukin-5 (IL-5) in lung tissue, the allergic immunoglobulin IgE in serum, the absolute eosinophil count (AEC) in bronchoalveolar lavage fluid (BALF), as well as the oxidative and nitrosative markers glutathione reduced (GSH) and superoxide dismutase (SOD) in lung tissue and nitric oxide end products (NOx) in BALF were assessed, followed by a histopathological study. **Results:** Cinnarizine administration significantly restored TV, PEFR, TNF- α , IL-5, IgE, AEC, GSH, SOD and NOx values back to normal levels,

and significantly decreased perivascular and peribronchiolar inflammatory scores. **Conclusion:** Cinnarizine may protect against experimental bronchial asthma. Suppressant effect of cinnarizine on pro-inflammatory cytokines release, IgE antibody production, eosinophil infiltration as well as oxidative and nitrosative stress may explain its anti-asthmatic potential.

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Introduction

Over 250,000 patients die from bronchial asthma each year, with a 50% increase every decade [1, 2]. Bronchial asthma is associated with infiltration of eosinophils, mast cells and activated T-helper lymphocytes, leading to increased release of allergic and inflammatory mediators including histamine, leukotrienes, tumor necrosis factor- α (TNF- α), interleukin-5 (IL-5) and nitric oxide [3, 4]. Eosinophils play a critical role recently reported as modulation of the responsiveness of CD4⁺ T-lymphocytes [5]. Massive amounts of histamine are released from mast cells upon their stimulation by occupied IgE antibodies [6]. Tidal volume (TV), peak expiratory flow rate (PEFR) and other indices of respiratory performance are massively ameliorated [7, 8].

Current treatments of bronchial asthma rely mostly on anti-inflammatory and bronchodilator activities, and so disease-modifying treatment lines are claimed. Cinnarizine is a piperazine derivative having potent histamine H₁ antagonistic and calcium channel blocking activities [9–12]. The roles of histamine release [13, 14] and calcium influx [15, 16] were previously studied regarding airway inflammation and hyper-responsiveness. However, no sufficient data are available regarding the beneficial effect of cinnarizine in experimental asthma.

Accordingly, this study was conducted to evaluate mechanistically the possible beneficial effect of cinnarizine against OVA-induced bronchial asthma in rats, where respiratory functions like TV and PEFR, inflammatory cytokines like TNF- α and IL-5 in lung tissue, IgE in serum, absolute eosinophil count (AEC) in bronchoalveolar lavage fluid (BALF), as well as oxidative and nitrosative markers like glutathione reduced (GSH), superoxide dismutase (SOD) and nitric oxide end products (NOx) were assessed.

Material and Methods

Animals

One-month acclimatized adult male albino rats, 150 ± 5 g, were handled according to the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 86–23, revised 1985).

Drugs, Chemicals and Reagent Kits

Cinnarizine, ovalbumin (OVA; grade III), aluminum hydroxide (Al(OH)₃), pyrogallol, tris buffer, N-(1-Naphthyl)ethylenediamine dihydrochloride and vanadium chloride were obtained from Sigma Aldrich Chemicals Co. (USA). Dexamethasone was obtained from Zhejiang Jingxin Pharmaceuticals Co. (China). IgE ELISA kit was obtained from Express Biotech Co. (USA). TNF- α ELISA kit was obtained from Ray Biotech Co. (USA). IL-5 ELISA kit was obtained from Cusabo Biotech Co. (China). GSH reagent kit was purchased from Bio Diagnostic Co. (Egypt).

Experimental Design

Rats were divided into a normal control group, an asthma control group subjected to OVA sensitization and challenge, a standard treatment group receiving dexamethasone (1 mg/kg/day, p.o.), and 2 treatment groups receiving cinnarizine (30 and 60 mg/kg/day, p.o.) for 14 days prior to OVA challenge in addition to 3 days during challenge, that is, starting from day 6 (fig. 1). Drug doses were adjusted based on pilot trials guided with published literature doses of dexamethasone [17] and cinnarizine [18, 19].

Methodology

Induction of Bronchial Asthma

Bronchial asthma was induced using OVA sensitization and challenge [20]. For sensitization, OVA was prepared in a concen-

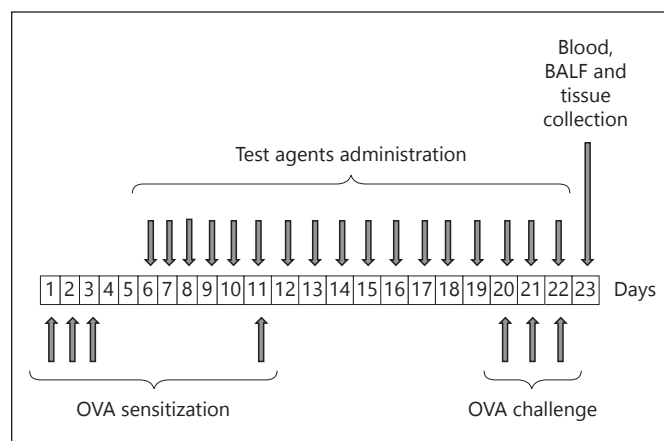


Fig. 1. A schematic diagram of experimental design, illustrating schedule of OVA sensitization and challenge, with timing of drug administration and sampling.

tration of 400 μ g/ml in saline and precipitated with an aqueous 20 mg/ml solution of Al(OH)₃ at 1:1 ratio, yielding a sensitizing preparation of 200 μ g OVA/10 mg Al(OH)₃. One ml of the sensitizing preparation was injected intraperitoneally at days 1, 2, 3 and 11. For challenge, 300 μ l of saline containing 1.5 mg of OVA was instilled into both airways of nasal cavity according to the installation technique described earlier [21], for 3 consecutive days starting from day 20. Blood, tissue and BALF were collected 1 day after the last intranasal challenge dose.

Assessment of Respiratory Functions

The respiratory functions TV and PEFR were assessed by the aid of a spirometer (AD Instruments, ML140, England) 1 h after the last intranasal OVA challenge as described by Salama et al. [22].

Sampling

Preparation of Serum. Twenty-four hours after the last intranasal OVA challenge, blood was collected from the retro-orbital plexus vein, left to clot at room temperature, then centrifuged at 1,500 rpm for 10 min for serum separation using a cooling centrifuge (Sigma 3–30K, USA). Serum was stored at -20°C and used later for analysis of IgE level [23].

Preparation of BALF. Soon after blood collection, animals were sacrificed by cervical dislocation and carefully dissected. Lungs were lavaged with 2 aliquots of 5 ml of ice-cold normal saline, yielding a total recovery volume per rat of about 8–9 ml. BALF was collected and centrifuged at 400 rpm for 10 min at 4°C using a cooling centrifuge [24]. The supernatant liquid was stored at -80°C and kept for analysis of NOx. Cellular pellets were re-suspended in 50 μ l of phosphate buffered saline for the assessment of AEC in the pellets.

Preparation of Lung Tissue Homogenate. Both lungs were removed, washed with ice-cold isotonic saline solution and dried with ash-less filter papers. The left lung was kept for the histopathological study, while the right lung was homogenized with 4 volumes of ice-cold saline to prepare a 25% homogenate using a ho-

mogenizer (yellow line, DI18 basic, Germany). The homogenates were centrifuged at 2,000 rpm for 20 min at 4°C using a cooling centrifuge, then stored in a -80°C deep freezer (Als angelantoni life science, Italy) for the time of assay of GSH, SOD, TNF-α and IL-5.

Assessment of AEC

The AEC was measured in the pellets within 1 h of BALF collection based on the principle described earlier [25] using a hemocytometer. Briefly, a fresh Dunger's diluting fluid was prepared with 0.5 g Eosin Yellow, 0.5 ml of 95% acetone, 0.5 ml of 40% formalin and 99 ml of distilled water. This solution can stain the eosinophilic granules brightly and distinctly, while lysing erythrocytes and other types of leucocytes.

Assessment of Biological Markers

Pulmonary tissue TNF-α and IL-5 were measured using ELISA kits according to the methods described by Brouckaert et al. [26] and Verhasselt et al. [27], respectively. Serum IgE was measured using ELISA kits based on the principle described earlier [28]. Pulmonary tissue GSH stores and SOD activity were measured according to the methods of Rahman et al. [29] and Marklund and Marklund [30], respectively. BALF NOx level was assessed according to the method described by Miranda et al. [31].

Histopathological Study

The dried left lungs were hardened in 10% formalin solution in saline and used for the preparation of paraffin sections, 5 μm thick. Routine hematoxylin and eosin (H&E) stain was used as described previously by Bancroft and Steven [32].

Perivascular and peribronchiolar inflammation scores were determined with a score of 0 assigned to normal sections, a score of 1 assigned to sections with few inflammatory cells, a score of 2 assigned to sections with a ring of inflammatory cells one cell layer deep, a score of 3 assigned to sections with a ring of inflammatory cells 2 to 4 cells deep, and finally a score of 4 assigned to sections with a ring of inflammatory cells of more than 4 cells deep [33, 34].

Statistical Analysis

Statistical analysis of the respiratory and biochemical parameters was performed using one-way analysis of variance (ANOVA) test followed by Bonferroni test to compare groups pairwise, with $p < 0.05$ considered significant, while statistical analysis of the inflammatory scores in the histopathological study was performed using chi-square test, with $p < 0.05$ considered significant, by the aid of Statistical Package for Social Sciences (SPSS) program, version 16 (SPSS Inc., Chicago, Ill., USA).

Results

Respiratory Functions

Cinnarizine administration in the lower dose level significantly increased the suppressed TV and PEFr but to values significantly lower than normal control or dexamethasone treatment values. However, cinnarizine administration in the higher dose level significantly restored TV and PEFr back to normal levels (table 1).

Table 1. Effect of cinnarizine administration on respiratory functions (TV and PEFr) in rats with OVA-induced bronchial asthma

Groups	Parameters	
	TV, ml	PEFr, ml/min
Normal control	0.1036±0.00279	11.8±0.20
Asthma control	0.0180±0.00071 ^a	2.1±0.14 ^a
Dexamethasone treatment	0.0919±0.00126 ^{a, b}	11.2±0.13 ^b
Cinnarizine lower dose treatment	0.0336±0.00112 ^{a-c}	8.2±0.60 ^{a, b}
Cinnarizine higher dose treatment	0.0968±0.00116 ^b	11.0±0.31 ^b

Each value represents the mean of 8–10 rats ± SEM.

^a Significantly different from normal control group at $p < 0.05$.

^b Significantly different from asthma control group at $p < 0.05$.

^c Significantly different from dexamethasone treatment group at $p < 0.05$.

Table 2. Effect of cinnarizine administration on pulmonary tissue cytokines (TNF-α and IL-5) production in rats with OVA-induced bronchial asthma

Groups	Parameters	
	tissue TNF-α, pg/g tissue	tissue IL-5, pg/g tissue
Normal control	7.9±0.27	15.7±1.21
Asthma control	55.0±2.90 ^a	73.9±3.86 ^a
Dexamethasone treatment	16.4±0.46 ^{a, b}	22.0±1.24 ^b
Cinnarizine lower dose treatment	38.2±1.50 ^{a-c}	48.0±2.70 ^{a-c}
Cinnarizine higher dose treatment	18.5±0.98 ^{a, b}	30.8±1.25 ^{a, b}

Each value represents the mean of 8–10 rats ± SEM.

^a Significantly different from normal control group at $p < 0.05$.

^b Significantly different from asthma control group at $p < 0.05$.

^c Significantly different from dexamethasone treatment group at $p < 0.05$.

Pulmonary Tissue Cytokines

Cinnarizine administration in the lower dose level significantly decreased TNF-α and IL-5 as compared with control asthma, but to values significantly higher than normal control and standard treatment values. With the higher dose level, TNF-α and IL-5 values were not significantly different from those that were obtained as a result of dexamethasone treatment (table 2).

Table 3. Effect of cinnarizine administration on serum IgE level and BALF AEC in rats with OVA-induced bronchial asthma

Groups	Parameters	
	serum IgE, ng/ml	BALF AEC, count/mm ³
Normal control	19.3±0.99	930±89
Asthma control	250.4±12.90 ^a	6,500±72 ^a
Dexamethasone treatment	31.9±1.78 ^b	1,800±28 ^b
Cinnarizine lower dose treatment	117.0±7.08 ^{a-c}	5,625±973 ^{a-c}
Cinnarizine higher dose treatment	44.4±3.17 ^b	1,590±160 ^b

Each value represents the mean of 8–10 rats ± SEM.

^a Significantly different from normal control group at $p < 0.05$.

^b Significantly different from asthma control group at $p < 0.05$.

^c Significantly different from dexamethasone treatment group at $p < 0.05$.

Table 4. Effect of cinnarizine administration on oxidative and nitrosative markers (pulmonary tissue GSH and SOD, and BALF NO_x) in rats with OVA-induced bronchial asthma

Groups	Parameters		
	tissue GSH, μmol/g tissue	tissue SOD, U/g tissue	BALF NO _x , nmol/ml
Normal control	177.3±7.90	75.0±3.72	21.8±2.03
Asthma control	37.8±5.60 ^a	53.3±2.03 ^a	52.7±4.50 ^a
Dexamethasone treatment	178.0±16 ^b	76.4±2.56 ^b	33.0±3.40 ^b
Cinnarizine lower dose treatment	142.5±11.40 ^b	67.7±5.55	38.3±3.40 ^{a, b}
Cinnarizine higher dose treatment	232.0±13.17 ^{b, c}	75.0±3.04 ^b	23.7±1.67 ^b

Each value represents the mean of 8–10 rats ± SEM.

^a Significantly different from normal control group at $p < 0.05$.

^b Significantly different from asthma control group at $p < 0.05$.

^c Significantly different from dexamethasone treatment group at $p < 0.05$.

Serum IgE and BALF AEC

Cinnarizine administration in the lower dose level significantly decreased the elevated serum IgE level, but not back to normal levels, without affecting AEC in BALF. On the other side, the higher dose level restored both serum IgE and BALF AEC to normal values (table 3).

Oxidative and Nitrosative Markers

Cinnarizine administration in the lower dose level significantly corrected tissue GSH and BALF NO_x levels, with the former being restored to normal levels. Alternatively, cinnarizine administration in the higher dose level significantly corrected tissue GSH and SOD as well as BALF NO_x, all back to normal levels, with SOD and NO_x levels not significantly different from the corresponding dexamethasone values, and with GSH value being significantly higher than the corresponding value with dexamethasone treatment (table 4).

Histopathological Study

Lung sections of OVA-treated rats co-treated with cinnarizine in the lower dose level showed some distorted pulmonary architecture with mild perivascular and moderate peribronchiolar inflammatory infiltration, while sections of rats treated with cinnarizine in the higher dose level showed more or less normalized pulmonary architecture with mild perivascular and mild peribronchiolar inflammatory infiltration (fig. 2).

Perivascular and peribronchiolar inflammatory scores were significantly decreased in the 2 cinnarizine treatment groups. With the higher dose of cinnarizine, the perivascular inflammatory score was not significantly different from that of the corresponding score in normal rats (tables 5, 6).

Discussion

The present investigation aims to evaluate the possible protective effect and the underlying mechanisms of cinnarizine against OVA-induced bronchial asthma in rats. Our results showed an efficient induction of bronchial asthma evidenced by significant and massive reductions in the respiratory functions TV and PEFR (table 1), which agreed with previous investigations in rats and mice [35–37]. Cytokines, particularly TNF-α, are involved in the initiation and progression of airway inflammation [38, 39], with IL-5 particularly reported to have a central role in the initiation of eosinophilic airway inflammation [40], strongly agreeing with our results (table 2). IgE plays a key role in the pathogenesis of extrinsic asthma, as in our model (table 3), through stimulating mast cell degranulation [41]. We also showed that OVA-induced bronchial asthma was associated with oxidative and nitrosative stress (table 4), which was also supported by the results of previous investigators [42, 43]. Nadeem et al. [44] suggested that such oxido-nitro-

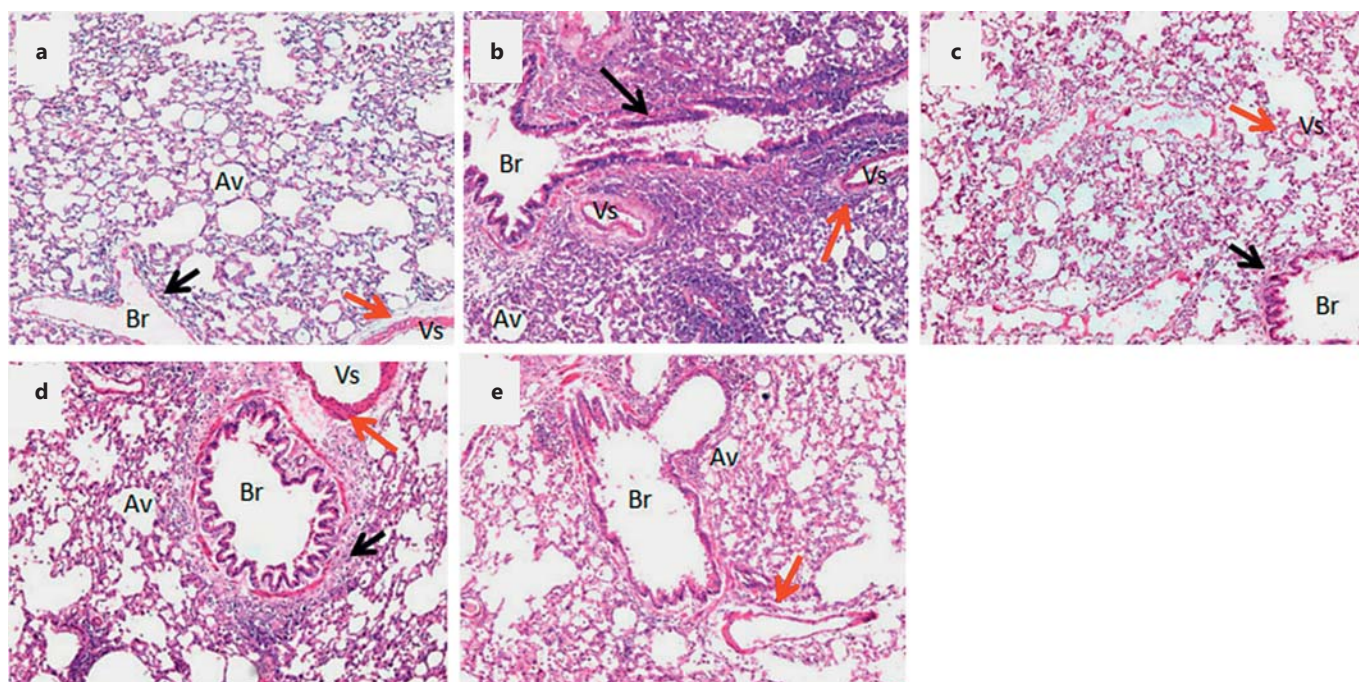


Fig. 2. Photomicrographs of lung sections obtained from different groups (H&E $\times 100$). **a** Normal control, showing normal pulmonary architecture of bronchioles (Br), alveoli (Av) and pulmonary vasculature (Vs). The peribronchiolar (black arrow) and perivascular (red arrow) regions are clearly defined. **b** Asthma control, showing abnormal pulmonary vasculature (Vs), distorted bronchial (Br) and alveolar (Av) architectures with severe perivascular (red arrow) and peribronchiolar (black arrow) inflammatory infiltrates. **c** Dexamethasone treatment, showing more or less normalized lung architecture and clearly defined bronchioles (Br) and blood vessels (Vs), with very mild perivascular (red arrow) and

peribronchiolar (black arrow) inflammatory infiltrations. **d** Cinnarizine lower dose treatment, showing improved pulmonary bronchiolar architecture (Br) and pulmonary alveoli (Av), with mild perivascular (red arrow, Vs) and moderate peribronchiolar (black arrow) inflammatory infiltrations with some distortion in the normal pulmonary architecture. **e** Cinnarizine higher dose treatment, showing more or less normalized bronchioles (Br) and alveoli (Av), with mild perivascular (red arrow) and peribronchiolar (black arrow) inflammatory infiltrations with more or less preservation of the normal pulmonary architecture.

Table 5. Perivascular inflammation score counts in different groups

Groups	Score					significance
	0	1	2	3	4	
Normal control	1	4	0	0	0	–
Asthma control	0	0	0	1	4	a
Dexamethasone treatment	4	1	0	0	0	b
Cinnarizine lower dose treatment	0	2	2	1	0	a–c
Cinnarizine higher dose treatment	0	4	1	0	0	b, c

^a Significantly different from normal control group at $p < 0.05$.

^b Significantly different from asthma control group at $p < 0.05$.

^c Significantly different from dexamethasone treatment group at $p < 0.05$.

Table 6. Peribronchiolar inflammation score counts in different groups

Groups	Score					significance
	0	1	2	3	4	
Normal control	3	2	0	0	0	–
Asthma control	0	0	0	1	4	a
Dexamethasone treatment	2	3	0	0	0	b
Cinnarizine lower dose treatment	0	0	2	2	1	a–c
Cinnarizine higher dose treatment	0	2	3	0	0	a–c

^a Significantly different from normal control group at $p < 0.05$.

^b Significantly different from asthma control group at $p < 0.05$.

^c Significantly different from dexamethasone treatment group at $p < 0.05$.

sative stress may also increase bronchial hyper-responsiveness.

In the present investigation, cinnarizine showed a very good anti-asthmatic potential comparable with the standard anti-asthmatic agent dexamethasone, evidenced from improved respiratory functions in rats with OVA-induced asthma (table 1). Histopathological examination of pulmonary sections strongly confirmed the results of the numerical data estimations (fig. 2). To date, only too old, few and incomplete studies were performed to assess the beneficial effects of cinnarizine on bronchial asthma [45, 46].

Interestingly, our results showed that the ability of cinnarizine to suppress the elevated OVA-induced TNF- α and IL-5 production in pulmonary tissue was not significantly different from that of dexamethasone (table 2). To support this finding, analysis of the perivascular and peribronchiolar inflammatory infiltration scores revealed that cinnarizine in the higher dose level showed a potent anti-inflammatory potential comparable with that of dexamethasone (tables 5, 6). No previous data showed an effect of cinnarizine on cytokine production or inflammation, but Abdel-Salam [47] reported an anti-inflammatory effect for cinnarizine on an experimental acetic acid-induced visceral inflammation model.

A second possible mechanism explaining the anti-asthmatic effect of cinnarizine may be concluded in the present study through its ability to suppress OVA-induced serum IgE antibody elevation (table 3). Although no previous data showed an effect of cinnarizine on serum IgE level, one old study reported a beneficial effect of cinnarizine on experimental asthma mediated by inhibition of IgE-mediated mast cell degranulation [46]. In another study, the ability of the cinnarizine analogue oxatamide to suppress mast cell degranulation was attributed to its ability to lower the cytosolic calcium elevation required for this degranulation [48]. More recently, special calcium channels were investigated in mast cells through where calcium ion influx was shown to play a vital role in mast cell degranulation [49]. In one study, the effectiveness of the essential oil of the Indian plant *Pistacia integerrima* was attributed to its effect on L-subtype calcium channels in mast cells [50].

The results of our study showed that cinnarizine administration could suppress eosinophil infiltration into BALF (table 3). No previous data were reported regarding the effect of cinnarizine on eosinophil activation, but the role of eosinophils in the progression of the inflammatory and immunological arms of bronchial asthma was recently reported [5, 51]. Eosinophils exert their deleteri-

ous effects on asthma progression through the release of several pro-inflammatory mediators including cytokines and others [52].

According to the results of this work, the anti-asthmatic effect of cinnarizine was coupled with an attractive antioxidant and thiol-preserving potential, even better than the standard agent dexamethasone (table 4). No sufficient data reported an anti-oxidant effect for cinnarizine, but anti-oxidants in general were recently reported to have good anti-asthmatic effects [53]. The anti-oxidant N-acetylcysteine was reported to decrease diesel-exhaust airway hyper-responsiveness clinically [54].

Finally, cinnarizine was reported to block histamine H₁ receptors in vivo and in vitro [55, 56], in addition to its calcium channel blocking activity [12, 57]. Histamine H₁-receptors play a major role in the pathogenesis of extrinsic asthma, and blockade of this receptor is well-known to protect against asthmatic attacks [58–60]. Moreover, calcium channel blockade in bronchial tree was reported to decrease airway remodeling [16], while inhibition of calcium influx into mast cells decreases their ability for degranulation and histamine release [49].

In conclusion, cinnarizine may be a promising agent for the management of bronchial asthma. Suppressant effect on pro-inflammatory cytokines release, IgE antibody production, eosinophil infiltration as well as oxidative and nitrosative stress, in addition to its well-known effect on calcium channels and histamine receptors, may explain its anti-asthmatic potential. Further clinical trials are needed to make more in-depth analysis of the study.

Disclosure Statement

No conflict of interest was evident regarding all authors.

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