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Anti-inflammatory effect of N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide and Gluconic acid on Allergic Rhinitis and Asthma controlling

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Abstract

Allergic rhinitis and asthma are main airway disease with a higher prevalence. Eosinophilic inflammation, airway hyper-responsiveness, mucus hyper-secretion, and reversible airflow obstruction are immunopathogenesis symptoms of rhinitis and asthma. Crotonic acid has bioactivity on the inflammation and Gluconic acid as chelator may protect crotonic acid activity in airway and with together may control allergic rhinitis and asthma.

Allergic rhinitis and asthma mice were treated with crotonic and gluconic acid. At least, total IgE, histamine, IL-4, IL-5, and IL-13 levels were measured. In lung tissues, goblet cell hyperplasia, mucus hyper-secretion, and inflammation were evaluated.

The level of IL-5, Goblet cell hyperplasia, perivascular and peribronchial inflammation were controlled by crotonic in asthma and allergic rhinitis groups. But total IgE, histamine, IL-4, IL-13 levels and Mucus hyper-secretion had no significant chenges between treated and non-treated asthma and rhinitis groups.

Crotonic acid can control eosinophilic inflammation via harnessing of IL-5 and prevent goblet cell hyperplasia. When it was used with gluconic acid, had strong effect on control of allergic rhinis and asthma immunopathology.

Introduction

Allergic rhinitis and asthma are complex and multi-factorial airway disease with a increased prevalence in the developed world that is characterized by airway inflammation, bronchoconstriction, dyspnea, wheezing and rhinoria. Allergy is an important problem of public health and afflicting more than 350 million individuals globally. A heightened allergo-immune response to environmental triggers is the dominant feature in patients with a genetic predisposition. In allergic subjects, Th2 response to the specific allergen leads to increased Th2 cytokines and total IgE levels. These factors can sensitize mast cells and basophils, and after re-trigger of allergen, clinical and allergic symptoms are initiated (1, 2).

Allergic reactions are the main orchestra of asthma. Asthma as a complicated bronchi disease and main problem of the respiration, is characterized by cough, breathlessness and wheezing. In the immunopathogenesis of asthma, infiltration of eosinophilic around bronchi and vessels, airway hyper-responsiveness, mucus hyper-secretion, and reversible airflow obstruction and harnessing of these problems are necessary in control of asthma (3, 4).

However, there is not complete cure for allergic diseases until recently. Actually, in worlds, heavy economic burden is imposed on countries national budget annually for providing healthcare services for patients with allergy and asthma. Some natural acids have significant effects on the immune system may be important in the development, severity and course of allergy and asthma can modulate innate and adaptive immune system responses and may therefore influence the development and the course of allergic rhinitis and asthma (1, 3).

Crotonic acid (2E-But-2-enoic acid) is a C4 short-chain unsaturated carboxylic acid. Crotonic acid is oligosaccharide low-molecular component and its bioactivity on the inflammation has been reported but there are no publications using of this component on inflammation of airways (5, 6). Gluconic acid as non-toxic, -volatile, -corrosive, and biodegradable chemical, is highly soluble in water, and its aqueous solution has found application as a catalytic medium for organic synthesis. Also, gluconic acid is powerful chelator (7, 8). The chelating ability of gluconic acid may protect crotonic acid activity in airway and lead to high potent bio-activity of crotonic acid in respiratory system. So, in this study, we used crotonic and gluconic acid to treatment and control of allergic rhinitis and asthma.

Material And Methods

Animal model and treatment schedule

BALB/c mice (7–8 weeks old) were divided in seven groups (n = 15) that include: negative control group or healthy that received no treatment (N), asthma group with no treatment (A), asthma group that was treated with N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide [Crotonic acid (A.Cro)], asthma group that was treated with Crotonic and Gluconic acid (A.Cro.Glu), allergic rhinitis group with no treatment (R), allergic rhinitis group that was treated with Crotonic acid (R.Cro), allergic rhinitis group that was treated with Crotonic and Gluconic acid (R.Cro.Glu). The allergic asthma and allergic rhinitis were induced by ovalbumin (OVA) according to a previously described protocol (3, 4). At least, the blood, broncho-alveolar lavage fluid (BALf), and lung tissue samples were collected.

Immunoglobulin E (IgE)

Blood samples were collected, centrifuged and then sera were separated. Total IgE level was measured by specific mouse ELISA kit.

Histamine level

The histamine level was determined in serum of the studied mice. After the last challenge, collected blood samples were used to the histamine level measurement by specific ELISA Kits.

Cytokines levels

After anesthetization of mice, BALf samples were collected via catheter and then, centrifuged and supernatant was stored for cytokine assay. Levels of interleukin (IL)-4, IL-5, and IL-13 were measured using specific mouse ELISA kits.

Histopathology

At the end of challenging period, the lung tissues were isolated, and then histopathological sections were prepared. Afterwards, the tissues were stained with Hematoxylin and Eosin (H&E) and periodic acid shiff (PAS) stain, and goblet cell hyperplasia, mucus hyper-secretion, peribronchiolar and perivascular inflammation were evaluated by with using a point scoring system as described before (4).

Statistical analysis

The experimental tests were repeated for three times. Result were presented as means ± SD and SPSS was used for analyses. GraphPad Prism was used for design and presentation of curves. The Student's t-tests was used to analyze differences between treated and non-treated groups and in this study, p value less than 0.05 was supposed to be significant.

Results

IgE level

The total IgE was significantly increased in A and R groups (2301 ± 21.2 and 2526.6 ± 38.2 ng/ml respectively) compared to N group (211.9 ± 9.2 ng/ml) ($p < 0.05$). The total IgE level had no significant difference ($p > 0.05$) between non-treated and treated asthma and rhinitis groups (A.Cro: 2284.3 ± 27.9, A.Cro.Glu: 2277.6 ± 29.1, R.Cro: 2490.1 ± 33.2 and R.Cro.Glu: 2484.5 ± 26.3 ng/ml).

Histamine level

The histamine was increased in A and R groups (579 ± 28 and 595 ± 19 ng/ml respectively) significantly ($p < 0.05$) compared N group (81 ± 5 ng/ml). There was no significant difference ($p > 0.05$) between non-treated and treated asthma and rhinitis groups (A.Cro: 522 ± 32, A.Cro.Glu: 526 ± 27, R.Cro: 553 ± 25 and R.Cro.Glu: 549 ± 36 ng/ml).

Cytokines levels

The levels of Th2 cytokines in A and R groups (IL-4: 96.2 ± 4.4 and 98.2 ± 5.5, IL-5: 89.1 ± 6.2 and 76.4 ± 6.2, IL-13: 136.3 ± 20.2 and 130.5 ± 21.1 pg/ml respectively) significantly ($p < 0.05$) increased compared with N group (IL-4: 41.6 ± 3.9, IL-5: 37.7 ± 4.8, IL-13: 66.2 ± 8.1 pg/ml). The levels of IL-4 and IL-13 had no significant difference ($p > 0.05$) between non-treated and treated groups of asthma and rhinitis, but the levels of IL-5 were decreased significantly in treated groups of asthma and allergic rhinitis ($p < 0.05$) compared with non-treated groups. Also, IL-5 level was decreased significantly in A.Cro.Glu group ($p < 0.05$) compared with A.Cro group (Fig. 1).

Histopathology

Inflammation in perivascular and peribronchial, goblet cell hyperplasia and mucus secretion were increased in A group significantly ($p < 0.05$) compared with N group. Goblet cell hyperplasia, perivascular and peribronchial inflammation were significantly decreased in A.cro and A.Cro.Glu groups compared to A group (Fig. 2). Perivascular inflammation was controlled significantly ($p < 0.05$) in A.Cro.Glu group compared to A.cro group ($p < 0.05$). Mucus hyper-secretion had no significant difference between non-treated and treated groups ($p > 0.05$).

Discussion

Asthma as worldwide problem of all countries has highly direct and indirect costs, and also, mortality, morbidity and health system involvement are main problems of asthma. On the other hand, allergic rhinitis as nasal and upper airways inflammation can reduce quality of life, especially in children. Therefore, controlling of the rhinitis and asthma attack is the main goal of asthma treatment (1, 4). Asthma is a complex inflammatory airways disease, which characterized by eosinophilic infiltration, mucus hyper-secretion, goblet cell hyper-plasia, airway hyper-responsiveness and reversible airflow obstruction and allergy comprises the main causes of asthma (2, 3). In our study, there was no significant difference between treated and non-treated asthma and rhinitis groups. May be crotonic acid has weakly effect on the allergopathology mediators.

Airway inflammation heterogeneity in asthma indicates there are different mechanisms involved. Inflammation, allergic reaction, and immune response dysregulation are main mechanisms. The main symptoms of asthma are mucus over secretion, bronchoconstriction, and airway inflammation that lead to cough, airways obstruction, wheezing, breathlessness, and chest tightness (2, 4). Crotonic acid was employed to the immunomycin production by *Streptomyces hygroscopicus* var. *ascomyceticus as* immunosuppressant agent (9). N-(trifluoromethylphenyl)-2-cyano-3-hydroxy-crotonic acid amide (A77 1726) has anti-proliferative effects and anti-inflammatory actions in animal models. Also, in psoriasis clinical trials, it has effect on the epidermal hyperproliferation and inflammatory cells infiltration (10, 11). Goblet cell hyperplasia, perivascular and peribronchial inflammation were cotrolled by crotonic treatment in asthmatic mice. Also, perivascular inflammation was controlled in A.Cro.Glu group better than A.cro group and showed that when crotonic acid was used with gluconic acid, it can have strong effect in control of inflammation. However, it had anti-proliferative and anti-hyperplasia effect on the goblet cells that is important in asthmatic patients.

Environmental triggers in genetically predisposed peoples activate Th2 immune response and orchestrate asthma pathophysiology. Some infections such as Linguatula serrata can change expression of some related molecules and immune responses that should be noted (12, 13). IL-13 plays important role in the mucus secretion and goblet cell hyperplasia. IL-5 activates eosinophils and migration to airways (bronchial inflammation). IL-4 induces IgE isotype switching and mast cells activation. IL-4, IL-5, and IL-13 are increased in the asthmatic patients (1, 3, 4). The g-Aminobutyric acid (GABA) receptors express in granule cells and its agonist cis-4-amino-crotonic acid evokes currents in granule cells (14). Honaucin A consists of 4-chlorocrotonic acid and (S)-3-hydroxy-g-butyrolactone that are connected via an ester linkage and can inhibit lipopolysaccharide-stimulated nitric oxide production. The nitric oxide decreasing is accompanied by transcription decreasing of several pro-inflammatory cytokines. It can be applied in therapeutic areas that are related with inflammation, such as asthma (15, 16). On the other side, cell signaling pathways are important targets for the asthma treatment and recognition of the new ligands (agonists or antagonists) for adaptor molecules can provide new therapeutic approach (1, 3). Crotonic acid may have targeted effect on the cell signaling and may control signaling pathways that are related in inflammation, cell migration and cell proliferation.

This should be considered that development of new anti-inflammatory therapies that target cyclooxygenases (COX) 1 and 2 in patients with allergic diseases are necessary. The crotonic acid agents can be new modulators of inflammation (17, 18). However, some metabolits can attenuate the immune response by inhibition of the transcriptional activity of peroxisome-proliferator-activated receptor gamma (PPARg) and may be explain role in immunomodulation and anti-inflammatory effects (19–21). In this study, the level of IL-5 was decreased in asthma and allergic rhinitis groups that were treated with crotonic acid and also crotonic with gluconic acid. IL-5 level was decreased significantly in A.Cro.Glu group compared with A.Cro group that was showed that when crotonic acid was used with gluconic acid, it had powerful effect in control of IL-5 releasing. May be gluconic acid had protective effect on crotonic acid in the airway and leaded to increasing of stability and efficacy of crotonic acid. Crotonic acid can control inflammation (especially eosinophilic inflammation) via harnessing of IL-5 around bronchial and vessels and also prevent goblet cell proliferation and hyperplasia. When it was used with gluconic acid, had strong effect on control of allergic rhinis and asthma immunopathology. Our study showed that crotonic acid had anti-inflammatory and immunomodulatory effects and its effect on control of inflammation is more powerful that anti-allergy effect, even was used with gluconic acid.

Some limitations were in this study. We did not study Th1 cytokines levels and could not study airway hyperresponsiveness. Some cellular signaling pathway such as NF-κB and the MAP kinase and inflammatory factors such as COX-2, iNOS, and PGs that have effect on asthma pathophysiology, were not studied that should be noted in future researches. Also, we could not find similar study (using Crotonic acid on allergy) and could no compare our result with publicized studies.

Declarations

Ethics approval and consent to participate

All methods were approved by the ethic committee of animal house of ix.med.vet.dep, 2021 (No. IX.MED.VET.DEP.REC.2021.3300011.4).

Consent for publication

Not Applicable.

Availability of data and materials

Not Applicable.

Competing interests

There is no competing interest.

Funding

Not Applicable.

Authors' contributions

XL, EMN and SSA participated in the laboratory testing and drafting the manuscript. XL and SSA supervised the study.

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Figures

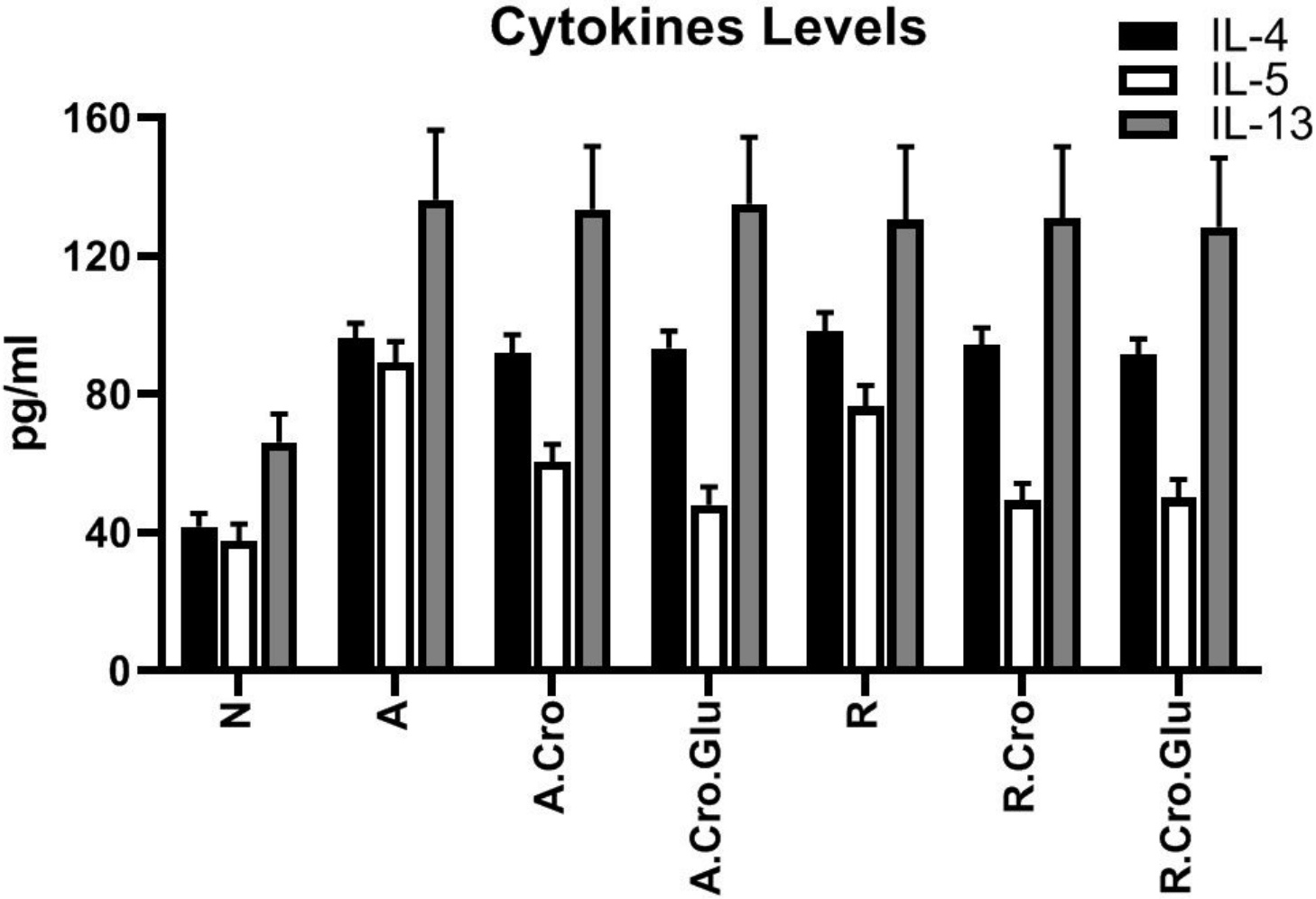

Figure 1

Cytokines levels. The IL-4, IL-5, IL-13 levels in BALf were evaluated by ELISA method in all groups.

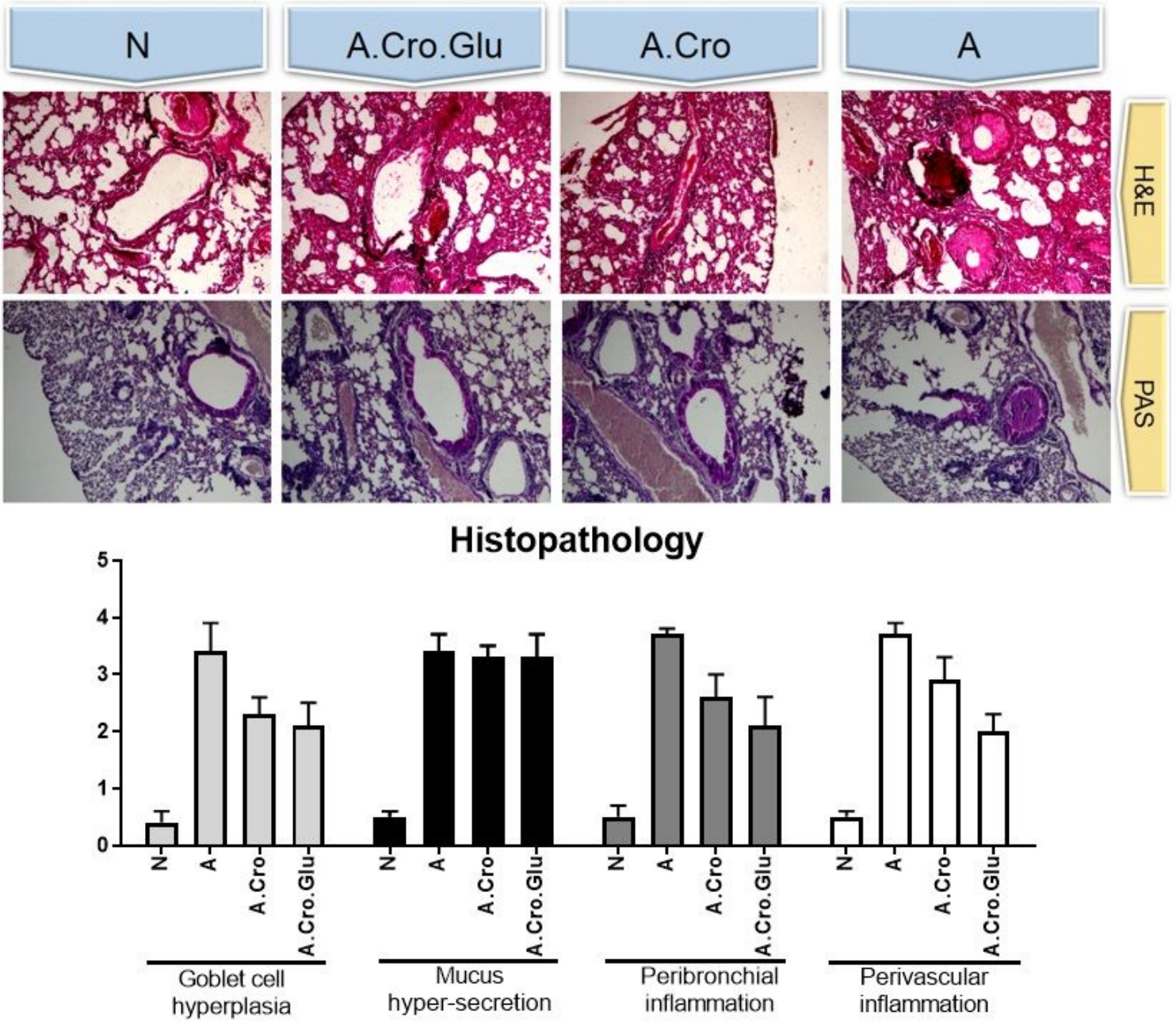

Figure 2

Lung Histopathology.

Lung tissues after staining (with H&E and PAS), were evaluated for the perivascular and the peribronchiolar inflammation, goblet cell hyperplasia and mucus hyper-secretion.