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Asthma is a public health problem worldwide deteriorating quality of life during most active phase. The social burden and cost to public and private health care system due to this disease are substantial. Incidence of bronchial asthma has increased to > 10% afflicting a sizeable population world over (Wong et al., 2004). The respiratory diseases together afflict about 20-30% of the world population. It is estimated that about 70-80% asthmatics are of atopic origin. The diseases like allergic rhinitis with an incidence rate of 26% and asthma with 8-15% incidence rate show rise in India and other developing countries (Anonymous 2000; Gaur et al., 2004). Asthma is an immune inflammatory disorder in which eosinophils play an important role as predominant inflammatory cells in the airways. The persistent inflammation causes an increase in airway hyperresponsiveness (AHR) that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing. The state of hyperresponsiveness and allergen-induced acute bronchoconstriction results due to IgE-dependent release of mediators from airway mast cells. These mediators include histamines, prostaglandins, and leukotrienes that affect on contraction of smooth muscle.

Presently, steroids and disodium cromoglycate are used as anti-inflammatory drugs for treatment of bronchial asthma. Bronchodilators such as β-agonists, anticholinergic and xanthines are used for symptomatic relief. Leukotriene modifiers, a new class of anti-asthma drugs and second-generation anti-histamines are used with some inhibitory effects on allergic responses. Controlled clinical trials have demonstrated that long term treatment with high doses of inhaled glucocorticosteroids may be associated with systemic side effects, including skin thinning, easy bruising, adrenal suppression, and decreased bone mineral density (Lipworth, 1999). At higher doses (10 mg/kg body weight/day or more), theophylline intoxication can occur involving multiple organs of the body. Further, theophylline intoxication in children and adults can result in seizures, arrhythmia and even death. Leukotriene modifiers have been associated with liver toxicity, and there are reports of Churg-Strauss syndrome associated with leukotriene modifier therapy (Health advisory for new asthma drug, 1998). The side effect of some second-generation antihistamines is sedation in the initial treatment period. The action of these drugs is short lived and once the drugs are withdrawn, the symptoms come back quickly because the underlying bronchial inflammation recurs. Therefore, a drug is required which can...
control immune inflammation for a prolong period with no or minimum side effects. Such drug can have a prophylactic role in asthma management.

Choline is a lipotropic agent, involved in maintaining cell structure and facilitates the movement of fats in and out of the cells (Blusztajn, 1998). It is widely distributed in foods, principally in the form of phosphatidylcholine (PC) and also as free choline. Choline is used as an appetizer for years as sorbiline and trichodol solution. Choline, a precursor to acetylcholine and CDP-choline elicits a variety of useful pharmacological effects in many diseases, including stroke, dementia, Alzheimer’s and Parkinson’s disease. Previous studies with choline showed anti-anaphylactic activity and anti-inflammatory activity in arthritis animal model (Smith, 1961). Choline magnesium trisalicylate had been used for treatment of asthmatics with aspirin hypersensitivity (Szczeklik et al., 1990). Studies with choline in asthmatics showed lysophosphatidyl choline lowering effect and improvement in symptoms and AHR (Gupta and Gaur, 1997; Gaur et al., 1997). However the molecule needs further evaluation as an anti-inflammatory agent with advanced methods of drug screening. Thus, the present study was undertaken to achieve the following objectives:

1. To evaluate the anti-inflammatory effect of choline in animal model.
2. To study the toxicity of choline in mouse model.
3. To evaluate the anti-oxidant activity of choline in mouse model of allergic airway inflammation.
4. To investigate anti-inflammatory activity of choline chloride in bronchial asthma patients.

1. To evaluate the anti-inflammatory effect of choline in animal model

Female Balb/c mice of 6-8 weeks were obtained from National Institute of Virology, Pune (India). Two different schedules of choline administration before (protocol A) and after (protocol B) ovalbumin (OVA) challenge were used to demonstrate the changes occurred after treatment in allergen induced mouse model of airway disease. In protocol A, mice were sensitized intraperitoneally (i.p.) with 100 µg OVA adsorbed on 2 mg of Al (OH)₃ in 100 µl of saline on days 0, 7 and 14. They were challenged with 2.5% aerosolized OVA in saline in a plexiglass chamber using nebulizer for 30 minutes on days 25-27. OVA sensitized and challenged mice were
treated with 100 µl saline, 1 mg/kg of choline in 100 µl saline orally 1 hour before each OVA challenge on days 25-27, 1 mg/kg of choline (i.n.) in 50 µl saline 30 minutes before each OVA challenge and 1 mg/kg of dexamethasone phosphate (i.p.) in 100 µl saline 30 minutes before each OVA challenge and control group was sensitized, challenged and treated with 100 µl of 0.9% NaCl (saline). After the last treatment/challenge, AHR in response to methacholine (Mch) was measured in mice using whole body plethysmograph on day 28 and sacrificed on day 29. Dexamethasone phosphate was used as a standard treatment for asthma.

In protocol B, mice were randomly divided in 5 groups and sensitized as mentioned in protocol A. Further, they were challenged with 2.5% aerosolized OVA in saline for 30 minutes on days 25-30. Twenty-four hours after the last challenge, mice were treated with choline on every alternate day from day 31-40 as mentioned above in protocol A. Finally, a booster OVA challenge was done on day 38. AHR was measured on day 41 after the challenge/treatment and mice sacrificed on day 42.

After sacrificing the mice, bronchoalveolar lavage (BAL) fluid was collected from mice lungs and blood was collected after cardiac puncture. Single splenocyte cell suspension was made, cultured and stimulated with OVA for 72 hours at 37°C in a CO2 incubator. Culture supernatant was collected to measure cytokines and eicosanoids. Total cell count and eosinophils were identified in BAL after choline treatment by oral or i.n. route in both the protocols. Treatment with oral choline before challenge exhibited a modest decrease in AHR, though it was not significant. However, i.n. treatment demonstrated significant reduction (p < 0.05) in Penh at Mch concentrations of 12-50 mg/ml. The choline treatment given orally or i.n. significantly reduced the eosinophil peroxidase (EPO) activity in BAL fluid and inflammatory infiltrates in mouse airways stained with hematoxylin and eosin (H & E) as compared to OVA challenged saline treated group. Oral and i.n. choline treatment resulted significant decrease in IgG1 and IgE (p < 0.05) levels in both the protocols A and B. IL-4 and IL-5 levels were significantly reduced with choline treatment by oral and or i.n. route (p < 0.05) in BALF and in culture supernatant. Leukotriene (LT) B4 level was also reduced in culture supernatant of oral choline treatment group mice. Besides LTB4, choline treatment strongly inhibited the release of cysteinyl leukotriene (Cys-LT) in BAL fluid as well as in culture supernatant. Choline treatment by either route (oral/i.n.) reduced the mucus production and goblet cell hyperplasia as compared to OVA challenged saline treated group stained with alcian blue-periodic
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acid Schiff. The results suggest anti-inflammatory activity of choline in mouse model of airway disease.

2. To study the toxicity of choline in mouse model
Acute toxicity assessment of choline chloride was performed by i.n., oral, and i.p. routes in Balb/c mice for 28 days. Choline chloride (200 mg/kg) was administered orally once daily till 28 day by oral gavage (Group 1). Mice received choline chloride (200 mg/kg) in 100 µl saline by i.p. at every alternate day for 28 days (Group 3). However, for i.n. dose (200 mg/kg), choline chloride was given in 50 µl vehicle for every alternate day for 28 days (Group 5). As control(s), saline was given in other three groups (Group 2, 4 and 6). Control mice were given 100 µl normal saline by oral gavage or i.p. or 50 µl saline through i.n. route for 28 days following the same protocol. Mice receiving i.n. dose were lightly anesthetized (3% isoflurane) before administering each dose.

Body weight, food and water consumption did not differ between mice treated with choline or saline (control) group. Hematologic and biochemical variables were not affected with no increase in serum toxicity marker enzymes (ALT and AST) indicating normal liver functioning. Choline administration did not affect total cholesterol and high density lipoprotein levels as compared to their respective controls. Urea and BUN levels in choline treated mice were not different than controls. Creatinine level was however, higher than control in i.p. treatment group, but did not reveal toxic effects in other parameters. The effect of choline on growth inhibition of mononuclear cells was examined to evaluate the choline induced immuno-suppression. In choline treated mice, by all the three routes, the number of mononuclear cells was comparable to its control. Repeated choline administration in mice did not affect the weight of lungs, heart, liver, spleen or kidney and was comparable with respective saline control groups. In conclusion, the repeated administration of choline chloride via i.n., oral or i.p. routes did not cause toxicity in mice in the toxicological endpoints examined.

3. To evaluate the anti-oxidant activity of choline in mouse model of allergic airway inflammation.
Oxidative stress plays an important role in allergic disorders and increased levels of oxidants are considered markers of the inflammatory process. Oxidative stress is
caused by a variety of free oxygen radicals collectively known as reactive oxygen species (ROS) (Reynaert, et al., 2007). ROS play a crucial role in the pathogenesis of airway inflammation and AHR. In the present study, effect of choline chloride on oxidative stress has been investigated along with a non-enzymatic anti-oxidant α-lipoic acid in a mouse model of airway inflammation.

Balb/c mice were immunized and challenged with OVA and administered choline via oral or i.n. route. Compared to OVA sensitized saline treated mice, choline administration by oral gavage or i.n. significantly reduced the inflammatory infiltrates in mouse airways stained with H & E. Choline treated mice had significantly reduced eosinophilic inflammation and EPO activity in BAL fluid. The enhanced ROS production in ovalbumin sensitized and challenged mice reduced markedly by choline treatment in both oral and i.n. groups (p < 0.05). The treatment with α-lipoic acid also decreased intracellular ROS levels in BAL fluid (p < 0.05). Antioxidant levels in BAL fluid were measured in terms of lipid peroxidation (formation of TBARS). Choline treatment by either route reduced the lipid peroxidation levels significantly as compared to OVA challenge saline treated mice (p < 0.05). The 8-isoprostanes levels were measured in BAL fluid as biomarker of oxidative stress by enzyme immunoassay (EIA). After the last challenge, 8-isoprostanes level was significantly elevated in OVA challenged saline treated group than saline control mice. Choline treatment by either route significantly reduced the level of isoprostanes (p < 0.05). NFκB p65 protein expression was measured in nuclear and cytosolic extracts from lung tissues. Western blot analysis revealed that levels of NFκB p65 in nuclear protein extracts from lung tissues were increased in saline treated OVA challenged mice. The increased NFκB p65 protein expression was inhibited after choline treatment by either route. In contrast, cytosolic NFκB p65 from lung tissues were decreased after OVA challenge as compared to normal control. The decreased NFκB p65 levels in cytosolic protein extracts were increased by administration of choline.

Th2 Cytokines (IL-4 and IL-5) level were decreased in BAL fluid with choline treatment. Intranasal choline treatment induced slight increase in IFN-γ level in spleen cells culture supernatant. Choline treatment significantly reduced the level of TNF-α but the decrease in IL-13 levels was not significant. The levels of IL-4 and IL-5 were also reduced by α-lipoic acid treatment (p < 0.05). Previous reports suggest that ROS
are needed for TNF-α induced NFκB activation (Garg and Aggarwal, 2002), so may be the anti-inflammatory effects of choline is partially mediated by the reduction of ROS generation. The present study demonstrates antioxidants activity of choline in mouse model of allergic airway inflammation.

4. To investigate anti-inflammatory activity of choline chloride in bronchial asthma patients.

Patients of asthma with or without rhinitis aged 15 to 45 years of both sexes were included in the present study on the basis of history, skin tests and other relevant investigations at the Dept. of Pulmonary Medicine, V. P. Chest Institute, Delhi. The presence of airway obstruction and >12% reversibility with 200 ml increase in FEV1 volume or FEV1 / FVC % < 80%, was considered to assign asthma as per American Thoracic Society (ATS) guideline (ATS, 1991). Diagnosis of rhinitis was assigned to patients having two or more of the symptoms- (1) watery nasal discharge, (2) sneezing, (3) itching of nose, and (4) nasal congestion, most of the times (Bousquet et al., 2001). Patients with associated cardiopulmonary disorders, other respiratory or systemic diseases, steroid dependent, non-cooperative, pregnant or lactating females and smokers were not included in the study.

At the first visit of patient, peripheral blood counts, chest radiogram and pulmonary function test (PFT) with reversibility were carried out. Skin tests with common allergen extracts were carried out at outpatient department (OPD), V. P. Chest Institute, Delhi. At the second visit, bronchial hyperreactivity (BHR) was measured after histamine challenge and result expressed as PC_{20} FEV1. The patients in Group A were given choline + standard pharmacotherapy or other drugs SOS as and when required. Group B patients were treated with standard pharmacotherapy as inhaled steroids + LABA or SABA/other drugs SOS as and when required. Subjects were randomly assigned respective group(s) and choline (Oral 1.5 gm/bd/day) given to the patients (Group A) initially for a period of 1 month. After the assessment, same therapy was continued for another 5 months.

Venous blood was collected from patients at 0, 3 and 6 month for analysis of various immunological and biochemical parameters. Leukotriene (Cys-LT and LTB4) and cytokines (IL-4, IL-5, IL-10, TNF-α, IFN-γ) estimation was done in peripheral blood mononuclear cell (PBMCs) culture supernatant. Total IgE levels were estimated
in serum. Clinical parameters such as symptom/drug score, peripheral blood eosinophils count, PFT (lung volume - FVC, FEV1, FEV1/FVC) and airway reactivity with histamine was recorded at 0 and 6 month. Skin tests with common aeroallergens were performed at 0 and 6 month of choline therapy. Skin tests were graded on the basis of wheal size of positive control i.e. histamine diphosphate and PBS served as negative control (Dreborg, 1989; Kumari et al., 2007).

Bronchial hyperreactivity was measured by administering aerosolized histamine diphosphate in phosphate buffered saline using a nebulizer. Spirometry was done before the treatment, to get the baseline PFT values. Histamine doses were given from 0.004 mg/ml to 8 mg/ml doubling the concentration in every next dose through nebulizer. Spirometry was done again after 6 months treatment and FEV1 was recorded within 30 and 90 seconds. Challenge was stopped when 20% fall in FEV1 or the highest dose of histamine is achieved. The patients were allowed to recover till baseline value reaches again. The choline response was assessed based on the clinical parameters such as 1) symptom scores, 2) drug scores, 3) airway reactivity, 4) skin test, and 5) immunological and biochemical parameters.

Ninety patients were screened by clinical history, PFT and skin tests. A total of 76 patients (inclusive of previous report) of bronchial asthma with or without rhinitis were included in the study. Of the total, 38 patients each were assigned to Group A and Group B. Out of 38 patients, 30 completed the study in Group A and 26 in Group B. After 6 months of therapy, choline group patients showed reduction in total symptom and drug score. Patients of choline therapy group required higher concentration of histamine to achieve 20% fall in FEV1 as compared to their baseline indicating improvement in PC<sub>20</sub> FEV1 (p < 0.01). Choline therapy induced significant reduction in total IgE levels (p < 0.01). There was also reduction of total IgE levels in pharmacotherapy group compared to its baseline. However, no change was observed in skin test reactivity with allergen extracts after 6 months of choline therapy as compared to baseline.

Choline treatment for 6 month substantially reduced cytokine levels (IL-4, IL-5 & TNF-α) in PBMCs culture supernatant as compared to baseline and standard pharmacotherapy. However, no change in IL-10 and IFN-γ level was observed after choline treatment. Choline treatment also reduced the level of Cys-LT, LTB4 and 8-isoprostanes in culture supernatant as compared to baseline (p < 0.01).
Conclusions

1. Choline treatment by oral/intranasal (i.n.) routes in sensitized mice prior to antigen (OVA) challenge significantly inhibited eosinophilic airway inflammation and eosinophil peroxidase activity.
2. Choline administration (oral/i.n.) in sensitized mice after antigen (OVA) challenge significantly inhibited allergic airway inflammation.
3. Choline treatment reduced airway inflammation and mucus production in allergic mice model.
4. The development of airway hyperresponsiveness was prevented effectively by i.n choline treatment.
5. Choline treatment reduced IgE and IgG1 secretion and inhibited the release of Th2 cytokines IL-4 and IL-5 and leukotrienes LTB4 and Cys-LT in mouse model.
6. Behavioral patterns of mice administered with high dose of choline did not differ compared to control for acute toxicity assessment.
7. Hematological and biochemical parameters were not affected in choline treated mice and were similar to control.
8. Histologic examination of the organs treated with high doses of choline did not show any alterations and were comparable to saline control group.
9. High doses of choline are well tolerated in mice and proved to be safe with no adverse effects by all the routes viz., oral, i.p. and i.n.
10. Choline treatment by both oral and i.n. route markedly reduced the reactive oxygen species (ROS) production and lipid peroxidation levels significantly as compared to OVA challenge saline treated mice.
11. Isoprostanes levels, a biomarker of oxidative stress, reduced significantly by choline treatment.
12. Choline administration by either routes downregulated nuclear factor- κB (NFκB) activity as compared to OVA sensitized saline treated mice.
13. Choline treatment significantly reduced the level of TNF-α as ROS are needed for TNF-α induced NFκB activation.
15. After 6 months of choline therapy, asthma patients showed reduction in symptoms and drug use.

16. There was an improvement in the PC$_{20}$ FEV1 of patients after 6 months of choline therapy as compared to baseline.

17. Choline treatment for 6 month also reduced the level of Th2 cytokines (IL-4 & IL-5) and TNF-$\alpha$ as compared to baseline and pharmacotherapy treatment.

18. Choline treatment also reduced the level of eicosanoids (Cys-LT & LTB4) and 8-isoprostanes as compared to baseline.

19. The choline exerts anti-inflammatory effect and can be used as an adjunct therapy for bronchial asthma.