Abstract

Asthma is a chronic immune inflammatory disease characterized by variable airflow obstruction and increased bronchial hyperreactivity (BHR). Inflammation is the hallmark of asthma and allergic disorders. The incidence of asthma has increased world over, which presents both public health and economic concerns. Asthma results from constriction or narrowing of bronchial tube caused by muscle spasm and airway narrowing due to immune inflammation. Efforts are therefore required to find out appropriate anti-inflammatory drug with no or minimal side-effect for management of the disease. The current asthma therapy is given to control symptoms such as coughing, wheezing, and or shortness of breath. However, the medications do not relieve the underlying inflammatory process of asthma completely. Inhaled glucocorticosteroids are at present the most effective controller medications. But 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 and easy bruising, adrenal suppression and decreased bone mineral density.

Choline, as a lipotropic factor is needed for maintaining cell structure and to facilitate the movement of fats in and out of cells. Choline, the major constituent of phosphatidylcholine (PC), is found in soybean, liver, oatmeal, cabbage and cauliflower. Previous studies with choline showed anti-inflammatory activity in arthritis animal model. But, whether choline had anti-inflammatory activity in the airways remains to be investigated. The present study (chapter 2) aims to evaluate the anti-inflammatory activity of choline in mouse model of allergic airway inflammation. Choline (1 mg/kg) was administered via oral gavage or intranasal (i.n.) route before and after ovalbumin (OVA) challenge in sensitized mice. Airway hyperresponsiveness (AHR) to methacholine was measured in mice by whole body plethysmography. Th2 cytokines and leukotrienes were estimated in bronchoalveolar lavage (BAL) fluid and spleen cells culture supernatant by ELISA. Eosinophil peroxidase (EPO) activity in BAL fluid supernatant was also determined. Choline treatment in sensitized mice before OVA challenge via oral/i.n. routes significantly inhibited eosinophilic airway inflammation and EPO activity. It also reduced IgE and IgG1 production and inhibited the release of Th2 cytokines and leukotrienes. However, the development of AHR was prevented effectively by i.n. choline treatment. Most importantly, choline treatment after OVA challenge by both routes could reverse already established asthmatic conditions in mice by inhibiting AHR,
eosinophilic airway inflammation and other inflammatory parameters. The results suggest that choline may be used for controlling as well as preventing asthma exacerbations.

Studies show that choline has potential to be used as a dietary supplement and as a drug for immune inflammatory diseases like asthma, rhinitis and others. But there are apprehensions regarding adverse effects of choline when given in high doses. To address this knowledge gap, present study (chapter 3) aims to assess the toxicity of choline chloride by intranasal (i.n.), oral and intraperitoneal (i.p.) routes in Balb/c mice for 28 days. Body weight, food and water consumption of mice were recorded daily. Haematology and clinical chemistry parameters were assessed to check hepatocellular functions and morphological alterations of the cells. Splenocyte counts were analyzed for evaluating cellular immunity. Liver function test was performed by assaying different enzyme systems in serum such as, urea, blood urea nitrogen, creatinine, alanine aminotransferase and aspartate aminotransferase. Body weight, food and water consumption did not differ between mice treated with choline and saline control group. Hematologic and biochemical variables were not affected without any increase in serum toxicity marker enzymes indicating normal liver functioning. Choline administration did not affect total cholesterol and high density lipoprotein levels as compared to their respective controls. Urea and blood urea nitrogen levels in choline treated mice were not different than controls. In conclusion, the repeated consumption of choline chloride via i.n. and oral or i.p. routes did not cause toxicity in mice in the toxicological endpoints examined.

Asthma is a complex multi-factorial immune inflammatory disease associated with increased oxidative stress and altered antioxidant defenses. The present study (chapter 4) aims to evaluate the effect of choline on oxidative stress more specifically in the production of reactive oxygen species (ROS). Balb/c mice were sensitized and challenged with OVA and administered with choline via oral gavage or i.n. route. Total cell counts, eosinophil counts and EPO activity were determined in BAL fluid. Levels of intracellular ROS, lipid peroxidation and isoprostanes were measured in BAL fluid obtained from mice lungs. Cytokine (IL-13 and tumor necrosis factor-alpha; TNF-α) levels were also measured in BAL fluid and spleen cells culture supernatant. Nuclear factor κB p65 protein expression was measured after last OVA challenge in nuclear and cytosolic extracts from lung tissues. Compared to OVA challenged mice, choline treated mice had significantly reduced eosinophilic infiltration and EPO activity in BAL fluid. Choline treatment reduced ROS production and isoprostanes level significantly in BAL fluid and thus suppressed
oxidative stress. Choline administration by either route decreased lipid peroxidation levels and downregulated nuclear factor-κB (NFkB) activity as compared to OVA challenged mice. A significant increase in pro-inflammatory cytokine TNF-α was observed in OVA challenged mice which were suppressed by choline treatment. The results suggest that choline modulates the redox status of the cell possibly by enhancing antioxidant defenses and could be beneficial for the treatment of bronchial asthma.

Anti-inflammatory effects of choline in mouse model of allergic inflammation were established (chapter 2 & 4). Further, choline administration in high doses did not show any adverse effects in mouse model (chapter 3). This gives the basis to study anti-inflammatory effect of choline chloride in asthma patients (chapter 5). The patients of asthma with or without rhinitis (n = 76) were recruited and choline (1500 mg bd/day) + pharmacotherapy or standard pharmacotherapy was administered in two separate groups for 6 months. Total IgE in serum and IL-4, IL-5, IL-10, IFN-γ and TNF-α were estimated at baseline and post-treatment in peripheral blood mononuclear cell (PBMCs) culture supernatant by enzyme immuno assay (EIA). Cysteinyl leukotrienes (Cys-LTs), leukotriene B4 (LTB4) and 8-isoprostanes were also measured in stimulated PBMCs culture supernatant by EIA. Clinical manifestations were assessed by determining bronchial obstruction, BHR to histamine, symptom/drug score and peripheral blood eosinophil count at baseline and post treatment. Choline administration reduced symptom/drug score, improved PC_{20} FEV1 (provocative concentration of histamine causing a 20% fall in forced expiratory volume in one second) compared to baseline and standard pharmacotherapy in asthma patients (p < 0.01). Choline therapy significantly reduced IL-4, IL-5 and TNF-α level as compared to baseline and standard pharmacotherapy (p < 0.01). Blood eosinophil count and total IgE levels were reduced in both the treatment groups compared to their baseline. Cys-LTs, LTB4 and 8-isoprostanes, a biomarker for oxidative stress were suppressed significantly by choline treatment compared to baseline and standard pharmacotherapy (p < 0.01). The results suggest that choline therapy modulates immune inflammation and improves lung functions in asthma patients. In conclusion, choline exerts anti-inflammatory effects on airways and can be used as an adjunct therapy in asthma patients.