CHAPTER 1

Introduction

Asthma is one of the most common chronic inflammatory diseases, and is ranked among the top 10 prevalent conditions. It affects about 300 million people worldwide and is projected to increase to 400 million by 2025 (1). Asthma prevalence increases globally by 50% every decade (1, 2). Prevalence rates for asthma around the world differ substantially. Asthma rates are officially low in India and an overall prevalence is 3% (30 million patients) among adults over the age of 15, a median prevalence of 2.4% (3). Prevalence of asthma is higher in rural areas (1,719 per 100,000 for women and 1,799 per 100,000 for men) than for urban areas and that it is more common among women than men (4). The complete causes of asthma are unknown. Allergic asthma is a result of an antigen\antibody reaction on mast cells in the respiratory tract. This reaction causes the release of inflammatory mediators from mast cells, which elicit the clinical response associated with an asthma attack. Onset of asthma between the ages of 5 to 15 years usually indicates asthma with an allergic basis (5, 6). People with asthma have symptoms when the airways are narrowed (bronchospasm), swollen (inflamed), or filled with mucus. Common symptoms of asthma include; coughing, especially at night, wheezing, shortness of breath, chest tightness, or pain (5). In the initial phase of asthma various chemical mediators such as histamine, prostaglandins, and leukotrienes are realizes which leads to contraction of airway smooth muscle, increase microvascular leakage, increase airway mucus secretion, and attract other inflammatory cells. Histamine is the first chemical mediator associated in the pathophysiology of asthma while cysteinyl-leukotrienes LTC4, LTD4, and LTE4 are potent constrictors of human airways and plays an important role in the acute inflammatory response in asthma (7).

Chronic asthma is characterized by airway obstruction, airway wall inflammation and edema, epithelial desquamation, mucous hypersecretion, bronchial hyperresponsiveness and in some case airway remodeling. Eosinophils, neutrophils, lymphocytes and degranulated mast cells play major role in airway inflammation and considered to be primary pathologic event in asthma and the inflammatory process (8). Many different inflammatory cells are involved in asthma (8). Mast cells play key role in asthma by
release of several chemical mediators such as histamine, leukotrienes, and prostaglandin; which may account for the variable bronchoconstriction in asthma. Mast cells also release cytokines that are linked to allergic inflammation, including interleukin (IL)-4, IL-5 and IL-13 (9). IL-5 induces terminal differentiation of immature eosinophils. IL-5 stimulates the release of eosinophils into the circulation and prolongs their survival. Challenge of the airway with allergen increases the local concentration of interleukin-5, which correlates directly with the degree of airway eosinophilia (10). The mature eosinophils are sources of inflammatory proteins, such as major basic protein, eosinophil-derived neurotoxin, peroxidase, and cationic protein. Major basic protein, can directly damage airway epithelium, intensify bronchial responsiveness, and cause degranulation of basophils and mast cells (11). On other hand neutrophils are also observed in more severe asthma (12). Neutrophils also secrete a variety of inflammatory mediators, including proteases, cytokines (e.g., tumor necrosis factor, transforming growth factor), and reactive oxygen species, which can cause airway epithelial injury and mucus hypersecretion. Lymphocytes involved in the allergic response of asthma and has role in degranulation of mast cells and basophils. T-lymphocytes releases cytokines, resulting in the recruitment and survival of eosinophils and in the maintenance of mast cells in the airway (13). There are four distinct subsets of CD4 T-lymphocytes, Th1, Th2, regulatory T cells (Treg), and T17 cells can differentiate from precursor T cells at the time of antigen presentation and influence cytokine production. Th1 cells drive cell-mediated immune responses and are characterized by the production of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and IL-2. Th2 cells mediate humoral responses and are characterized by their secretion of IL-4, IL-5, IL-9, and IL-13. Th17 cells, a newly discovered subset, produce IL-17 and IL-22. In contrast to Th1, Th2, and Th17 cells, Treg cells act directly or indirectly to suppress the function of effector T cell responses in allergic asthma (13-15).

Macrophages are another inflammatory cell that plays a significant role in asthma by the releasing of a certain pattern of cytokines. Macrophages act as antigen-presenting cells which process allergen for presentation to T-lymphocytes, although alveolar macrophages are far less effective in this respect than macrophages from other sites, such as the peritoneum (16). There may be subtypes of macrophages that perform different inflammatory, anti-inflammatory or phagocytic roles in allergic disease.
Nitric Oxide (NO) is synthesized by NO synthases in several cells in the airways (17). NO is a potent vasodilator that increases plasma exudation in the airways and amplify the Th2-lymphocyte–mediated response. Many inflammatory cells produce oxygen-derived free radicals which causes contraction of airway smooth muscle and changes in receptor function, which could influence airway reactivity and may activate NF-κB to increase the expression of multiple inflammatory genes (18).

Phospho-inositol-3-kinase (PI3K) family also plays an important role in inflammatory lung diseases particularly asthma. PI3K control inflammatory cells growth, differentiation, survival, migration, proliferation, and mediator production (19). PI3K signaling is integral to both mast cell and eosinophil function (20). Inhibition of PI3K signaling with the nonselective inhibitor LY294002 in an ovalbumin (OVA)-challenged murine model of asthma reduced inflammatory cell influx into the lung as well as reduction of IL-5, IL-13 and CCL11 (eotaxin) (21). In addition, tissue eosinophilia, airway mucus production and airway hyper-responsiveness (AHR) to inhaled metacholine were all suppressed. Collectively, these studies clearly demonstrate that direct targeting of PI3K in the lung reduces allergic inflammation. PI3Kγ knockout mice have impaired eosinophil recruitment and survival in an OVA challenge model of allergic inflammation (22). This may relate to effects on the maintenance of eosinophilic inflammation, since PI3Kγ knockout mice have reduced eosinophilic inflammation at later (48 h) rather than early time points (6 and 24 h) (23). Taken together, these studies clearly show that selective pharmacological inhibition of PI3Kδ/γ isoforms is a potential therapeutic strategy for suppressing allergic inflammation in asthma.

Medications to treat asthma can be classified as controllers or relievers. Controllers are medications taken daily on a long-term basis to keep asthma under clinical control chiefly through their anti-inflammatory effects. They include inhaled and systemic glucocorticosteroids, leukotriene modifiers, long acting inhaled β2-agonists in combination with inhaled glucocorticosteroids, sustained-release theophylline, cromones, and anti-IgE. Inhaled glucocorticosteroids are the most effective controller medications currently available. Relievers are medications used on an as-needed basis that act quickly to reverse bronchoconstriction and relieve its symptoms. They include rapid-acting inhaled β2-agonists, inhaled anticholinergics, short-acting theophylline, and short-acting oral β2-agonists (24).
Glucocorticoids are the most effective therapy available for patients with asthma and now become first line treatment option for asthmatic patients. Studies have shown that when inhaled corticosteroids are used consistently, they improve asthma symptoms more effectively than any other single long-term control medication in both children and adults (5). Patients with mild to moderate persistent asthma treated with inhaled corticosteroids has shown improved in symptom like scores, lower exacerbation rates, and reduced symptom frequency (25). Glucocorticoids may have direct inhibitory effects on many of the cells involved in airway inflammation in asthma, including macrophages, T-lymphocytes, eosinophils, and airway epithelial cells (26). This process may explain the reduction in the number of eosinophils in the circulation and airways of patients with asthma during glucocorticoid therapy (27, 28) particularly the fraction of eosinophils with low density (29). Glucocorticoids may not inhibit the release of mediators of allergic reactions from mast cells, but they do reduce the number of mast cells within the airway (26-28). However; long term use of corticosteroid is known to induce various side effects like hyperglycemia, hypertension, cardiometabolic abnormalities, psychiatric adverse effects, weight gain and osteoporosis (30). Moreover chronic use of corticosteroid induces vitamin D deficiency and low level of vitamin D contributes to increase in asthma severity (31).

Corticosteroid are the most common cause of drug-induced diabetes. Though the exact prevalence is not known, a few observations suggest that glucocorticoid-induced diabetes or hyperglycemia. Corticosteroid use causes hyperglycemia that may relate to the inhibition of glucose metabolism or insulin resistance mainly interfere insulin signals cascade and inhibitory effect on β cells (32, 33). Besides insulin resistance, visceral obesity is another major side effect of corticosteroid treatment. Corticosteroid have been found to be both lipolytic as well as adipogenic (34) and corticosteroid can regulate differentiation, function and distribution of adipocytes. Corticosteroids have multiple potential deleterious effects on cholesterol metabolism, including an increase in the activity of acetyl-coenzyme A carboxylase and free fatty acid synthetase, down regulation of LDL receptor activity, increase in the activity of HMG-CoA reductase and inhibition of lipoprotein lipase (35). A high cumulative dose of corticosteroids is associated with increased levels of very low-density lipoproteins (VLDL), total cholesterol (TC), and triglycerides (TG), as well as a decrease in high-density lipoprotein (HDL) levels. This has been confirmed in early corticosteroid withdrawal analyses that show beneficial effects in reducing dyslipidemia.
The long term use of corticosteroid induces various psychiatric adverse effects; such as mood alterations, hyperactivity, insomnia, and even frank psychosis (37). The prevalence of such condition occurs in between 2% to 57% of corticosteroid-treated patients (38). It has been hypothesized that steroid psychosis may result from the induction of tyrosine hydroxylase synthesis (39) or via direct disruption of hippocampus-dependent behavior by some as yet unknown mechanism (38). Psychotic reactions, should they occur, develop most frequently within the first 5 days of use of corticosteroids (40, 41).

Combinations of inhaled corticosteroids and long-acting β2 agonists are effective in most (about 90%), but not all, asthmatic individuals. Indeed, even patients whose asthma is apparently well controlled by existing therapies might benefit from more efficacious therapies that are easier to comply with. Improved compliance in the mild-to-moderate asthmatic patient could be achieved by the development of safe oral versions of conventional treatments or of new, more efficacious treatments, particularly if these agents altered the course of the disease or pointed towards a cure (36).

Recently, newer physiologic functions for vitamin D have been identified. Epidemiologic and genetic studies as well as research using animal models suggest vitamin D plays a vital and complex role in immune system function and regulation. Multiple immune cell types express vitamin D receptors, including activated T and B cells, macrophages and dendritic cells. Vitamin D inhibits the function of T lymphocytes both directly and via effects on antigen presenting cells (APCs). It has potent antiproliferative effects on CD4+ T cells. Moreover, numerous clinical studies have shown that low vitamin D level (vitamin D deficiency) associated with asthma exacerbation and supplementation with vitamin D has significant improvement in asthma symptoms (42, 43).

The recent data has shown that vitamin D has direct effects on activated helper T cells, regulatory T cells, activated B cells and dendritic cells (DC) (44). Vitamin D inhibits the polarization of naive Th0 cells to Th1 and to a lesser extent Th2 cells, it shifts the balance of Th1/Th2 T cells toward the latter ones and up regulates Treg cells, that is, the T cells population known to inhibit both Th1 and Th2 cells (45, 46). In asthma, reduced vitamin D levels are associated with impaired lung function, increased airway hyperresponsiveness, increased expression of TNF-α (47, 48) and reduced glucocorticoid response (49, 50). More recently, vitamin D inhibit Th17 associated cytokines have been shown both in vitro
Chapter 1

Introduction

and in vivo in experimental which may be important in steroid refractory airway disease (51, 52). Th17 produced more inflammatory cytokines such as IL-1β, IL-6 and IL-17 (53) which plays a mechanistic role in increasing asthma severity and reducing corticosteroid sensitivity.

Treg cells are immunosuppressor cells of the body, maintain the self-tolerance that is, they protect the organism from autoimmune reactions (diseases) in mice and humans, among others by inhibiting IL-2 production (54) Vitamin D have alone can induce Tregs and enhance proliferation (55). Moreover, vitamin D increased both IL-10-secretion and toll-like receptor (TLR)-9 expression by Tregs (56). Both in vivo and in vitro shown that vitamin D also enhances production of anti-inflammatory cytokine IL-10 by human T cells (57). Dendritic cells seem to be the key cells for antigen presentation in asthma (58). Vitamin D inhibits the maturation of monocyte-derived DCs and induced synthesis of IL-10 (59). Therefore, vitamin D decreased DC maturation and concomitant enhancement of suppressor or Treg cell.

Some patients with severe asthma have poorly respond to systemic high-dose of glucocorticoids and this condition is termed “steroid-resistant asthma” (60, 61). Proinflammatory cytokine such as IL-2 and IL-4 are markedly increased in bronchoalveolar lavage samples from steroid resistance asthmatic patients and steroid treatment does not decrease the levels of these cytokines compared with steroid sensitive asthma (62). Increase in steroid resistance may be due to high level of IL-2 and IL-4 which reduced glucocorticoid affinity for T-lymphocytes (63). Tregs supress the activation of immune response, inflammatory cytokine IL-2, express immunomodulator IL-10, and potentially convert effector T cells to hyporesponsive or regulatory forms (55). Reduction in Tregs is responsible for steroid resistance (64). Vitamin D has been shown to promote and increase the synthesis of Treg (46) whereas in the absence of vitamin D; number of Tregs are reduced (55). Vitamin D significantly inhibited inflammatory cytokine expression, IL-1α, IL-1β, IL-12, IL-4 and tumour necrosis factor (65, 66). Treatment with vitamin D to glucocorticoid-resistant asthmatic patients enhance subsequent responsiveness to dexamethasone by restoring the defective IL-10 response to glucocorticoids by CD4+ T cells in these individuals (57). In vitro glucocorticoids induce dose dependent synthesis of IL-10 by stimulation of CD4+ T cells. This steroid-induced IL-10 synthesis can over-come by the addition of vitamin D to the T cell culture. In a small
a pilot study, administration of vitamin D to steroid resistant asthmatic patients enhanced their response to dexamethasone for the induction of IL-10 (57). Moreover, vitamin D addition can decrease the active dose of dexamethasone greater than 10-fold (31).

Number of epidemiological evidence has shown of a link between vitamin D levels and asthma. Vitamin D deficiency is associated with poorer lung function (67), severe asthma exacerbations (68) and increased odds of asthmatic state (69). In asthmatic patients, 25-hydroxy vitamin D levels had direct and significant correlations with both predicted FEV1 and FEV1/FVC (70-72). Searing et al.,(31) observed a significant inverse correlations with vitamin D levels and use of inhaled steroids (P = .0475), use of oral steroids (P = .02), and total steroid dose (P = .001). Vitamin D levels were significantly and inversely associated with total IgE, allergic disease and eosinophil count. Therefore; the increased serum 25-(OH)D(3) level may inhibit total IgE expression, suggesting that increasing serum 25-(OH)D(3) level might be a new option for the prevention and treatment of asthma (73, 74).

Moreover, altered vitamin D homeostasis is associated with increased risk of developing glucose intolerance (75), metabolic syndrome (76), cardiovascular events (77), psychiatric effects (78), obesity (79) and hypertension (80).

In the light of above mentioned facts, the present study was undertaken to attempt the efficacy, adverse events profile of vitamin D alone and in the combination with corticosteroids in asthma and study the possible mechanism of action of vitamin D in asthma.

**Objectives of the Study**

1. To study the spasmolytic and anti-inflammatory activity of vitamin D.
2. To study efficacy and adverse effects profile of corticosteroid-vitamin D combinations and monotherapies in asthma.
3. To study mechanism of action of vitamin D; dependent or independent from corticosteroid receptor.