

Salicylic acid derivatives as potential anti asthmatic agents using disease responsive drug delivery system for prophylactic therapy of allergic asthma [☆]



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ABSTRACT

Asthma is a multi-factorial and complicated lung disorder of the immune system which has expanded to a wider ambit unveiling its etiology to be omnipresent at both ends of the spectrum involving basic pharmacology and in-depth immunology. As asthma occurs through triggered activation of various immune cells due to different stimuli, it poses a great challenge to uncover specific targets for therapeutic interventions. Recent pharmacotherapeutic approaches for asthma have been focused on molecular targeting of transcription factors and their signaling pathways; mainly nucleus factor kappa B (NFκB) and its associated pathways which orchestrate the synthesis of pro-inflammatory cytokines (IL-1β, TNF-α, GM-CSF), chemokines (RANTES, MIP-1a, eotaxin), adhesion molecules (ICAM-1, VCAM-1) and inflammatory enzymes (cyclooxygenase-2 and iNOS). 5-aminosalicylic acid (5-ASA) and sodium salicylate are known to suppress NFκB activation by inhibiting inhibitor of kappa B kinase (IKKβ). In order to target the transcription factor, a suitable carrier system for delivering the drug to the intracellular space is essential. 5-ASA and sodium salicylate loaded liposomes incorporated into PEG-4-acrylate and CCRGGC microgels (a polymer formed by crosslinking of trypsin sensitive peptide and PEG-4-acrylate) could probably suit the needs for developing a disease responsive drug delivery system which will serve as a prophylactic therapy for asthmatic patients.

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Introduction

Asthma is a complex chronic lung inflammation that is characterized by airway obstruction which leads to many clinical complications like coughing [1], wheezing [2], and/or uneasiness in breathing [3]. In the last few decades, there has been a drastic increase in the severity and prevalence of asthma worldwide [4]. So far asthma affects 300 million people globally, with a 50% increase in prevalence every decade due to exposure to airborne

allergen or air pollutants or irritants, or certain medications and/or respiratory tract infections [4]. About 80% of deaths were reported from low and lower-middle income countries [4]. With the proportion of the world's urban population projected to increase from 45% to 59% by 2025, there is likely to be a marked increase in the number of asthmatics worldwide over the next decade [5]. It is estimated that there may be an additional 100 million people suffering from asthma by 2025 [5] (Fig. 1). Asthma creates a substantial burden on individuals and families as it is more often under-diagnosed and under-treated [6–8].

The understanding of pathophysiological mechanism of asthma is incomplete; however, it leads to a variety of nonspecific spasmogenic stimuli [9]. Various studies have shown excessive airway mucus production due to aggravation of the disease in asthmatics with an elevated serum IgE levels, which have been linked to

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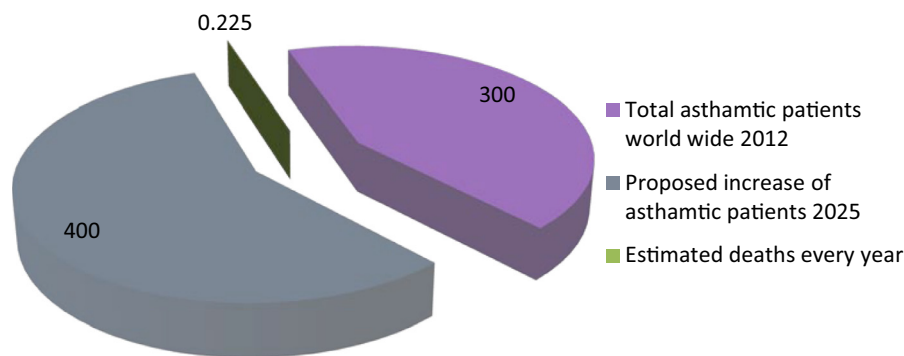


Fig. 1. Schematic representation portraying the statistics of incidence of asthma across the world. Data expressed in millions [4,5].

aberrant T helper (T_H2) cell responses against commonly inhaled allergens [10,11]. Allergen-specific T_H2 cells exert their critical effector functions by producing a unique repertoire of cytokines, the most important of which being IL-4, IL-5, and IL-13 [11]. The cytokines IL-4 and IL-13 regulate class switch recombination in B-cells to produce abnormal IgE antibodies to inhaled allergens [12,13] whereas IL-5 cytokine helps to recruit eosinophils to the lung tissues which play a crucial role in aggravating asthmatic condition due to the expression of chemokine receptors [14].

Until today no specific therapies are available to cure asthma; the current treatment options only aim to suppress the disease severity [15]. The clinical management of allergic asthma has been a challenging task in the early decades of 20th century [16]. Anti-inflammatory corticosteroids have emerged as principal anti-asthma medications for suppressing the aggravated immunological responses in 1980's and have been successful ever since. However, increasing number of cases are being reported with the development of drug resistance followed by non-responsiveness on long term exposure to corticosteroids such as dexamethasone, thereby posing a serious threat to clinical management of asthma [15]. This implicates a necessity to explore new chemical moieties or repurposing of existing drug candidates in addition to identifying potential drug targets for promising therapy [15].

Commencement of the 21st century has led to the discovery of transcription factor nucleus factor kappa B's (NFκB) role in allergic asthma [17,18]. NFκB is a pleiotropic transcription factor that plays an important role in regulating the expression of multiple genes including pro-inflammatory cytokines (IL-1β, TNF-α, GM-CSF), chemokines (RANTES, MIP-1α, eotaxin), adhesion molecules (ICAM-1, VCAM-1) and inflammatory enzymes [cyclooxygenase-2 and inducible nitric oxide synthase (iNOS)] involved in immune responses [19]. The NFκB family is composed of five structurally related DNA-binding proteins, designated NFκB1/p50, NFκB2/p52, RelA/p65, Rel/c-Rel, and RelB [20]. Although the most common NFκB complex is a heterodimer of the p65 and p50 subunits, the different family members can associate in various homo or heterodimer combinations [20]. Inactive NFκB complexes are sequestered in the cytosol by inhibitory proteins of the IκB family. Following various stimuli, IκB proteins are phosphorylated, ubiquitinated and degraded, leading to NFκB activation and nuclear translocation, initiating transcription of NFκB dependent genes [20].

Here we propose to study a target-specific approach to deliver drugs directly to epithelial cells and macrophages of the inflammatory lung tissue. This formulation not only targets specific inflammatory sites in the lung, but also can improve disease severity with fewer side effects. Several different formulations for specifically targeting inflammatory lung tissues in allergic asthma have been developed over the past few years for the treatment, but can cause side effects due to long term or overuse of these drugs. Despite

their many advantages, there exist several design challenges that need to be addressed to achieve efficient and effective delivery of drugs to combat allergic asthma (see Fig. 2).

Hypothesis

Our hypothesis is to provide an alternative prophylactic therapy for corticosteroid resistant asthmatic patients. For this purpose, we propose a treatment approach for allergic asthma patients using salicylic acid (SA) derivatives (5-amino-salicylic acid and sodium salicylate). The approach is to target nucleus factor Kappa B (NFκB) in TNF-α signal transduction pathway using trypsin sensitive disease responsive microgels containing drug loaded liposomes. The overview of the hypothesis is shown in Fig. 3.

Entailment of the hypothesis

Our hypothesis postulates that salicylic acid derivatives can be used as possible alternatives for treating allergic asthma replacing corticosteroid therapy. The principal problem prevalent in therapies for allergic asthma is the development of drug resistance to corticosteroids which serve as a first line treatment for allergic asthma [15]. The effect of 5-aminosalicylic acid against allergic asthma has shown promising results in a murine model implicating the possibility of employing it for treating allergic asthma [21]. Transcription factor NFκB plays an important role in aggravating the allergic response by orchestrating the synthesis of various signaling molecules and enzymes. The selected drug candidates are known to inhibit the inhibitor of kappa kinase B (IκkB) which is a key enzyme involved in activating NFκB [18]. The prospective approach for delivering a drug into the most extensively immune protected organ in the body requires a precise design evaluation. Our intention is to develop a possible drug delivery system that effectively reaches the deep lung and evades alveolar macrophage clearance in addition to drug release in response to the allergic reactions initiated during the preliminary phase of allergic asthma [22]. This can be accomplished utilizing PEG-4-acrylate microgels that are optimal for deep lung delivery; they also meet the predicted criteria for pulmonary drug delivery [to maintain an aerodynamic diameter (da) of 0.5–5 μm [23–25] and geometric diameter (dg) of greater than 6 μm] [25,26]. Incorporating trypsin sensitive peptide (CCRGCC) into PEG-4-acrylate by means of a crosslink produces a disease responsive microgel which aids in the break down and release of the drug [22]. The final challenge in this study is to deliver the drug to intracellular spaces for effective drug targeting. This can be accomplished with drug loaded liposomes recognized as a biocompatible drug delivery system which can effectively evade identification by the immune system and can deliver the drug candidates to intracellular spaces [27].

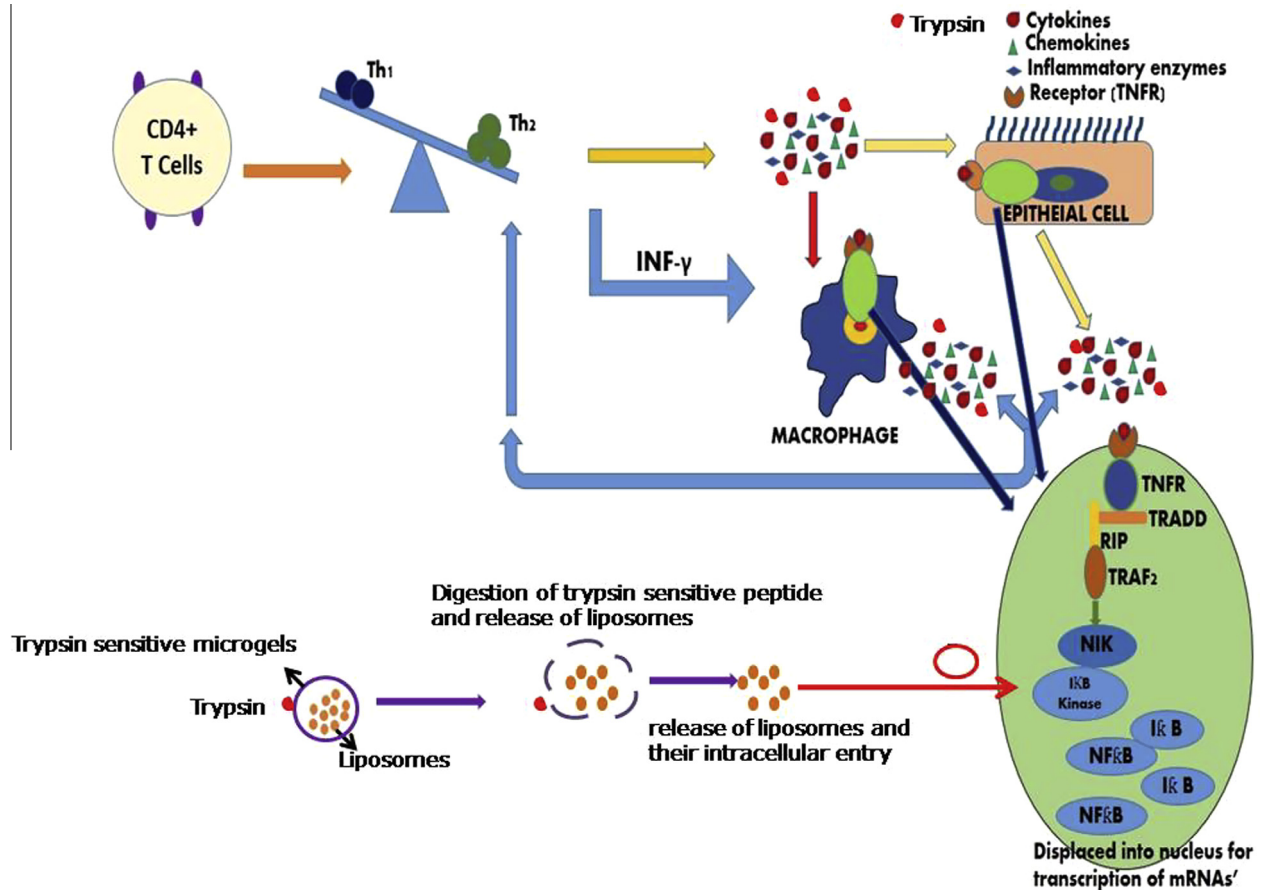


Fig. 2. Activation of CD4+ cells results in an imbalance in the T_H cells differentiation leading to increased expression of T_H2 . T_H2 cells on exposure to allergen releases a multitude of inflammatory precursors such as cytokines, chemokines, inflammatory enzymes (COX, iNOS) and adhesion molecules (ICAM, VCAM), which in turn stimulate inflammatory cells leading to type II hypersensitivity reaction. This picture primarily focuses of TNF- α induced NF κ B activation pathway which is predominant in macrophages and lung epithelium. It illustrates the steps involved in activation of NF κ B and subsequent nuclear translocation for initiating inflammatory protein synthesis. Also, it depicts the hypothesis of delivering the drugs to the targeted site of action using disease responsive trypsin sensitive PEG 4-ACR micorgels for achieving deep lung delivery overcoming the challenges faced in pulmonary drug delivery as mentioned in the text.

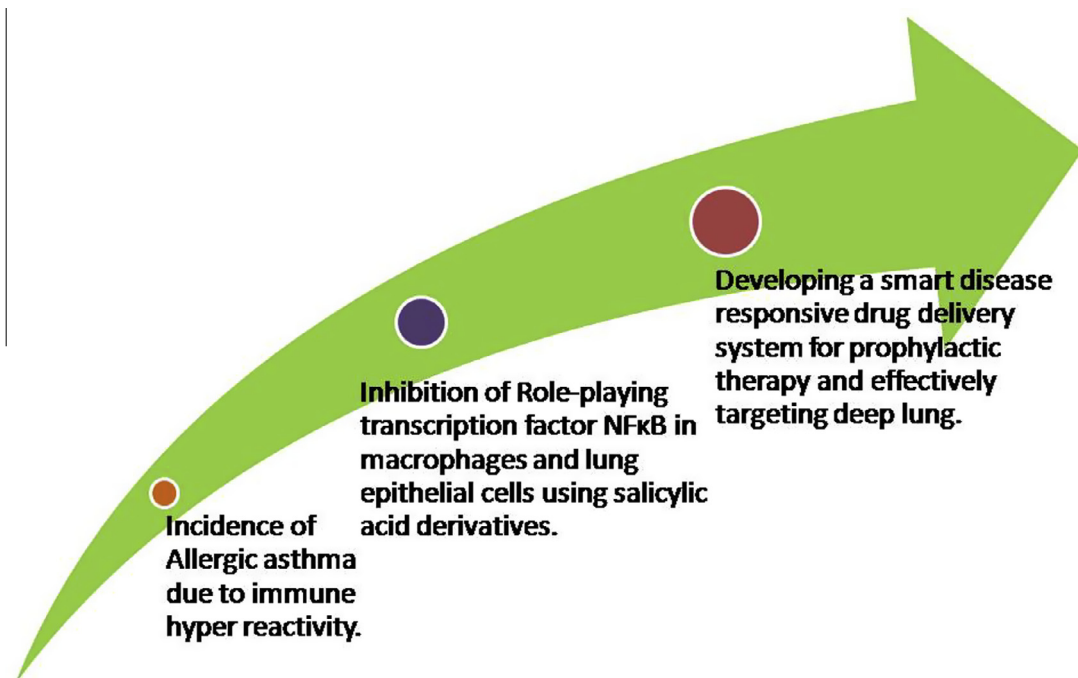


Fig. 3. Schematic illustration of the hypothesis.

Table 1

Table showing the optimal conditions for a formulation to effectively deposit drug candidates in deep lung [22].

S. no.	Property	Range	Reason
1.	Aerodynamic diameter (da) of microgels	0.5–5 μm	To effectively reach deep lung <0.5 μm leads to elimination of particles by exhalation >5 μm leads to deposition of particles in throat
2.	Geometric diameter (dg) of microgels	>6 μm	To evade clearance by alveolar macrophages
3.	Geometric diameter (dg) of liposomes	<0.2 μm	To deliver the drug candidates in intracellular space

Discussion

It had been recognized for many years that patients who die from acute asthma attacks have grossly inflamed airways [9]. Biopsies of lung tissues have revealed evidence for increased numbers of activated mast cells, macrophages, eosinophils and T-lymphocytes [11,12]. The chronic inflammation of asthma has been found to be due to increased expression of multiple inflammatory proteins (cytokines, enzymes, receptors, adhesion molecules). In most cases these inflammatory proteins are induced by transcription factors and DNA binding factors that increase the transcription of selected target genes [17]. Several studies indicate that the transcription factor that may play a crucial role in asthma is NF κ B, which can be activated by multiple stimuli, including protein kinase C activators, oxidants and pro-inflammatory cytokines (such as IL-1 β and TNF- α) [18]. Evidence from literature shows an increased activation of NF κ B signaling in asthmatic airways, particularly in epithelial cells and macrophages [28]. NF κ B regulates the expression of several key genes that are over-expressed in asthmatic airways [18].

Considering the above challenges, it is essential to showcase a molecule with inhibitory potential towards NF κ B activation. In recent years, there has been increasing interest in developing systems for the controlled delivery of therapeutic molecules to the lungs, especially to intracellular targets in the deep lung. Pulmonary drug delivery has great potential for both local and systemic treatments, especially with emerging biological drugs, proteins, peptides and nucleic acids [23]. Apart from its appeal as a non-invasive route of administration, delivery via pulmonary inhalation offers several advantages including a large surface area with high vascularization for drug absorption, thin epithelial layer, low enzymatic activity, and shunt of first-pass metabolism [24,29].

Further, there is also a necessity for bringing out a suitable drug delivery system to sustain localized delivery in the lungs without getting cleared by alveolar macrophages. It would also be an additional factor if a disease responsive drug delivery system were to be developed as a viable prophylactic therapy for asthma [30].

Established findings have shown that 5 amino-salicylic acid and sodium salicylate inhibit the TNF- α mediated activation of NF κ B [31,32]. Studies on 5 amino-salicylic acid have revealed that it suppresses T_H2 cytokines and oxidative stress in Balb/C mice induced with allergic asthma using ovalbumin [21]. These findings have led us to explore the reason for the activity attained and the necessity of a suitable drug delivery system.

The most widely accepted nano-carriers in the present scenario for pulmonary drug delivery are liposomes [27]. They are prepared using biopolymers which camouflage them from being recognized as xenogens by the immune system. This characteristic feature allows them to be counted as one among other successful nano-carriers. This aside, they also enhance the delivery of therapeutic agents into the intracellular spaces [33]. The established range for optimal aerodynamic particle diameter to achieve efficient deep lung deposition (following inhalation) is between 0.5 and 5 μm . Particles with aerodynamic diameters (da) less than 0.5 μm are prone to fail deposition in the deep lung and are exhaled, whereas particles greater than 5 μm deposit in the mouth

and throat [23–25]. However, this optimum aerodynamic diameter range is prone to clearance by alveolar macrophages once they deposit in the deep lung [25,26,34]. Geometric diameter (dg) comes into play at this stage and particles must typically be more than 6 μm to avoid this rapid clearance and have hydrophilic, rather than hydrophobic, surface chemistry [33,35]. Apart from this, drugs that are targeted to intracellular pathways require nanoscale carriers in the range of 0.2–0.4 μm to improve mucus penetration [36] and cellular uptake in airway epithelial cells [37,38]. These contravening requirements make it a complex design space where the ideal carrier should possess an aerodynamic diameter (da) in the range of 0.5–5 μm during inhalation, geometric diameter (dg) of above 6 μm following lung deposition, and dg of 0.2–0.4 μm to penetrate the mucus and deliver drugs into the cytosol. In addition to this, the trypsin sensitive disease responsive polymer degenerates within 15–18 min upon exposure to the enzyme, extending its advantage to be used as prophylactic delivery system [22] (see Table 1).

Additionally, due to the elaborate distribution and presentation of different pathologies in the lungs, it should also be beneficial for pulmonary drug carriers to incorporate pathophysiologically-triggered drug release mechanisms to minimize non-specific side effects on normal cells [39,40].

In conclusion, 5-ASA and sodium salicylate loaded liposomes incorporated into trypsin (CCRGCC) sensitive PEG-4-acrylate microgels could inhibit IK κ B; thereby, evading the activation of NF κ B and eventually leading to reversion of aggravated autoimmune responses in allergic asthma. It may also serve as an efficient alternative approach for patients with corticosteroid resistance. The design of our hypothesis involves the development of drug loaded liposomes incorporated into disease responsive microgel formulation and their *in vivo* evaluation in standardized murine models for allergic asthma.

Conflict of interest

The authors declare no potential conflicts of interest.

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