Sources of actions and efficacy of antiallergic drugs

Bernard Panaszek, Jerzy Liebhart

Department of Internal Medicine and Allergology, Wrocław Medical University, Traugutta 57/59, PL 50-417 Wrocław, Poland

Correspondence: Bernard Panaszek, e-mail: panaszek@allergolam.wroc.pl

Abstract:
Actions of antiallergic drugs depend on their ability to inhibit a particular stage of allergic reaction and to control symptoms of allergic inflammation. Drugs effective in early stage of allergic reaction prevent allergic cascade by acting on dendritic cells, by means of DNA-based therapies resulting both in down-regulation of T-helper cell type 2 (Th2) cytokine profile release and immunoglobulin E (IgE) production, or by blocking IgE mediated activation of mastocytes and basophiles using monoclonal anti-IgE antibodies. Adhesion molecules and cytokines are also a potential target for therapeutic intervention in allergy. The relationship between drug concentration and pharmacologic response or effect is the essence of clinical pharmacology and the major mechanism of this relationships is that drugs or chemicals bind to or interact with macromolecules on cell surfaces (receptors) or in the cytoplasm to produce the effect. After receptor binding, the drug can activate and/or intensify a normal physiologic function and is termed an agonist, such as β2-receptor agonists and corticosteroids or can inhibit any intrinsic activity by competing for endogenous regulatory substances at the receptor and are called antagonists, such as antihistamines and anticholinergics. Through molecular biology techniques, most of the receptors for β2-agonists, corticosteroids, H1 antihistamines, anticholinergics, antileukotrienes (montelukast, pranlukast, zafirlukast) as well as enzyme inhibitors (methylxanthines, zileuton), which are of interest for asthma and allergy treatment, have been identified and described, and much is still being learned about their molecular activity and interactions.

Key words:
allergic inflammation, DNA-based vaccines, monoclonal anti-IgE, adhesion molecules, cytokines, receptor agonists, receptor antagonists, enzyme inhibitors

Introduction

Sources of actions of anti-allergic drugs refer to their abilities to inhibit a particular stage of allergic reaction and control symptoms of allergic inflammation. This phenomenon is initiated by allergen presentation, which causes cell differentiation with cytokine activation, then specific antibody production and mediator release from sensitive effector cells. At the first stage of allergic reaction, allergen is engulfed by antigen presenting cells (APC), mainly dendritic and Langerhans cells, where it is degraded in endoplasmic reticulum into peptide particles which subsequently form immunogenic complexes with newly synthesized class II MHC molecules [88]. Afterwards, allergen peptide-MHC-II complexes present on APC surface, come into contact with T lymphocyte receptor (TCR) followed by activation of this cell. During this stage of allergic reaction, additional signals appear as the consequence of cooperation between adhesion mole-
cules. Activation of lymphocyte T by specific peptide-MHC-II complex has different consequences for CD4+ cells, which produce cytokines giving them possibility to impact other immune competent cells and towards CD8+ cells which disclose their cytotoxic ability [22].

On the basis of a profile of cytokines produced by CD4+ lymphocytes, phenotype of cells which play a crucial role in allergic reaction (hypersensitivity) is established. They are divided into T helper cell type 1 (Th1) phenotype, releasing mainly interleukin (IL)-2 and interferon-gamma (INF-γ) cytokines, and into Th2 cells producing mainly IL-3, IL-4, IL-5, IL-6 and IL-13 cytokines. These two subpopulations of CD4+ (T) cells determine type of allergic reaction against external and internal allergens. Thus, Th1 lymphocytes are responsible for late type of allergic hypersensitivity (cell-mediated), while Th2 cell phenotype corresponds with specific antibody-mediated (IgE) immediate type of allergic reaction. Positive regulation by IL-4, IL-13 on lymphocytes B and receptor interaction between Th2 cell and lymphocytes B is a condition sine qua non for specific IgE production [40].

Specific IgE interact with active immune cells, which have receptors for this antibody on the cell surface and belong to effector phase of allergic reaction. Among two receptors to be found on cell membrane, the high affinity FcεRI receptor of mastocyte and basophils enables the contact of this cell with allergen by bridging two IgE molecules fixed on the membrane causing activation and degranulation of the cell. Active mastocytes release many mediators (histamine, serotonin, proteoglycans, proteases, enocansoides) and chemotactic factors (eosinophil chemotactic factor of anaphylaxis: ECF-A, neutrophil chemotactic factor-NCF), moreover, they are an abundant source of cytokines (tumor necrosis factor-alpha: TNF-α, IL-4, IL-5, IL-6, IL-8). The second, low affinity receptor for IgE – FcεRII occurs on the surface of many other than mastocyte effector cells, like eosinophils and platelets, but opinion that these cells may be activated via FcεRII is controversial, because convincing data emphasize rather its supportive regulatory role in IgE-dependent cells activation [14].

Eosinophils are very important effector cell of allergic inflammation as a source of cationic proteins (major basic protein-MBP, eosinophil cationic protein-ECP, eosinophil derived neurotoxin-EDN, eosinophil peroxidase-EPO), lipid mediators (leukoriens, prostaglandins, platelet activating factor-PAF), cytokines (granulocyte-macrophage colony-stimulating factor: GM-CSF, TNF-α, IL-3, IL-5, IL-6), enzymes (glucuronidase, phospholipase, ribonuclease, collagenase) and reactive oxygen metabolites (O₂⁻, OH⁻, H₂O₂) [87]. Active platelets, in turn, release from α-grains bronchial hyperreactivity (BHR)-inducing factor, fibroblast growth factor (FGF) and histamine-releasing factor (HRF). Furthermore, PAF, which originates from membrane phospholipids, triggers platelet aggregation by affecting prostaglandin and thromboxane synthesis [60, 81].

Thus current effort and trials in development of effective drugs for allergy treatment are focused on every stage of allergic reaction [14]. Many chemical entities are still in experimental stage, a long way from putting them into practice. Practically, a few groups of efficient medicines, mainly active in effector phase of allergic response, are at physicians disposal in management of allergic diseases. Among them, corticosteroids, which control allergic inflammation, receptor blockers for mediators, stabilizers of active immune cells, enzyme inhibitors and relatively large group of molecules with symptomatic function may be taken into account. An allergic disease is dependent on a multitude of factors, therefore, many events should be taken into consideration in planning treatment, so therapy is usually complex in this case. Factors influencing therapeutic efficacy can be categorized broadly as either drug or patient factors. Among drug factors, pharmacologic properties, structure-activity relationships, physicochemical properties such as lipophilicity or hydrophilicity, formulation or delivery system, and mechanism of elimination from the organism play a pivotal role. Patient factors include both normal physiological and pathophysiologial differences. Physiological variables include age, genetic, and sex differences, as well as normal diurnal patterns and dietary differences. Pathophysiologial differences include state of a disease and its severity and comorbidities demanding other therapies [38].

**Drugs effective at early stage of allergic reaction**

Currently made attempts include at least two directions of research efforts to prevent allergic cascade at
the level of dendritic cells, by means of DNA-based therapies resulting in both down-regulation of Th2 cytokine profile release and IgE production, or at the level of blocking IgE-mediated activation of mastocytes and basophiles using monoclonal anti-IgE antibodies.

Among several types of DNA-based therapies, that are currently being evaluated in the treatment of allergy, the DNA vaccines encoding the expression of allergens, immunostimulatory DNA sequences containing a cytosine-guanosine DNA motif (CpG DNA) that biases the immune response away from a Th2 or proallergic response, and antisense oligodeoxynucleotides that inhibit the translation of specific host cellular messenger ribonucleic acid (mRNA) may be mentioned [37].

DNA vaccines are circular, extrachromosomal pieces of plasmid DNA that can be modified to carry genes of interest (e.g., grass allergen for allergen immunotherapy) and have generally been associated with the induction of Th1 as opposed to Th2 immune response. The studies on DNA vaccines encoding an allergen to modify the Th2 immune response to that allergen developed in mice, showed either IgE or IL-5 cytokine down-regulation response [63]. The other studies using a mouse model of asthma confirmed that mice sensitized to develop Th2 response to ovalbumin (OVA) and pretreated with intradermal injections of plasmid DNA encoding OVA protein developed less bronchoalveolar lavage (BAL) fluid eosinophils, lung tissue eosinophils, and bone marrow eosinophils after inhalation of OVA protein compared with mice injected with a control plasmid DNA construct [11]. Further studies using a rat model of asthma have shown that the IgE response, histamine concentration in BAL fluid, and BHR after provocation with aerosolized dust mite allergen Der p 5 were repressed in rats immunized with a plasmid DNA encoding the Der p 5 allergen [32]. DNA vaccines also prevent anaphylactic reactions to peanut allergen (Ara h 1) in mouse models of anaphylaxis [65], furthermore, they can also significantly reduce mortality associated with anaphylaxis [29], as well as prevent allergic responses to birch pollen [26] and latex allergens [73].

An alternative method of DNA-based immunization that has gained significant attention lately is the use of CpG-rich immunostimulatory DNA sequences as inducers of Th1 responses to allergen. The mechanism by which CpG DNA may influence cells and mediate intracellular signaling is at present incompletely understood, however, Toll-like receptors (TLR) expressed on dendritic cells and DNA protein kinase (DNA-PK) [12] as well as mitogen-activated protein kinase (MAPK) may participate in this process [86]. Several studies have established that CpG DNA can inhibit Th2 cytokine responses, as well as eosinophilic inflammation of airways and BHR in mouse models of asthma [75]. In addition CpG DNA also significantly reduces blood eosinophilia, suggesting the decrease in the bone marrow production of eosinophils which is associated with a significant inhibition of cytokines (IL-5, GM-CSF, IL-3) derived from T cells, very important in allergic inflammation [10]. Evidence for the ability of CpG DNA to activate human cells is derived from several in vitro studies demonstrating that human monocytes, dendritic cells, B cells, and NK cells are activated by CpG DNA [27] with production of antiallergic cytokine profile (IFN-γ, IL-6, IL-12, TNF-α). Moreover, CpG DNA directly activates human dendritic cells to express increased levels of co-stimulatory molecules, which makes the dendritic cells more effective inducers of allogeneic T cell proliferative responses [28]. In research on mononuclear cells derived from dust mite sensitive patients, CpG DNA decreased Der p 1-induced IL-4 production and increased Der p 1-induced IFN-γ production [7].

At present there is no published data on the use of antisense therapy in the treatment of human allergic disease or asthma but several gene products important to asthma and allergic inflammation have been targeted with antisense oligodeoxynucleotide in animal models of allergy. These include cell surface receptors (adenosine A1, IL-5 receptor) [62], cytokines (IL-4) [52], intracellular signaling molecules (Syk protein tyrosine kinases) [42], and transcription factors (GATA-3) [21]. GATA-3 is a transcription factor preferentially expressed in Th2 lymphocytes that regulates the expression of Th2 cytokine genes important to allergic reaction. Studies with antisense targeted to GATA-3 in mouse models of asthma have demonstrated that GATA-3 antisense inhibits eosinophilic airway inflammation, BHR, and cytokine IL-4 and IL-5 expression. Antisense introduced directly to the airways inhibits stem cell factor, a mast cell growth factor thus suppresses airway inflammation and IL-4 production.

A number of intensive studies currently in progress aim to evaluate the therapeutic potential of a variety of anti-IgE antibodies and attempt to generate high-
affinity humanized antibodies capable of preventing IgE binding to the receptor. Among many potentially active monoclonal antibodies tested so far, only omalizumab has currently been introduced in clinical practice [64]. Omalizumab is a recombinant DNA-derived humanized IgG1κ monoclonal antibody that has a molecular weight of approximately 149 kD and selectively binds to human IgE inhibiting its fixing to the surface of mast cells and basophilic granulocytes and subsequently prevents the release of pro-inflammatory mediators that induce an allergic inflammation. The antibody is produced by a Chinese hamster ovary cell suspension culture in a nutrient medium containing the antibiotic gentamicin [66]. Therapeutic anti-IgE antibodies are able to reduce free IgE levels and to block the binding of IgE to FcεRII without crosslinking IgE and triggering degranulation of IgE-sensitized cells. Omalizumab is the non-anaphylactogenic anti-IgE monoclonal antibody that binds to the C epsilon3 domain of immunglobulin IgE at the same site as these antibodies bind FcεRI and FcεRII. Therefore, omalizumab inhibits IgE effector functions by blocking IgE binding to high-affinity receptors on IgE effector cells and does not cause mast cell or basophil degranulation because it cannot bind to IgE on cell surfaces where the FcεRI receptor already masks the anti-IgE epitope [16]. Studies in patients with atopic asthma showed that omalizumab decreases number of lymphocyte B, producing serum IgE and allergen-induced bronchoconstriction during both the early and late-stage responses to inhaled allergen [25]. By removing free IgE, omalizumab also markedly down-regulates the expression of high-affinity receptors on basophils, mast cells and dendritic cells [4]. After subcutaneous administration, its absorption is slow, reaching the peak concentration in serum after an average of 7–8 days. At recommended doses, serum free IgE levels decrease within 1 hour following the first dose and are maintained between doses. A dose and dosing frequency are adjusted according to body mass and serum total IgE concentration before commencing the treatment [17]. In several clinically controlled trials, omalizumab proved effective in reducing asthma-related symptoms, decreasing corticosteroid use and improving quality of life of asthmatic patients [74]. Recent studies show the benefits of omalizumab as add-on therapy in patients with severe persistent asthma who are inadequately controlled by optimal pharmacotherapy [56, 74]. The anti-IgE approach to asthma treatment has several advantages, including concomitant treatment of other IgE-mediated diseases such as allergic rhinitis [15, 17], conjunctivitis [64], atopic dermatitis [43], latex allergy, food allergy and chronic urticaria [51] with a favorable safety profile and a convenient dosing frequency.

Adhesion molecules and cytokines as a potential target for therapeutic intervention in allergy

Cell adhesion molecules (CAM) are a numerous, heterogeneous group of cell surface proteins, which are both receptors and ligands for receptors. Their functions include adhesion, recognition, cell-to-cell interaction and communication between intermediate cells and extracellular matrix. Among groups of CAM, selectins, integrins, cadherins and other isoforms, including CD44 can be distinguished [9].

Selectins are a family of transmembrane molecules, expressed on the surface of leukocytes and activated endothelial cells. Selectins contain an N-terminal extracellular domain with structural homology to calcium-dependent lectins, followed by a domain homologous to epidermal growth factor, and two to nine consensus repeats (CR) similar to sequences found in complement regulatory proteins. Each of these adhesion receptors is inserted via a hydrophobic transmembrane domain and possesses a short cytoplasmic tail. The initial attachment of leukocytes, during inflammation, from the blood stream is due to the activity of the selectin family (L-selectin, P-selectin, E-selectin) and causes a slow downstream movement of leukocytes along the endothelium via transient, reversible, adhesive interactions called leukocyte rolling. Each of the three selectins can mediate leukocyte rolling given the appropriate conditions [35]. Selectin interactions evoke allergic inflammation in the lung and skin, thus, targeting selectin interactions to treat inflammation may have variable effects depending on the site and origin of the inflammatory response [35]. The latest efforts were undertaken to develop anti-selectin antibodies, anti-selectin receptor antibodies, recombinant selectin counter-receptors, low molecular weight selectin antagonists (glycomimetics), induction of selectin tolerance and selectin-targeted imaging agents [41].
Integrins are heterodimers formed from the alpha and beta chains. The alpha subclass is responsible for a specific binding to ligands and defines the specific features of these molecules. The 8 chain participates in the integration with cytoskeleton proteins. It determines the functions of the integrin receptor. The best recognized integrins include: integrin beta1 with such ligands as VCAM-1, MAdCAM-1, TSP-1, laminin, fibronectin, tenascin, furthermore beta2 with such ligands as ICAM-1, ICAM-2, ICAM-3 and beta3 with fibronectin, thrombospondin and vitronectin as their ligands [34]. The expression and activity of integrins have been found to be affected by a variety of factors being either activators or inhibitors and therapeutic attempts are focused on disturbing their cell-to-cell and cell-to-extracellular matrix interaction to decrease allergic inflammation [20, 58, 72].

Cytokines produce their biological activities through their linkage to signal transduction pathways mediated by high affinity receptors on cells involved in allergic reaction. The signaling cascade stimulated by receptor binding forms a complex network with cross-communication. Thus, a single receptor may couple to multiple transduction systems or multiple receptors may couple to the same pathway. Most cytokine receptors are members of four families so therapeutic intervention in this complex network is very difficult [8]. However, much effort has been undertaken to affect these cytokines, which play crucial role in allergic reaction. Interleukin-4 is believed to be an important mediator of asthma and allergic diseases. Blockade of IL-4 could potentially inhibit the cellular pathways that lead to the production of other inflammatory cytokines and IgE antibodies, which are important in the development of asthma. For this purpose, humanized anti-IL-4 antibody, a high-affinity, neutralizing antibody was used, which has been demonstrated to effectively block binding of IL-4 to its receptor and to inhibit the synthesis of IgE antibodies in vitro [85]. Neutralizing antibodies for interleukins (IL)-2, -5 and -8, RANTES and leukemia inhibitory factor (LIF) when added to the BAL fluid from patients with birch-pollen allergy, inhibited the chemotactic activity leading to eosinophil accumulation in the lung of pollen-allergic asthmatics [82]. Inhibition of the soluble receptors (TNRF2/p75) of TNF-α and another inhibitor of this cytokine – monoclonal antibody may also be useful in the treatment of severe asthma [31].
extended side chain reacting with a specific site within the β2-adrenergic receptor, the so-called exosite, resulting in repeated stimulation of the portion of the receptor connected with adenyl cyclase [53].

Apart from the beneficial effects of the β2-adrenergic agonists on relaxation of bronchial smooth muscle they have also nonbronchodilator activity, because β2-adrenergic receptors are widely distributed in the lungs and cells associated with the asthmatic inflammatory response. Introduced in the treatment of asthma β2-adrenergic agonists improve mucociliary clearance by increasing ciliary beat frequency and chloride ion and water secretion into bronchial lumen which makes respiratory epithelium more resistant to viruses and bacteria [6]. These drugs are also able to suppress microvascular permeability and swelling of mucous membrane, and diminish cholinergic neurotransmission by prejunctional β2-adrenergic receptors, but more remarkable is their capability to stabilize many cells involved in asthmatic inflammation (basophils, mast cells, eosinophils, macrophages/dendritic cells, T lymphocytes, bronchial epithelial cells) inhibiting their function and mediator release (histamine, leukotrienes C4 and D4, prostaglandin D2, TNF-α, ECP, IL-12, IL-4, thromboxane B2-TBX2, GM-CSF) [67]. Capacity of priming the glucocorticoid receptor by long acting β2-adrenergic agonists is also a very interesting phenomenon. The encouraging clinical results of the combination of LABA and inhaled corticosteroids (ICS) led to in vitro investigations of their possible interactions which displayed enhanced translocation of the glucocorticoid receptor into the nucleus of cells when the LABA are added to suboptimal concentrations of ICS [1]. The mechanism appears to be through priming of the glucocorticoid receptor by mitogen-activated protein kinases (MAPKs) generated as a result of prolonged stimulation of the β2-adrenergic receptor [48].

The glucocorticoid hormones of the adrenal cortex with their synthetic analogues (corticosteroids) represent the most effective class of drugs in therapy of inflammatory diseases including allergic inflammatory reaction. The drug formulations comprise systemic (hydrocortisone, prednisone, prednisolone, methylprednisolone, triamcinolone, dexamethasone), inhaled (beclomethasone, budesonide, fluticasone, flunisolide, mometasone, pro-drug cycloesone) and topical (hydrocortisone, betamethasone, dexamethason) forms of chemicals. Corticosteroids first penetrate the plasma membrane then bind to a specific receptor forming the activated receptor complex that is translocated to the nucleus, where it works by inducing gene transcription, exerting the effect eliciting transactivation and transrepression of various genes and transcription factors, such as nuclear factor kappa B (NF-κB) and activated protein 1 (API) [57]. In consequence, corticosteroids repress expression of genes effectuating proinflammatory activity including cytokines, chemokines, interleukins, metalloproteases, enzymes synthesizing prostanoids, inflammatory peptides, nitric oxide, and enhance expression of genes perpetuating anti-inflammatory action, that embrace soluble interleukin receptors, membrane functional proteins, membrane regulatory proteins, intracellular regulatory proteins [63].

Antihistamines were discovered more than 60 years ago, about 30 years after histamine was identified. Recent efforts in histamine research include improved understanding of its important role as a chemical messenger involved in physiological responses in the brain and other organs, and in pathological responses, including immunomodulatory effects in the allergic reaction [30]. The mechanism of action of histamine depends on a class of its receptors (H1, H2, H3, H4) among which H1 plays pivotal and H2 minor role as a target for therapeutic intervention in allergic diseases [44]. All histamine receptors described at present are heptahelical structures that transduce extracellular signals through various G proteins, which function as mediators between the cell surface receptors for histamine and the intracellular second messenger systems. H1 antihistamines present the most commonly used medications in the world. The majority of the first-generation H1 antihistamines, such as chlorpheniramine are built of one or two heterocyclic or aromatic rings (AR1, AR2) connected by nitrogen, carbon, or oxygen (X) to the ethylamine group. The tertiary nitrogen of this ethylamine group has two substituents (R1, R2) and may be contained in a piperazine or piperidine ring. The character of the linkage atom (X) has been used to divide H1 antihistamines into six groups: ethanolamines, ethylene diamines, alkylamines, pipерazines, piperidines, and phenothiazines. Two of the second-generation H1 antihistamines, cetirizine and levocetirizine, are piperazines, but most of the other second-generation drugs (desloratadine, ebastine, fexofenadine, levocabastine, loratadine, mizolastine) contain a piperidine ring [71]. The H1 antihistamines seem to act not only as simple pharmacologic antagonists of histamine at H1 receptor sites,
but by binding to H1 receptors and preventing the agonist from binding, thus blocking the H1 response. For the most part of H1 antihistamines, this binding was competitive and readily reversible, therefore, recently, this view has been challenged by experimental studies. Data obtained from these experiments showed the inactive and active conformations of H1 receptors existing in equilibrium, so two subdivisions (agonists and antagonists), were supplemented by three other subdivisions, namely agonists, inverse agonists, and neutral agonists [46]. Inverse agonists combine with and stabilize the inactive conformation of the receptor to shift the equilibrium towards the inactive state. Thus, they may down-regulate constitutive receptor activity, even in the absence of histamine [45]. For most H1 antihistamines, antiallergic and anti-inflammatory effects have been documented in vitro and in vivo, showing their efficacy in patients with allergic disorders in whom nasal, ocular, bronchial, and skin allergy challenge models have been introduced for quantifying the antiallergic effects of H1 antihistamines [24, 70]. Clinical usefulness of H2 antihistamines (ranitidine, famotidine) in allergy may be less valuable then H1 blocking drugs, but H2 antagonists administered concomitantly with H1 antihistamines, reveal significant efficacy in the prophylaxis and treatment of urticaria and anaphylaxis [47]. Interestingly enough H2 antihistamines show also immunomodulatory property resulting in reduction of activity of endogenous histamine on lymphocytes and other immune active cells, as well as cytokines and adhesion molecules, mediated reaction [33, 89].

**Anti-leukotrienes** interfere with arachidonic acid metabolism and these drugs are now available mainly for the treatment of the complex inflammatory disease that is asthma. Modulation of leukotriene activity has focused on two segments of the pathway of asthmatic inflammation. The new drugs, specifically developed to interfere with these two inflammatory pathways, are the cysteinyl leukotriene receptor antagonists (cysLTRAs) and 5-lipoxygenase (5-LO) inhibitors [84]. The main pharmacologic difference between the cysLTRAs and the 5-LO inhibitors is that cysLTRAs inhibit the activity of the cysteinyl leukotrienes (cysLTs) only, while 5-LO inhibitors block the production of both LTB4 and cysLTs. There are practically three cysLTRAs acting as cysteinyl leukotriene 1 (CystLT1) receptor antagonists (montelukast, pranlukast, zafirlukast), whereas zileuton inhibits the 5-LO [68]. Antileukotriene therapy in asthma prevents the effects of allergen-induced inflammation, nocturnal asthma, exercise-induced bronchospasm /bronchoconstriction and aspirin-induced asthma [77]. For many years, ability of these drugs to inhibit asthmatic inflammation in chronic asthma have been discussed. Several published reports have supported an anti-inflammatory effect of antileukotriene therapy in chronic asthma based on the observation that blood eosinophils decreased over time after treatment with both cysLTRAs and 5-LO inhibitors [54]. Moreover, many studies carried out recently suggest that antileukotrienes may interact with inhaled corticosteroids and improve their anti-inflammatory properties by blocking this pathway of asthmatic inflammation which depends on cysteinyl leukotrienes thus completing an anti-inflammatory effects [76]. In addition, the combination of an antihistamine (loratadine) and cysLTRA (montelukast) has also been evaluated in allergic rhinitis and the combination provided greater improvement in total daytime symptom score, rhinorrhea, nasal itching, and sneezing than either of the two individual treatments [13]. Cysteinyl LTs are also believed to play a role in atopic dermatitis, but little research has been published about efficacy of cysLTRAs (montelukast) and 5-LO inhibitors (zileuton) in this very complex, from pathological point of view, disease [36].

**Methylxantines**, phosphodiesterase inhibitors (PDEs), especially theophylline remains one of the most widely prescribed drugs for the treatment of asthma worldwide because it is inexpensive and widely available. However, in many industrialized countries theophylline has become a third-line treatment that is only used in poorly controlled patients. Despite the fact that theophylline has been used in asthma therapy for more than 60 years, many doubts still surround its molecular mode of action and its logical place in therapy [2]. Theophylline is a weak and nonselective inhibitor of PDEs, which break down cyclic nucleotides in the cell, thereby leading to an increase in intracellular cyclic 3',5'-adenosine monophosphate (cAMP) and cyclic 3',5'-guanosine monophosphate (cGMP) concentrations [5]. Apart from inhibition of PDEs, possible mechanisms of action of theophylline involves adenosine receptor antagonism (A1, A2A, A2B), stimulation of catecholamine release, mediator inhibition (prostaglandins, TNF-α), inhibition of intracellular calcium (Ca2+) release, inhibition of NF-κB (decreased nuclear translocation), increased histone deacetylase activity (in-
creased efficacy of corticosteroids) [2]. When given at low doses (plasma concentrations of 5 to 10 mg/l), theophylline demonstrates anti-inflammatory and immunomodulatory effect without producing numerous side effects. Recently, there has been an increasing interest in selective phosphodiesterase inhibitors, which may enhance the beneficial effects and reduce the adverse effects of theophylline. To date, 11 PDE families of enzymes have been identified by molecular cloning. Among selective PDE inhibitors, several PDE4 inhibitors are currently under research and are tested in clinical trials in asthma and allergic rhinitis. The studies demonstrate an anti-inflammatory effect that includes eosinophils, neutrophiles and T lymphocyte activity [3, 55].

The cromone group of compounds with similar physicochemical properties enclose cromolyn sodium (sodium cromoglycate), administered with the use of a dry powder inhaler (Spinhaler) and nedocromil sodium (nedocromil) delivered by a metered-dose inhaler (MDI). Mechanism of action of cromones arises from abolishing or inhibiting calcium channel activation of mast cells and basophils degranulated by an allergen [80]. Animal studies have demonstrated that both drugs inhibit chloride transport in a variety of cells, reduce the open-channel availability of single Cl^- channels and inhibit the whole-cell Cl^- current activated in pulmonary endothelial cells by exposure to hypotonic saline [90]. Although many studies developed in the past have shown anti-inflammatory properties of these compounds, such as a significant reduction in EG2+ eosinophils, AA1+ mast cells, and CD4+, CD8+, CD3+, and CD68+ lymphocytes as well as adhesion molecules (ICAM-1, VCAM-1) in bronchial biopsies, at present cromones are being perceived as less effective medicines in treatment of asthma and other allergic diseases [69]. Nevertheless, there are still physicians, especially pediatricians who prefer cromones as safe and efficient enough drugs administered in allergy. It should be also emphasized, that an oral formulation of cromolyn sodium is effective in the management of food allergy and may be used either to prevent acute reactions to foods in planned deviations from an elimination diet or to allow for some relaxation of a restricted diet [59].

The narrowing of bronchial lumen is mediated partly by an increased release of acetylcholine from the parasympathetic nerves what is demonstrated also in asthma. Therefore, anticholinergic drugs that are muscarinic receptor blockers were introduced in the treatment of asthmatic patients to achieve effective airway relaxation. Muscarinic receptors have now been divided into five subtypes M1-M5, all of which are blocked nonselectively by atropine, but in the lung, there are mostly M1, M2, and M3 receptors. Several studies conducted in allergic asthmatics have shown that intravenous atropine (1.5 to 2.5 mg) or inhaled atropine (1.5 mg) completely blocks antigen-induced increases in airway resistance [19]. Similar results have been reported when more selective anticholinergic compounds as ipratropium bromide and oxitropium bromide were inhaled into bronchial tree of patients with asthma [23]. The main problem with this group of drugs is the fact that all the anticholinergics used at present are not fully selective, hence, they block both the M3 receptor, which mediates bronchial smooth muscle contraction, and the M2 receptor, which reduces the release of acetylcholine from the nerve endings. Thus, the effects of any nonselective antimuscarinic drug depend on a balance between blockade of the inhibitory neuronal M2 receptor and blockade of the postjunctional M3 receptor. Recently introduced in clinical practice selective M3 receptor antagonist tiotropium bromide a quaternary ammonium compound, related to ipratropium, gives a hope for better understanding the role of anticholinergics in asthma management [78]. Pharmacokinetic selectivity of tiotropium bromide for M3 receptors depends on its dissociation from muscarinic receptors so this compound dissociates from the M2 receptor nearly 10 times faster than from the M3 receptor (T1/2 3.6 h vs T1/2 34.7 h) although its affinity for M2 and M3 receptors remains approximately equal. In addition, tiotropium dissociates from M1 and M3 receptors 100 times more slowly than ipratropium [18], thus a special type of balance between muscarinic receptors determines its selectivity. The difficulty of designing antagonists that are selective for M3 receptors over M2 receptors consists in their structural similarity that arises from the degree of amino acid sequence homology. It ranges from 71% to 86% among the five subtypes of muscarinic receptors compared with less than 50% homology among most other families of G protein-linked receptors [79]. The M2 and M3 receptors share 77% amino acid sequence homology which reflects the degree of complexity of developing highly selective ligands for the receptors. It is well documented that anticholinergics are less effective than β-agonists as single-agent therapy in the treatment of asthma. However, acute exacerbations of asthma re-
result from an accumulation of factors, some of them, which promote increased cholinergic transmission to the airways, are susceptible to anticholinergic therapy especially in combination with β2-agonists [83]. Clinical trials of nasal ipratropium spray have demonstrated that it is effective in relieving symptoms, mainly rhinorrhea, in patients with allergic rhinitis, nonallergic rhinitis, and the common cold and such treatment is well tolerated [39].

References:

33. Itoh H, Takahashi HK, Iwagaki H, Yoshino T, Morimoto Y, Saito S, Yagi T, et al.: Effect of histamine on intercel-
34. Janson C, Ludviksdottir D, Gunnbjornsdottr M, Bjorn-
sson EH, Hakansson L, Venge P: BHR-study group. Cir-
37. Kavanaugh A, Broide D: Immunomodulators. In: Midd-
44. Leonardi A, DeFrancis G, De Paoli M, Fregoni I, Plebani M, Secchi A: Histamine-induced cytokine production and ICAM-1 expression in human conjunctival fi-
47. Lin RY, Curry A, Pesola GR, Knight RJ, Lee HS, Bakal-
48. Magne S, Couchie D, Pecker F, Pavoine C: Beta(2)-adre-
nergic receptor agonists increase intracellular free Ca(2+) concentration cycling in ventricular cardiomyo-
50. Mandon B, Siga E, Champagnolle A, Imbert-Teboul M, Elalouf JM: Molecular analysis of beta-adrenergic recep-
52. Molet S, Ramos-Barbon D, Martin JG, Hamid Q: Adop-
tively transferred late allergic response is inhibited by IL-4, but not IL-5, antisense oligonucleotide. J Allergy Clin Immunol, 1999, 104, 205–214.
55. Nejman-Gryz P, Grubek-Jaworska H, Glapiniski J, Chazan R: Effect of phosphodiesterase 4 inhibitor (roli-


Received:
March 15, 2007; in revised form: August 06, 2007.