



## Review Article

# Delivery Systems Using Glycyrrhizic Acid for Allergen-specific Immunotherapy



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## Abstract

There has been a recent increase in the incidence of allergies and the severity of allergic reactions worldwide. Most allergy treatments, such as antihistamines, only treat the symptoms of the allergy. In contrast, allergen-specific immunotherapy (ASIT) refers to a pathogenetic treatment that prevents the progression of allergies. ASIT can also reduce the risk of mild reactions, such as allergic rhinitis, worsening into more serious conditions, such as allergic bronchial asthma. ASIT is performed by administration of an allergen, usually either subcutaneously or sublingually. Sublingual administration is regarded as safer than subcutaneous administration because of a reduction in the risks associated with systemic effects. The uptake of allergens by the mucous membranes in sublingual administration can be improved using delivery agents, such as liposomes, dendrimers, and nanoparticles. Glycyrrhizic acid can self-associate to form micelles and thus, form complexes to enable the delivery of poorly soluble drugs. In addition, glycyrrhizic acid has been shown to have anti-inflammatory effects and is itself a potential treatment for allergic diseases such as allergic rhinitis and asthma. Thus, the development of an ASIT using glycyrrhizic acid is of interest. Herein, we review allergic bronchial asthma and allergic rhinitis and the use of ASIT in the treatment of these conditions. We then discuss glycyrrhizic acid and the potential development of delivery agents using glycyrrhizic acid for use in ASIT.

## Introduction

Allergic conjunctivitis and allergic bronchial asthma (BA) are among the most common manifestations of an allergic reaction.<sup>1</sup> Studies show that allergic rhinitis (AR) may be associated with an enhanced risk of promoting BA.<sup>2,3</sup> The only known method that can modify the immune response to treat allergies is allergen-specific immunotherapy (ASIT).<sup>4,5</sup> This method involves the introduction of gradually increasing doses of allergens until immunological tolerance is achieved.<sup>6</sup> Among the various types of ASIT, the safest method is intranasal administration of an allergen.<sup>7</sup> There are many options for allergen delivery systems for ASIT, including synthetic polymers (polyesters, such as lactic acid polymers, and polyglycol), natural polymers (polypeptides and polysaccharides), liposomes, virus-like particles, and dendrimers.<sup>8</sup> One

candidate for a delivery vehicle for ASIT is glycyrrhizic acid (GA). GA can form micelles as well as having immunomodulatory properties.<sup>9</sup> This article reviews general information regarding allergic BA, AR and ASIT and presents data on the properties of GA and the possibility of its use as a delivery agent for ASIT.

## Allergic rhinoconjunctivitis

AR is one of the most common allergic diseases, and AR diagnoses appear to be on the rise worldwide.<sup>10</sup> The main symptoms of allergic rhinoconjunctivitis include itching in the nose and eyes, sneezing, rhinorrhea, lacrimation, and conjunctival hyperemia.<sup>3</sup> Allergic rhinoconjunctivitis symptoms affect all aspects of daily life and cause decreased sleep quality and decreased performance. Sleep was reported to be disturbed in approximately 50% of the surveyed AR patients, 61% reported fatigue, 38% had increased irritability, and 23.5% reported feeling unwell.<sup>11</sup> A diagnosis of AR is made from anamnestic data and patient symptoms. Important factors in making a correct diagnosis are if relief of clinical symptoms occurs with the use of antihistamines, as well as the occurrence of symptoms under certain conditions, e.g., seasonality, being in a dusty room, and being in contact with animals. Diagnosis of AR is likely if two or more classic symptoms of AR are confirmed, lasting for more than 1 h during the day. When a rhinoscopy is performed, an edematous pale mucous membrane is found in patients with AR. Skin testing is an important diagnostic method for AR. A skin test

**Keywords:** Bronchial asthma; Allergic rhinoconjunctivitis; Allergen-specific immunotherapy; Glycyrrhizic acid.

**Abbreviations:** ASIT, allergen-specific immunotherapy; AR, allergic rhinitis, BA, bronchial asthma; GA, glycyrrhizic acid; IL, interleukin; PRRSV, porcine reproductive and respiratory syndrome virus; SCIT, subcutaneous immunotherapy; SLIT, sublingual immunotherapy; Treg, regulatory T cell.

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is a simple, effective, and safe way to identify allergies. The results of a skin test are available 15–20 m after the start of the procedure, and several different allergens can be assessed in one session.<sup>12</sup>

Treatment of AR involves the use of drugs, such as corticosteroids, antihistamines, antagonists of leukotriene receptors, cromones, and decongestants.<sup>13</sup> Oral antihistamine are drugs that treat allergy symptoms by blocking the effects of histamine. Currently, preference is given to the use of second-generation antihistamines, because first-generation antihistamines have many side effects on the central nervous system, as these compounds can freely pass through the blood-brain barrier to act on different receptors. Second-generation antihistamine drugs have an improved safety profile over the first generation. However, it has been reported that even second-generation antihistamines can cause central nervous system effects at the recommended doses. Cromones, which are stabilizers of mast cell membranes, are used for the prevention and treatment of seasonal AR, and the treatment only of year-round AR symptoms, because these drugs do not have a sufficient suppressing effect on nasal obstruction. The membrane stabilizing effect of these drugs develops slowly, and the drugs need to be used four times a day, which creates a considerable inconvenience for patients. Another drawback is the need to use it four times.<sup>11</sup> Decongestants and vasoconstrictors are used to treat AR formulated as drops or sprays. These treatments effectively and quickly restore nasal breathing for a short time. With short courses of use (up to 10 days), these drugs do not cause changes in the mucous membranes of the nasal cavity, but with longer-term use, rebound syndrome occurs resulting in the development of persistent edema, profuse rhinorrhea, and changes in the morphological structure of the mucous membrane.<sup>14</sup> Intranasal corticosteroids are recommended as the first line of treatment for allergic rhinoconjunctivitis in patients with mild-to-moderate persistent symptoms. The high efficiency of the use of intranasal topical steroid drugs as a means of basic therapy for AR in conjunction with antihistamines, and as a monotherapy, has been convincingly demonstrated. However, the use of topical steroids only has a stopping effect and these drugs need to be used regularly.<sup>11</sup>

## BA

BA is the result of a complex interaction between genetic susceptibility and environmental factors that only results in the disease in susceptible individuals.<sup>15</sup> The most frequent phenotype is allergic asthma, which is caused by inhaled allergens, such as house dust mites, epidermal allergens, and grass pollen allergens. The prevalence of allergic asthma has increased greatly over the past decades and now affects more than 330 million people worldwide. Approximately 50% of asthma patients are sensitized to house dust mite allergens.<sup>1,2,16,17</sup> The most common asthma symptoms are choking, shortness of breath, squeezing in the chest, and heaviness in the chest. Before an attack, the patient begins to cough and discharge sputum, and distinct wheezing occurs.

An attack of BA is a sudden deterioration in a patient's condition, which occurs mainly at night or in the early morning. The patient wakes up with a paroxysmal obsessive dry cough and takes a characteristic posture—leaning forward and with the weight resting on hands. The attack is accompanied by shortness of breath, increased heart rate, cough, and wheezing on exhalation.<sup>18</sup> To diagnose BA, it is necessary to consider anamnesis, physical examinations, and laboratory and instrumental data. In the laboratory analyses of patients with BA, eosinophilia will be observed, and the titer of specific IgE will be increased in the serum. In spirometry analysis, a patient with BA will have a Tiffno index < 0.75. In late asthma with long-term BA, the patient's X-rays will show signs of emphysema. An increase in FEV1 of >12% in a bronchodilator test also indicates BA.<sup>15</sup>

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Treatment of BA is via stepwise therapy using inhaled glucocorticosteroids and  $\beta$ -2 agonists with various durations of action. However, despite clinical efficiency in achieving asthma control, current BA treatment regimens cannot cure the disease because of the ongoing remodeling of the airway walls, even in well-controlled patients, and the chance of developing asthma attacks is unchanged.<sup>19,20</sup> In general, to prevent the development of exacerbations of allergic diseases, it is recommended to avoid or minimize exposure to causative allergens and irritants, such as dust mites, mold, pollen, pets, and tobacco smoke. Allergen-impermeable bedding and maintaining the indoor relative humidity below 50% are effective in protecting against house dust mites. Pollen exposure can be minimized by keeping windows closed and limiting time spent outdoors. Removing pets from the home can reduce symptoms within 4–6 months for patients sensitized to epidermal allergens.<sup>11</sup> It has been shown that in patients with existing asthma and sensitivity to dust mites, bronchospasm, and bronchial hyper-reactivity worsen when exposed to a tick-borne allergen and decrease in a tick-free environment.<sup>21</sup> However, in some cases minimizing contact with an allergen is not always possible.<sup>22,23</sup>

## Indications and contraindications of ASIT

The use of modern pharmacological drugs can control the symptoms of atopic diseases, but the only method that changes the nature of the immunological reactivity is ASIT.<sup>4,5</sup> The basis of the ASIT technique is to change the pathological immune response to a physiological response and involves achieving clinical tolerance to allergens by administering extracts of allergens to patients with allergic diseases.<sup>20</sup> For ASIT to be effective, the amount of the injected allergen must greatly exceed the amount of similar allergens that enter the body under natural conditions.<sup>24</sup>

The main indications for ASIT are persistent and intermittent AR (rhinoconjunctivitis), controlled atopic BA, hay fever (including in combination with allergic rhinoconjunctivitis and BA), and insect allergies, such as toward hymenoptera insects (including anaphylactic reactions). Contraindications for ASIT can be absolute (children under 2 years of age, starting ASIT during pregnancy, uncontrolled BA, active autoimmune diseases, acquired immunodeficiency syndrome, and malignant neoplasms) and relative (children 2–5 years of age, partially controlled BA, autoimmune diseases in remission, beta-blocker therapies, cardiovascular diseases, and human immunodeficiency virus infections). The final decision on ASIT is made individually for each patient, taking into account all factors.<sup>25</sup> The optimal duration of treatment is 3 to 5 years, and the clinical benefits can persist for many years after discontinuation.<sup>4,5</sup>

## Mechanisms of ASIT

Immunological processes occurring in the organism during ASIT include the rearrangement of the immune response from T2-type to T1-type and the induction of T-regulatory cells that have an immunosuppressive effect. As a result of these changes, there is an increase in the synthesis of blocking IgG and IgG4, which compete with IgE on mast cells for binding to the allergen. It has also been shown that the use of ASIT helps to reduce the number of mast cells and eosinophils, and their mediators in tissues.<sup>3,6,26</sup> In addition, ASIT induces significant changes in allergen-specific T cell subsets,

namely, stimulating Th0/Th1 lymphocytes with a resulting increase in interferon-gamma and interleukin (IL)-2 production. ASIT can contribute to the suppression of Th2 cells and the development of immunological tolerance. In addition, the use of ASIT reduces the activation and release of mediators (histamine, prostaglandin D2, and eosinophilic cationic protein), all of which contribute to the development of immune tolerance, resulting in long-term changes in the body even after discontinuation of the treatment.<sup>20</sup>

Allergen-specific immunotherapy is usually carried out in two ways, subcutaneous administration of an allergen (subcutaneous immunotherapy; SCIT) or sublingual administration (sublingual immunotherapy; SLIT) using single or multi-allergenic extracts in tablet or drop form.<sup>27,28</sup> The main target for SLIT is the immune system of the oral mucosa. The specificity of the introduction of the allergen to the oral mucosa prevents the systemic spread of the allergen, resulting in fewer side effects than other methods of administration. The dendritic cells of the oral mucosa are involved in the capture of antigens, both by acting as antigen presenting cells and by potentiating the development of tolerance through the production of IL10 and transforming growth factor-beta. Within 12–24 h after contact of the dendritic cells with an antigen, the cells migrate to the cervical lymph nodes, where dendritic cells, in the role of antigen presenting cells, interact with naive CD4-T cells and induce Treg cells.<sup>10</sup>

The use of sublingual ASIT can assist in reducing clinical symptoms, as well as reducing the need for the use of pharmacological drugs, in both patients with AR and patients with moderate, persistent BA.<sup>17,29</sup> In BA, a tendency for the reversal of bronchial hyperreactivity has been observed after 3 years of SLIT.<sup>10</sup> The safety of ASIT in asthma has not always been certain; accordingly, the fear of asthma exacerbations has made physicians somewhat reluctant to prescribe ASIT in this context. However, studies have shown that sublingual ASIT was well tolerated and safe in adult patients.<sup>30</sup> For example, very few serious side effects have been recorded with SLIT in patients with mild to moderately persistent BA.<sup>31</sup> In general, SLIT is one of the safest ASIT methods. Local reactions in the form of itching, a burning sensation in the mouth, and edema of the oral mucosa and tongue occur rarely and usually disappear a few days after the start of therapy.<sup>30</sup> Cases of systemic reactions with the use of SLIT are extremely rare, and there have been no reported life-threatening conditions.<sup>25</sup> Currently, sublingual ASIT is approved by European regulatory authorities for many allergens and is widely used throughout the world.<sup>32</sup>

SCIT has been used to treat allergic diseases for approximately 100 years. Numerous studies have demonstrated the effectiveness of this technique, which is manifested by a decrease in clinical symptoms and a decrease in the dose and number of drugs used, for patients with mild-to-moderate symptoms of allergic asthma or persistent AR.<sup>19,20</sup> In patients with allergic rhinoconjunctivitis, the beneficial effects associated with SCIT have been shown to persist for at least 10 years, even though the therapy was discontinued after 3 years of treatment.<sup>4,5</sup> The risk of both local and systemic reactions with SCIT is slightly higher than with SLIT. In ASIT administered by injection, local reactions are observed in most patients and are expressed in the form of redness, itching, and edema at the injection site of the allergen. Generally, local reactions resolve within a day, but in some cases can persist for up to 3 days.<sup>25</sup> Systemic reactions in the form of nasal congestion, sneezing, itching in the nose, itchy eyelids, redness of the eyes, lacrimation, dry cough, itching, and body rashes occur with SCIT for the treatment of mild-to-moderate BA in approximately 0.1% of patients, while severe reactions in the form of anaphylactic shock are rare (1 per

1 million injections).<sup>30</sup> There are serious risks when using SCIT to treat uncontrolled BA. Of the two published reviews of deaths caused by SCIT, 62% of all deaths were associated with uncontrolled asthma. Currently, uncontrolled BA is an absolute contraindication for SCIT. In general, the risk of severe allergic reactions caused by SCIT has decreased in recent years, because medical staff is better prepared to prevent dangerous complications associated with the occurrence of severe reactions to SCIT.<sup>33</sup> Although not completely risk-free, SCIT has an excellent safety profile, provided the treatment protocol is followed precisely.<sup>34</sup>

Intranasal administration of an allergen is a promising method for ASIT. Studies on animals with induced AR have shown that the intranasal route of allergen administration in liposomes led to a decrease in the severity of allergic symptoms and a shift from the T2 immune response toward T1.<sup>1,35</sup> Dosage forms that are applied to nasal mucosa (mucosal vaccines), appear to have especially promising safety profiles because of the absence of risks associated with the injection of an allergen.<sup>7,36</sup>

### Advantages of ASIT

Allergen-specific immunotherapy is currently the only method of treating allergic diseases that shows evidence of a disease-modifying effect capable of inducing remission of the disease and reducing the dose of drugs required for the symptomatic treatment of allergic diseases.<sup>19,20,22,27</sup> Studies of patients with respiratory allergies have shown that 3-year ASIT was sufficient to induce long-term remission of symptoms after discontinuation of treatment. For example, after SLIT with house dust mite allergen extract, patients with AR showed remission lasting for 7 and 8 years after 3 or 4 years of ASIT.<sup>20</sup> The positive effect of ASIT has also been confirmed to persist for 10 years or more after the end of treatment in studies in children with seasonal AR and BA.<sup>4</sup> ASIT should be considered as a treatment in cases where contact with the allergen cannot be controlled, as well as when there is a constant need for medication. In addition to the above advantages of ASIT, the prophylactic effect of ASIT in allergic asthma and the reduction of other concomitant diseases, such as recurrent sinusitis, can be beneficial.<sup>22</sup> Studies have shown that ASIT prevents sensitization to new types of allergens.<sup>3</sup> This prevention could lead to a new ASIT indication with the possibility to reduce the growing prevalence of allergic diseases. However, additional research is required.<sup>37</sup> An important aspect in the treatment of allergic diseases is the economic cost. The results of individual studies and data from systematic reviews have shown that allergen-specific immunotherapy (both SLIT and SCIT) is more economically beneficial than symptomatic drug therapy. ASIT has been shown to reduce medication and medical service costs by up to five-fold, which is equivalent to roughly 80% savings.<sup>38</sup>

### GA: biological properties

Licorice glabra (*Glycyrrhiza glabra*) is a perennial rhizome herb from the legume family (Fabaceae). The roots and rhizomes of licorice contain up to 23% of the saponin glycyrrhizin (a mixture of potassium and calcium salts of GA); 27 flavonoids, including liquiritin, liquiditoid, isoliquiritin liquiditoid, quercetin, kaempferol, and apigenin), the total content of which can reach 4%. Others are glabra, glycyrrhetic acid, steroids, essential oils, asparagine, ascorbic acid (up to 30 mg%), tannins (8.3–14.2%); pigments, resin gums, asparagine, higher aliphatic hydrocarbons and alcohols, higher fatty acids, and alkaloids.<sup>39</sup>

Glycyrrhiza has a broad history of use and has been known since ancient times in China, Egypt, and Japan.<sup>40</sup> In Eastern traditional medicine, licorice root was used to treat stomach diseases (including stomach ulcers), lung diseases (bronchitis and tuberculosis), was included in treatments for rheumatism, impotence, and nephritis and was prescribed to the elderly as a rejuvenating and life-prolonging agent.<sup>39</sup> Glycyrrhiza is widely used in traditional medicine to this day.<sup>40</sup> Licorice extract is used in formulations recommended for diseases of the upper respiratory tract because it has an expectorant and anti-inflammatory action. In addition, licorice is used in diuretic and laxative preparations, because of its antacid and enveloping properties, and is used to treat hyperacid gastritis and gastric and duodenal ulcers. This medicinal plant is also used for the treatment of BA, neurodermatitis, allergic and occupational dermatitis, eczema, rheumatism, gout, and hemorrhoids. Licorice powder is also used in pharmaceutical practice as a base for pills to improve the taste and odor of drugs.<sup>39</sup> GA is a triterpenoid saponin from licorice root extract and is responsible for many of the beneficial properties of the plant.<sup>41</sup>

Glycyrrhizin has been reported to have antiviral activity. Treatment of porcine reproductive and respiratory syndrome virus (commonly known as PRRSV) in animals using drugs containing glycyrrhizin has been demonstrated to reduce the intensity of clinical manifestations. This effect was associated with the ability of the drug to reduce the invasion and replication of PRRSV, increase antiviral innate immune responses, and prevent the accumulation of intracellular reactive oxygen species caused by PRRSV infection.<sup>42</sup> Glycyrrhizin has also been shown to inhibit the replication of viruses.<sup>40</sup> It has been suggested that inhibition of viral particle replication is not the only mechanism responsible for the antiviral properties of GA. GA has also been shown to prevent the penetration of viral particles into cells, as well as prevent the dissemination of the virus by decreasing the release of viral particles from infected cells.<sup>43</sup> It was thought that the membrane-modifying ability of GA may be responsible for this antiviral activity.<sup>40</sup> Recent studies have shown that drugs containing GA can be used as a SARS-CoV-2 treatment because glycyrrhizin has a destructive activity against the receptor-binding domain and angiotensin-converting enzyme 2, which are proteins that are involved in the entry of the virus into the cell.<sup>44</sup>

GA is known to be an excellent antioxidant, as supported by the results of numerous studies. In particular, a hepatoprotective effect mediated by the antioxidant properties of glycyrrhizin has been observed.<sup>40</sup> The antioxidant effect of supramolecular complexes containing the monoammonium salt of GA was studied in the homogenate and mitochondria of rat liver using a model of paracytomolc hepatitis. The administration of the supramolecular complexes to animals with hepatitis reduced the content of primary and secondary lipid peroxidation products.<sup>45</sup> The effectiveness of GA as an antioxidant has been shown in a rat model of induced acute myocardial infarction in which treatment with GA reduced the degree of oxidative stress in the damaged myocardium. In addition, it has been shown that supramolecular complexes of carotenoids with glycyrrhizin exhibited a higher antioxidant activity toward hydroperoxyl radicals, compared with carotenoids without GA.<sup>46</sup> In addition, GA, similar to other terpenic acids, exhibited antioxidant activity in relation to H<sub>2</sub>O<sub>2</sub> in human bronchial epithelium cells.<sup>47</sup>

### Immunomodulatory properties of GA

The antiallergic properties of GA are especially important given

the interest in using GA as a delivery agent for allergen-specific immunotherapy. It is generally accepted that the antiallergic activity of GA is caused by three mechanisms. First, GA regulates Th cell differentiation, which reduces the increased production of the T2-related cytokine, IL4, to restore the T1/T2 immune balance. Second, GA reduces the activity of B cells that produce IgE. Third, GA acts as a mast cell stabilizer, inhibiting the synthesis and release of both histamine in mast cells and  $\beta$ -hexosaminidase, a biomarker of mast cell degranulation.<sup>48</sup> There is also evidence that GA can activate Treg cells.<sup>9</sup> In addition, GA has been shown to exhibit a powerful immunoregulatory effect, activating chicken macrophages against *Salmonella in vitro*, through the expression of the genes responsible for the production of nitric oxide and interferon-gamma.<sup>49</sup> In a model using rabbits with atrazine-induced immune toxicity, glycyrrhizin exhibited protective properties because of the suppression of caspase-3 in the splenocytes of the rabbits.<sup>50</sup>

The anti-inflammatory activity of glycyrrhizin has been known for a long time and many studies have investigated this effect.<sup>40</sup> For example, glycyrrhizin successfully suppressed inflammation caused by *Leishmania donovani* by inhibiting the release of prostaglandin E2 from activated macrophages. GA has also been shown to suppress inflammatory processes by decreasing the production of inflammatory cytokines. In addition, a GA licorice extract has been demonstrated to be effective in relieving the symptoms of atopic dermatitis, such as pruritus, edema, and erythema.<sup>46</sup> Licorice extract and its components, in particular, GA, are effective for the treatment of BA and AR. In an *in vitro* study using cells from patients with AR, GA decreased the levels of IL-4, IL-10, and interferon- $\gamma$  and significantly inhibited the augmented T cell proliferation induced with anti-OX40 mAb.<sup>9</sup> It was shown that GA may have a therapeutic effect on AR, partly by modulation of the T1/T2 balance through suppression of OX40 and increasing the immunosuppressive activity of Tregs. In a mouse model of BA, GA reduced airway inflammation and remodeling via the transforming growth factor  $\beta$ 1/Smad signaling pathway.<sup>51</sup> In a clinical trial, licorice nasal irrigation was superior to steroid and saline nasal irrigation for treating AR.<sup>52</sup> Overall, the anti-inflammatory properties of glycyrrhizin are a result of immunomodulatory, anti-apoptotic, and antioxidant effects. Several studies have linked the anti-inflammatory properties of GA with the antioxidant activity. Especially, it has been shown that glycyrrhizin, along with other components of glycyrrhiza extract, inhibited the production of nitric oxide and inflammatory cytokines in a model using microglial cells activated by lipopolysaccharides.<sup>53</sup>

### Drug delivery systems using GA

One of the major problems in medicinal chemistry at present is the low bioavailability of different medicinal compounds. Low bioavailability is mainly associated with low solubility, low permeability, or both. The solubility of drugs intended for the oral route is especially important. A possible way to deal with the problem is the use of drug delivery systems in the form of complexes with compounds that are easily dissolved in liquid. In many cases, this method allows a significant reduction in the effective dose while maintaining the therapeutic effect. A lipophilic drug molecule can pass through the lipid layer through passive transport (against the concentration gradient), but it is necessary to achieve a sufficiently high concentration of the drug in the extracellular environment. However, 30% of the released drugs are insoluble in water. Considering that approximately 85% of the most commonly used drugs are taken orally, this is a promising trend.<sup>40</sup> GA is an interesting

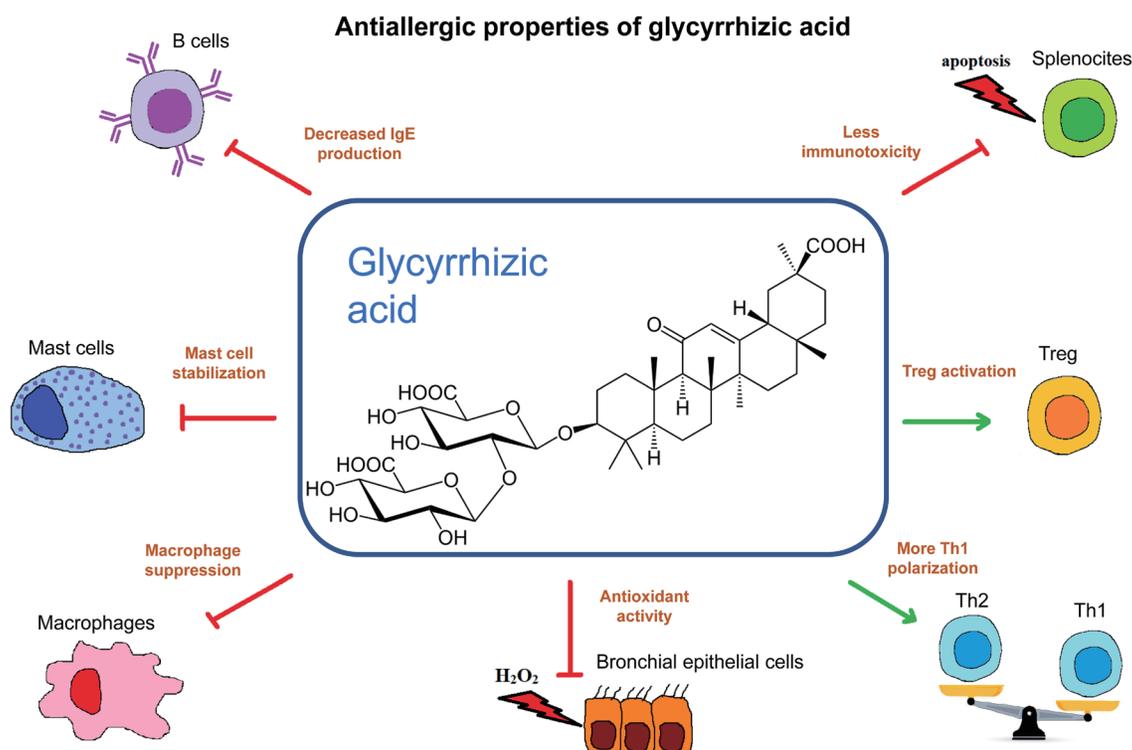


Fig. 1. Antiallergic properties of glycyrrhizic acid. Treg, regulatory T cell.

candidate for drug delivery. GA is a large amphiphilic molecule, containing hydrophilic glucuronic acid moieties and a hydrophobic triterpene core. GA self-associates in liquid media and micelle formation take place through the hydrophobic interactions of the triterpene core of GA.<sup>41,54</sup>

There is evidence that GA can form insoluble complexes with cholesterol and its oxidation products. Cholesterol is a main component of cell membranes that is responsible for the physical properties of the membranes, such as viscosity and permeability. Thus, it can be assumed that one of the mechanisms of the biological activity of GA as a drug carrier is its effect on the properties of cell membranes. Two possible mechanisms have been suggested. First, GA is incorporated into the membranes and locally pushes the lipid bilayer, which increases the diffusion permeability. Second, GA extracts cholesterol from the membrane, thereby reducing the membrane rigidity and facilitating the penetration of molecules through the membrane.<sup>55</sup>

The self-assembling ability and drug complexation in aqueous solutions, as well as its membrane-modifying properties, make GA a promising means of drug delivery.<sup>40,41</sup> Several physicochemical studies have shown that the complexation of hydrophobic drugs with GA can increase the drug's solubility 10-fold compared with the free drug.<sup>40</sup> Studies have shown that GA can enhance the therapeutic effect of medical compounds, and in some cases even change the mechanism of action of the drug. Physicochemical studies of GA have been performed to identify the molecular-cellular mechanism of action of GA. It was found that GA can form stable complexes. Because of the hydrophilic and hydrophobic parts of the molecule, GA formed independent complexes in aqueous and aqueous-alcoholic solutions.<sup>55</sup> Animal studies have shown that phenibut (a nootropic and anxiolytic drug) in combination with GA had a similar effect at a

16-fold lower dose, compared with phenibut alone. In addition, glycyrrhizin increased the mnemonic abilities of the animals and reduced the side effects of phenibut, such as allergic reactions and drowsiness. In addition, the toxicity of phenibut was significantly reduced, and the therapeutic index was increased. Similar effects have been observed for GA in combination with other classes of drugs. For example, a complex of nifedipine with GA exhibited antihypertensive activity at a 10-fold lower dose compared with the free drug.<sup>56</sup> In general, studies of GA as a delivery vehicle for drugs have shown promising results.<sup>57,58</sup> In the case of a complex with a house dust mite major allergen Der p 1, the results of assessing the effect of the GA complex with Der p 1 on the composition of lymphocyte subpopulations and cytokine production of peripheral blood mononuclear cells from healthy donors indicate a change in the T1/T2 immune balance towards the cellular immune response, which can decrease allergic reactions to house dust mite allergen during ASIT.<sup>59</sup>

#### Advantages and disadvantages of GA-mediated delivery of allergens

As mentioned above, GA has an anti-allergic effect due to its immunomodulatory, anti-apoptotic and antioxidant properties (Fig. 1) and has a direct effect in improving the symptoms of allergic airway diseases. As a drug vehicle, GA enhances the membrane fluidity and permeability and increases drug stability, which is of great importance for peptide drugs, including allergens. Therefore, the use of GA for allergen delivery has a number of advantages. On the other hand, GA is associated with a risk of lowering the concentration of potassium ions in the blood.<sup>60</sup> Side effects of GA include hypokalemia, hypertension, hypertensive encephalopathy, rhabdomyolysis, and cardiac arrest. However, GA leads to side

effects after long-term ingestion, and the concentration of GA in food is much higher than required for ASIT. GA are widely used in food and pharmaceutical products, and their safety has been acknowledged in international evaluations.<sup>61</sup> The upper safe dose of GA is 100 mg per day.

As the GA enhances the T1 immune response, the use of the GA can lead to allergic contact dermatitis.<sup>62</sup> Additionally, GA may increase autoimmune reactions according to the T1 type of immune response. However, GA ameliorated the clinical disease severity of experimental autoimmune T1 disease in animal models.<sup>63</sup> Considering the antiallergic properties, efficacy as a drug carrier, and complications of glycyrrhizin, the use of GA as a vehicle for ASIT can cause its health benefits to outweigh its side effects.

### Future directions

ASIT is the only method that can relieve patients of allergies and the need to take stopping therapy. Currently, many studies are in progress to improve this method of treatment and minimize the side effects of the therapy. One of the promising areas of research is the search for new safe delivery agents for ASIT. GA has shown potential as a drug carrier. Further studies of the properties of GA and its interactions with the immune system may enable the use of GA in intranasal ASIT that does not cause systemic side reactions. Moreover, the development of GA-based drug delivery systems for ASIT will likely lead to more effective and safe treatment of allergic diseases.

### Conclusions

GA has anti-inflammatory, antioxidant, and immunomodulatory effects, which allow it to achieve several benefits in the treatment of allergic diseases such as BA and AR. Drug delivery systems based on GA can enhance the delivery and increase the effectiveness of ASIT through the effect of GA on the immune balance.

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### Conflict of interest

The authors have no conflict of interests related to this publication.

### Author contributions

Conceptualization (EP), original draft preparation (EP, MB), review and editing (OK, VK), and supervision (VK).

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