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REVIEW

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Clinical efficacy of sublingual immunotherapy tablets for allergic rhinitis is unlikely to be derived from *in vitro* allergen-release data

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ABSTRACT

Introduction: Allergen bioavailability underpins the efficacy and safety of SLIT tablets. Three product-related factors are likely to influence this: tablet potency, formulation and sublingual holding time.

Areas covered: Tablet formulation determines the rate and extent of solubilized allergen release. Using validated *in vitro* dissolution assays, the two licensed grass pollen SLIT tablets are shown to release ≥85% of their total allergenic activity within several minutes. Sublingual holding time affects the contact duration between solubilized allergens and sublingual tissue. Maximal uptake of allergens by sublingual tissue requires ~5 minutes, with little uptake occurring within the first minute. A higher potency tablet with longer sublingual holding time would provide higher bioavailability, while faster rates of allergen release *in vitro* are unlikely to translate to a greater increase in bioavailability. Differences in dissolution times cannot serve as a surrogate of *in vivo* bioavailability, and are not related to differences in efficacy at the marketed tablet dosages. Rapid *in vitro* dissolution is likely not a key requirement for inducing a potent immune response.

Expert opinion: *In vitro* dissolution cannot predict the clinical efficacy of SLIT tablets but could be important in immune tolerance and safety. In addition, a discontinuous administration regimen may have benefits for adherence and cost without compromising efficacy.

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Administration; allergic rhinitis; efficacy; formulation; grass pollen; *in vitro* dissolution; safety; sublingual immunotherapy tablet

1. Introduction

Sublingual immunotherapy (SLIT) using natural allergen extracts from grass or tree pollen, or house dust mite (HDM), has become a mainstay of treatment for immunoglobulin (Ig) E-dependent respiratory allergies. Two formulations of grass-pollen SLIT tablets are currently licensed for the treatment of allergic rhinitis (AR) in both Europe and the USA [1]. The 5-grass pollen SLIT tablet (Oralair®; Stallergenes Greer, Antony, France) contains purified, calibrated freeze-dried extract of pollen from sweet vernal grass (*Anthoxanthum odoratum*), cocksfoot/orchard grass (*Dactylis glomerata*), perennial rye grass (*Lolium perenne*), meadow grass (*Poa pratensis*) and timothy grass (*Phleum pratense*), and is used at a 300 index of reactivity (IR) dose, equivalent to ~20–25 µg/mL of the group five major allergens [1]. The compressed tablet contains allergen extract plus mannitol, microcrystalline cellulose, croscarmellose sodium, colloidal anhydrous silica, magnesium stearate and lactose monohydrate [1]. The 1-grass pollen SLIT tablet (Grazax®/Grastek®; ALK-Abelló, Hørsholm, Denmark) is a 75,000 SQ-T (standardized quality tablet units) freeze-dried SLIT tablet containing an allergen extract from timothy grass (*P. pratense*), plus fish gelatin, mannitol and sodium hydroxide [1].

SLIT tablets can be easily administered at home and do not require injections [1]. The sublingual route is often used for the administration of small molecule drugs (e.g. nitroglycerine or opioid analgesics) to achieve fast absorption into the plasma. In contrast, proteins of large molecular size do not cross the mucosa to reach the bloodstream, but instead are captured and processed by oral antigen-presenting cells (APCs) within 15–30 minutes [2], as assessed *in vivo* in animal models. APCs loaded with allergen-derived peptides migrate to the cervical lymph nodes within 12–24 h, where they interact with naive CD4 + T cells to induce Th1 and regulatory T cells with suppressive activity within 2–5 days. These CD4 + T cells subsequently migrate into the blood and tissues, resulting in long-term allergen-specific tolerance [3]. The sublingual route offers a special advantage over other mucosal routes, because the antigen can be captured and processed by APCs prior to any significant proteolytic degradation occurring, thereby preserving the integrity of T and B cell epitope repertoires [2].

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Article highlights

- Differences in AIT tablet dissolution times cannot be assumed as a surrogate of *in vivo* bioavailability.
- Clinical efficacy of AIT tablets is unlikely related to a very rapid *in vitro* allergen release at the marketed tablet dosages.
- The current knowledge of the oral immune response supports tablet formulations with dissolution times superior to one minute.
- The relevant aspect for allergists is 6 versus 12 months of treatment which is very important for long term compliance.
- Pre- and co-seasonal administration scheme of AIT tablets appears to be associated with lower annual costs compared to perennial administration.

Allergen bioavailability (in this case, the availability to uptake by APCs) underpins the efficacy and safety of SLIT tablets, and at least two product-related factors, in addition to tablet potency, are likely to influence this, namely tablet formulation and sublingual holding time. Here, we examine how the 5- and 1-grass pollen SLIT tablets differ with regards to these factors and other key characteristics, and whether differences between the products and formulations are of clinical relevance.

2. Product and formulation characteristics of licensed SLIT tablets for grass pollen-associated AR

The 1- and 5-grass pollen SLIT tablets have several differing characteristics, as illustrated by Table 1.

2.1. Tablet potency and administration regimen

The clinical effectiveness of SLIT and wider allergen immunotherapy (AIT) is clearly dose-dependent [6], so clinicians should be able to assess and compare the potency of the various products available. Conversion to the bioequivalent allergy unit (BAU), the only allergen unit approved and recognized by a regulatory agency (the US Food and Drug Administration), permits direct comparison of allergenic potency in this way [7]. The optimal dose of the 5-grass pollen tablet (300 IR) has been confirmed as 9,000 BAU, with higher doses yielding no additional benefit [8,9]. This dose has demonstrated long-term efficacy when prescribed in a discontinuous, pre- and coseasonal regimen (starting approximately 4 months prior to the pollen season) [10], and the high potency of the 5-grass pollen tablet does not appear to be associated with any safety signals or poor tolerability [11].

The approved 75,000 SQ-T dose of the 1-grass pollen tablet equates to 2,800 BAU; this dose is shown to be more effective than the 2,500 and 25,000 SQ-T doses [12], and has also demonstrated long-term efficacy when prescribed continuously during the entire year [13]. In contrast to the clinical development of the 5-grass pollen SLIT tablet, the optimal dose of the 1-grass pollen SLIT tablet has not been confirmed in dose-finding studies. Using the BAU, the 5-grass pollen SLIT tablet appears to have >3-fold greater allergenicity than the 1-grass pollen SLIT tablet.

Economic and cost-effectiveness studies from Germany [14] and Canada [15] have shown that the pre- and coseasonal administration schedule of the 5-grass pollen SLIT tablet appears to be associated with lower annual costs versus perennial administration of the 1-grass pollen SLIT tablet, with annual relative savings estimated at €1,142 and Canadian $1,168, respectively [14,15]. Moreover, compliance with the pre-/coseasonal regimen is high [16]. This has even been demonstrated in a study that administered the 1-grass pollen SLIT tablet to allergic patients according to a pre- and coseasonal schedule instead of the recommended perennial schedule [17]; in this study, compliance ranged from 72% in the first year of treatment to 90% by the third year of treatment [17]. The discontinuous administration regimen of the 5-grass pollen SLIT tablet, in part because of its associated excellent compliance, has therefore been proposed as inherently more effective than perennial SLIT administration regimens [18].

2.2. Grass-pollen epitope coverage

Grass pollen has ubiquitous representation and significant clinical importance worldwide [19]; the distribution of Pooideae species varies across regions of Europe and North America, meaning that pollen allergen exposure to specific

Table 1. Product and formulation characteristics of the 5-grass pollen and 1-grass pollen SLIT tablets.

Characteristic	1-grass pollen SLIT tablet	5-grass pollen SLIT tablet
Administration [4,5]	Perennial	Pre-/coseasonal
Dosing		
Optimal [8,9]	Not determined	300 IR
Licensed [4,5]	75,000 SQ-T	300 IR
Tablet potency [7]	2,800 BAU	9,000 BAU
Dissolution time *in vitro* [27,28]		
Physical disintegration of tablet	2 s	>31 s
To release ≥85% of allergenic activity	~15 s	2 minutes
Clinical onset of action [40]	Not determined	1 month
Grass pollen epitope coverage [20]	+	+++++
Efficacy in clinical trials [40]	+++	+++
Safety in clinical trials [40]	+++*	+++

*Epinephrine was administered during clinical trials of the 1-grass pollen SLIT tablet [n = 1 adult receiving placebo, n = 1 adult receiving 1-grass pollen SLIT tablet (both cases judged as treatment-related) and n = 2 pediatric subjects (of which 1 case was judged as treatment-related)] [40].
BAU, bioequivalent allergy unit; IR, index of reactivity; SLIT, sublingual immunotherapy; SQ-T, standardized quality tablet unit

grasses differs between countries [20,21]. The pollen allergens from different Pooideae species exhibit some shared T and B cell epitopes, while group 1 and group 5 allergens display species-restricted IgE epitopes [22]. The heterogeneity in grass pollen epitopes across species can be differentiated by the immune system, which may support the use of a mixed pollen allergen extract over a single pollen allergen preparation [21]. The ability of the 5- and 1-grass pollen SLIT tablets to mimic the range of epitopes that may be encountered in Europe has been assessed by testing sera from adult patients living in Spain (n = 19) or Sweden (n = 22) [20]. The 5-grass pollen SLIT tablet demonstrated significantly greater inhibition of IgE binding to pollen allergens from 12 grasses common to Europe (bent grass, Bermuda grass, brome grass, cocksfoot, false oat-grass, meadow fescue, meadowgrass, rye-grass, sweet vernal-grass, timothy, wild oat and Yorkshire fog) than did the 1-grass pollen SLIT tablet ($p < 0.0001$), both for the total tested population and when stratified by country. This difference between SLIT tablets was significantly greater for Spanish than Swedish patients ($p < 0.05$). Fewer epitopes associated with grass pollen allergy were present in the 1-grass pollen SLIT tablet, compared with the 5-grass pollen SLIT tablet, and this difference was especially pronounced when comparing sera from patients in the two geographical regions [20]. In agreement with previous research [22], the authors concluded that the 5-grass pollen SLIT tablet has superior epitope coverage to the 1-grass pollen SLIT tablet, especially for patients from Spain and other Southern European countries, who may be less exposed to Timothy grass than other grass species [20].

2.3. Tablet dissolution and allergen release

Absorption of small molecules through biological membranes, including the transmucosal membrane, is positively related to lipophilicity and negatively related to ionization and molecular size. Transmucosal formulations of the small molecule, fentanyl, achieve rapid dissolution and absorption across the mucosa and are therefore more likely to match the time-course of transitory flares of breakthrough pain than oral formulations in patients with stable, controlled persistent pain [23]. Conversely, the superior immunogenicity of a novel intranasal influenza vaccine, based on a novel polycationic lipid-ceramide carbamoyl-spermine, is thought to be attributed to long retention of the vaccine at the administration site, eliciting strong local and systemic immune responses [24]. Pharmacokinetic and biodistribution studies following intranasal administration of the vaccine in mice showed that both the lipids and antigens were retained in the nose and lung for at least 24 h, and this retention appeared to be correlated with enhanced immunogenicity [24]. Therefore, rapid dissolution is not necessarily a key factor for induction of a potent immune response to medications administered via the nasal route. This might also apply to oral administered agents.

SLIT relies on the efficient delivery of soluble allergens to the sublingual mucosa, in a form that is appropriate for capture by the immunocompetent cells that reside there. After entering the oral mucosa, allergens are taken up by resident APCs, which subsequently migrate to the draining cervical lymph nodes; here, they elicit allergen specific Th1/Treg responses, thus suppressing the established allergic Th2 response, and leading to the gradual development of immune tolerance [3,25]. As mentioned previously, allergen bioavailability within the sublingual mucosa is the foundation of both the safety and efficacy of AIT products. The European Medicines Agency defines bioavailability as 'the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action' [26]. Therefore, any rigorous assessment of allergen bioavailability from SLIT tablets should consider several parameters, including allergen content and release kinetics after tablet dissolution, the effect of salivary components on allergen degradation, and the extent of allergen uptake by epithelial cells and APCs in the sublingual mucosa [27].

Depending on the method used, *in vitro* dissolution assays of SLIT tablets can produce dramatically different results. Using a method advocated by the Japanese Pharmacopoeia, the 1-grass pollen SLIT tablet was shown to dissolve completely within 1 s versus incomplete dissolution of the 5-grass pollen SLIT tablet after 30 s, and this difference between dissolution times was significant ($p < 0.001$) [28]. Grass group 5 major allergen content was measured by enzyme-linked immunosorbent assay using a proprietary monoclonal antibody against Phl p 5, after dissolution of either grass pollen SLIT tablet in a phosphate buffer approximating human saliva in pH, ionic strength and total protein content (but not containing salivary enzymes or any other components of saliva). Full recovery of the 1-grass pollen SLIT tablet allergen content was achieved after ~15 s, whereas only ~16% of Phl p 5 content was released from the 5-grass pollen SLIT tablet after 120 s ($p < 0.0001$ for comparison of released Phl p 5) [28]. It should be remembered that quantification of Phl p 5 can be affected by the specificity and sensitivity of the antibodies used for detection, and/or whether they are mono- or polyclonal antibodies; moreover, quantification of Phl p 1/grass group 1 major allergens has not been assessed for either SLIT tablet formulation.

Comparable assays to those used for grass pollen have been used to evaluate SLIT tablets containing house dust mite (HDM) allergens (12 SQ HDM or 300 IR HDM). Disintegration times were ~1 s versus 45 s for the 12 SQ HDM and 300 IR HDM SLIT tablets, respectively ($p < 0.0001$). Complete release of Der f 1, Der p 1 and Der 2 from the 12 SQ-HDM SLIT tablet was obtained within 30 s, whereas for the 300 IR HDM SLIT tablet, soluble HDM major allergen content continued to increase over time, but did not reach complete release, even after 10 minutes [29,30]. In contrast, using an *in vitro* dissolution assay endorsed by the European Pharmacopoeia (Ph. Eur. 2.9.3 dissolution test for solid dosage forms, apparatus 4 flow-through cell [31]), ≥85% of total allergenic activity is shown to be released from 300 IR and 100 IR HDM and 5 grass SLIT tablets within 2 minutes (Figure 1) [27].

Rapid complete release of grass pollen or HDM major allergens (e.g. within seconds) may contribute to a faster allergen saturation of APCs and prevent optimal allergen targeting

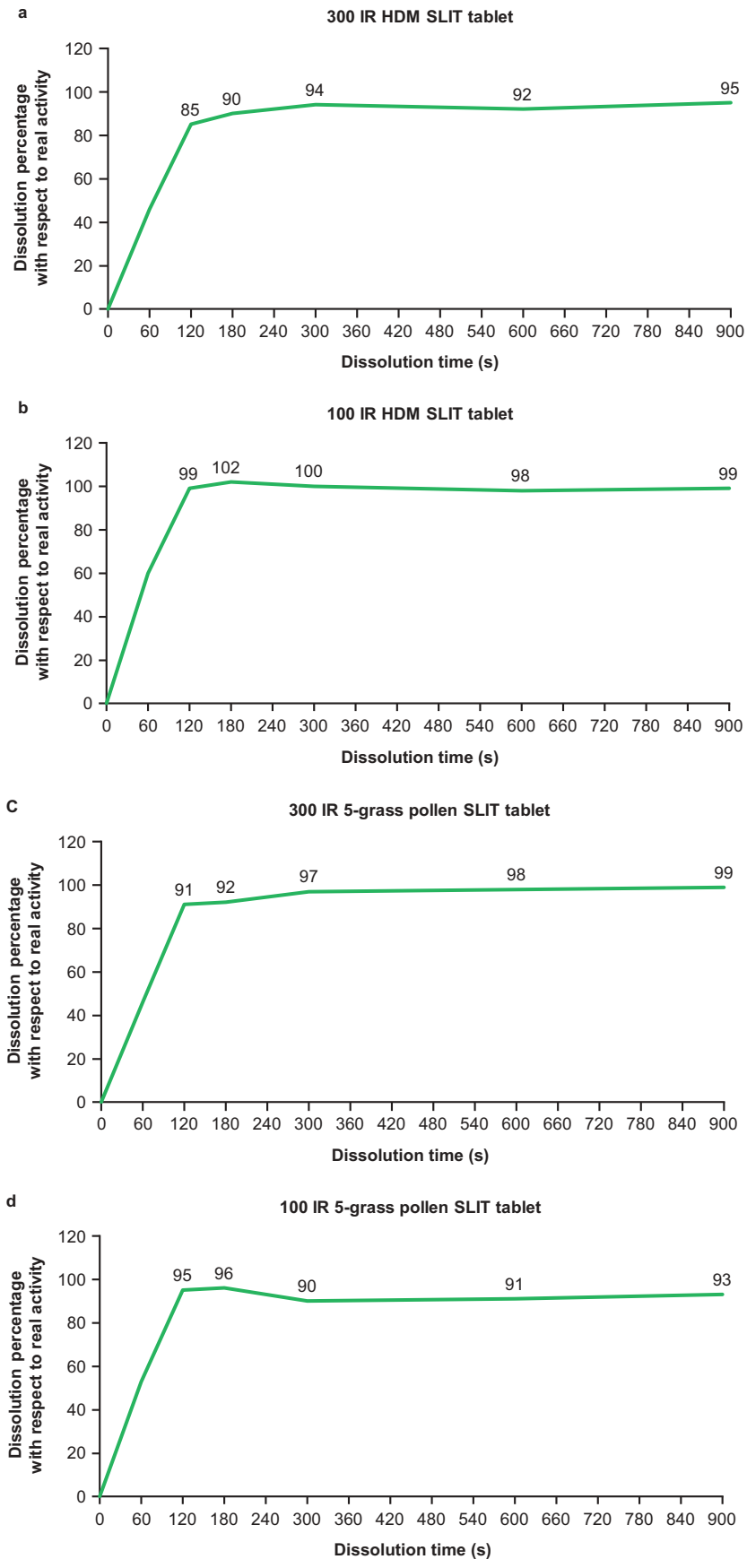

Figure 1. Kinetics of allergen activity release and dissolution of 300 IR and 100 IR HDM and 5 grass SLIT tablets *in vitro*.

An *in vitro* dissolution assay endorsed by the European Pharmacopoeia (Ph. Eur. 2.9.3 dissolution test for solid dosage forms, apparatus 4 flow-through cell [31]) was used to investigate the dissolution time of 6 replicate samples of the 300 IR or 100 IR HDM and 5 grass pollen SLIT tablets, and the amount of allergenic activity released at each time point was measured using an ELISA-based assayELISA, enzyme-linked immunosorbent assay; HDM, house dust mite; IR, index of reactivity; SLIT, sublingual immunotherapy.

[27]. The kinetics of allergen uptake by APCs in the sublingual mucosa are dose-dependent up to the point of saturation, and therefore are an important contributor to allergen bioavailability [32]. The 5-grass pollen and 300 IR HDM SLIT tablets have been designed to sustain allergen release over 2–3 minutes, with the aim of promoting allergen uptake by APCs without triggering rapid activation of local pro-inflammatory mast cells [33]. It is unclear whether the rapid release of allergen from the 1-grass pollen SLIT tablet offers an advantage over the more gradual release from the 5-grass pollen SLIT tablet, because the production of high local concentrations of proteins may overwhelm immune cell uptake mechanisms. Unlike small molecule therapeutics (e.g. fentanyl, nitroglycerin), allergen uptake into sublingual tissue is comparatively slow and can be saturated, because it involves the transport of bulky proteins across cellular barriers.

Proteins that are swallowed are rapidly degraded by digestive enzymes and absorbed [34,35], and this process may alter the T and B cell epitope repertoire from that seen with oromucosal or nasal uptake. In a study that investigated the *in vivo* distribution kinetics of radiolabelled *Parietaria judaica* allergen after sublingual, oral and intranasal administration in healthy, non-allergic humans, no degradation of the labelled allergen occurred in the sublingual space for up to 20 minutes after sublingual administration, and there was no absorption of the radiolabelled allergen through the sublingual mucosa into the bloodstream for up to 30 minutes, but swallowed labelled allergen underwent rapid hydrolysis and absorption in the gastrointestinal tract [34]. These findings are consistent with the large size and relatively high molecular weight of the protein, which also possesses a hydrophilic surface [34]. Studies in allergic subjects using the radiolabeled allergens, Par j 1 and Der p2, administered sublingually have demonstrated that sensitization to the allergen affects at some extent its biodistribution and kinetics, especially at mucosal level [35,36].

The sublingual holding (swallowing) time affects the time of contact between solubilized allergens and sublingual tissue, because swallowing will remove the majority of unabsorbed allergens from the sublingual space, preventing further uptake. It takes 5 minutes for maximal uptake of allergens by the sublingual tissue, with little uptake within the first minute [32] (Figure 2). In light of this, the sublingual holding time should be at least several minutes. Early swallowing (e.g. at 1 minute) may reduce the uptake of allergens.

Human saliva contains >3,000 distinct proteins, including enzymes, hormones, antibodies and cytokines [37], any of which may promote allergen degradation, decrease allergen potency, and preclude optimal targeting onto epithelial cells and APCs. Such effects are difficult, if not impossible, to model and assess using *in vitro* dissolution assays. For these reasons, although differences in dissolution times can be demonstrated using *in vitro* dissolution assays, these cannot serve as a surrogate of bioavailability, because they lack any direct correlation with an *in vivo* sustained effect [27].

3. Clinical efficacy and safety of the 1- and 5-grass pollen SLIT tablets

The optimal allergen dose for SLIT should achieve the highest therapeutic effect without compromising safety or leading to unacceptable allergen-mediated discomfort during treatment [29]. Both the 1- and 5-grass pollen SLIT tablets have been extensively studied during clinical development, and their favorable efficacy and safety profiles are well established (Table 1). Perennial treatment with the 1-grass pollen SLIT tablet leads to clinical improvement in adults suffering from rhinoconjunctivitis that is sustained over the long term [13,38]. The 5-grass pollen SLIT tablet, administered in a pre-coseasonal schedule, is effective in reducing allergic symptoms and symptomatic medication use, and improving a patient's quality of life [1,39]. Improvement in symptoms upon allergen exposure is detectable after 1 month of treatment with the 5-grass pollen SLIT tablet [40], demonstrating that although it may have a slower *in vitro* dissolution time than the 1-grass pollen SLIT tablet, allergen release is sufficient to achieve clinical efficacy within only 1 month. The clinical onset of action has not been determined for the 1-grass pollen SLIT tablet.

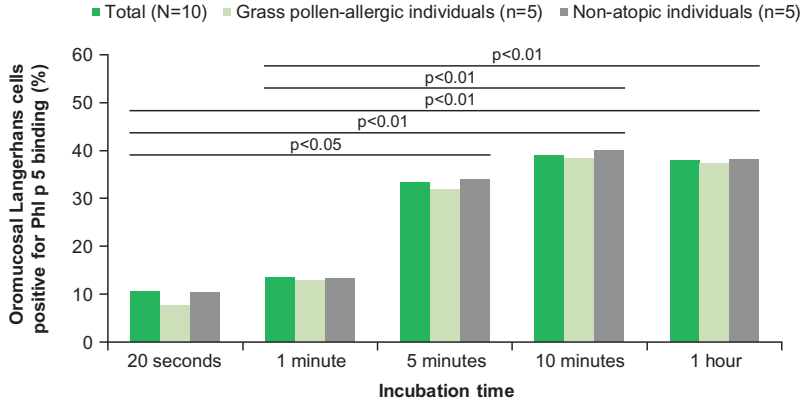

Figure 2. Kinetics of grass pollen allergen binding by oromucosal Langerhans cells *ex vivo*.

This was an *ex vivo* study not related to SLIT tablet use. Using oromucosal tissue from individuals with grass pollen allergy (n = 5) or non-atopic individuals (n = 5), incubated with fluorescein isothiocyanate-coupled Phl p 5 (1 mg/mL) at 37°C for the specified times, significant binding of Phl p 5 to Langerhans cells was detected from 5°minutes of allergen exposure onwards, and binding capacity was comparable between tissue samples. Figure adapted from Allam *et al*. 2010 [32].SLIT, sublingual immunotherapy.

The safety profiles of both SLIT tablet products in clinical trials are similar, with the majority of adverse events being local, mild-to-moderate reactions that resolve without sequelae in the first 1–2 weeks of treatment. Neither SLIT tablet products caused anaphylactic shock or fatalities in clinical trials, although some grade 1–3 systemic AEs were observed. Notably, while no use of epinephrine was documented in trials of the 5-grass pollen SLIT tablet, 4 cases of epinephrine autoinjector use occurred during clinical trials of the 1-grass pollen SLIT tablet conducted either with adult or pediatric patients [40–42].

4. Conclusion

The optimal dose of the 5-grass pollen tablet (300 IR; 9000 BAU) has demonstrated long-term efficacy when prescribed in a discontinuous, pre- and coseasonal regimen. Importantly, the high potency of the 5-grass pollen tablet does not appear to be associated with any safety signals or poor tolerability. The optimal dose of the 1-grass pollen SLIT tablet has not been confirmed, but the approved dose (75,000 SQ-T; 2800 BAU) has demonstrated long-term efficacy when prescribed continuously during the entire year. The pre-/coseasonal regimen of the 5-grass pollen SLIT tablet appears to be associated with excellent adherence, and lower annual costs versus the 1-grass pollen SLIT tablet.

Differences between the licensed 1- and 5-grass pollen SLIT products are evident. A recent report has implied that *in vitro* dissolution kinetics of SLIT tablets correlate with bioavailability and clinical efficacy [28]; however, it is clear that this is not the case, and *in vitro* dissolution rate cannot be used as a reliable indicator of clinical efficacy. Based on the current knowledge of the oral immune response, a formulation encompassing a very rapid dissolution may not be optimal for sublingual administration of allergens. A direct head-to-head comparison between the two grass pollen SLIT tablet products, to assess any differences in the extent of their efficacy, has yet to be performed.

5. Expert opinion

The biovailability of sublingual AIT in tablets is certainly of central importance, and likely related also to efficacy. It is true that the unique mode of action of SLIT (modulation of the immune response) and the complexity of events following sublingual administration make it difficult to study in detail all of the aspects involved. Nonetheless, immunological studies on allergen uptake by APCs in relation to the dissolution of tablets would allow us to further improve the efficacy of SLIT.

It is clear that the primary endpoint to be evaluated when comparing different SLIT formulations should be clinical efficacy supported by analysis of systemic biomarkers (for example, IgG4 or IgE), but this will require expensive and complex head-to-head trials, and this is not immediately feasible.

As already discussed, it is impossible to draw conclusions on the bioavailability and clinical efficacy of SLIT tablets, based on their *in vitro* dissolution time. However, the different dissolution times between the grass pollen SLIT tablet products could potentially influence administration.

Further investigation of the relationship between allergen retention at the administration site, and the elicited local and systemic immune responses, as well as the optimal frequency and duration of administration to gain an appropriate and sustained immune response is needed. Additionally, a better understanding of the possible differences in, and magnitude and extent of, humoral and mucosal immune responses provided by monovalent or multivalent formulations may pave the way for improving and optimizing the efficacy of future SLIT. One can speculate that tablets designed to sustained allergen release over 2–3 minutes, without triggering rapid activation of local proinflammatory mast cells, are behind good safety profile.

Real-life data collected from registries of patients using SLIT and other treatments for grass pollen allergy are starting to provide more insights about the long-term effects of SLIT, and in the near future, will likely play a greater role in guiding treatment, especially regarding the most appropriate and effective administration regimen. Pre- and coseasonal administration appears to have advantages over perennial administration in terms of a reduced frequency and duration of administration, and the benefits for adherence and cost savings arising from this.

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