

Bioavailability of House Dust Mite Allergens in Sublingual Allergy Tablets Is Highly Dependent on the Formulation

Katsuyo Ohashi-Doi^a Hirokazu Kito^a Weibin Du^a Hiroshi Nakazawa^a
Henrik Ipsen^b Pernille Gudmann^b Kaare Lund^b

^aTorii Pharmaceutical Co. Ltd., Tokyo, Japan; ^bALK A/S, Hørsholm, Denmark

Keywords

Sublingual immunotherapy · Sublingual immunotherapy tablet · Bioavailability · Release kinetics · Dosage · Administration · House dust mite · Allergen extracts · Allergen-specific immunotherapy

Abstract

Background: In sublingual immunotherapy (SLIT), the immune system is addressed by solubilized allergen that interacts with immunocompetent cells of the oral mucosa, the efficiency of which is governed by 2 main factors of SLIT allergen bioavailability: the allergen concentration and the mucosal contact time. Recently, 3 house dust mite (HDM) SLIT tablets were developed that differ with regard to allergen content, nominal strength (maintenance doses: 6 SQ-HDM/10,000 Japanese Allergen Units [JAU], 12 SQ-HDM/20,000 JAU, and 300 IR/57,000 JAU), and formulation (freeze-dried/compressed). Here, the importance of the SLIT tablet formulation for HDM major allergen bioavailability is examined. **Methods:** The HDM major allergen content, tablet disintegration times, and allergen release kinetics were determined. Dissolution kinetics (allergen concentration vs. time) of Der f 1, Der p 1, and Der 2 were measured. Area under the curve (AUC) was used as a surrogate parameter for allergen

bioavailability. **Results:** The release of HDM major allergens from the freeze-dried tablets was complete after 30 s, while only partial release was achieved with the compressed tablets, even after prolonged dissolution. At 1 min, i.e., the recommended sublingual holding time for the freeze-dried tablets, the allergen bioavailability (AUC) of the compressed 300 IR/57,000 JAU tablet was 4.7-fold (Der f 1), 10.8-fold (Der p 1), and 23.6-fold (Der 2) lower than that of the freeze-dried 12 SQ-HDM/20,000 JAU tablet and similar to (Der f 1) and 5.3-fold (Der p 1) and 12.5-fold (Der 2) lower than that of the freeze-dried 6 SQ-HDM/10,000 JAU tablet. **Conclusions:** SLIT tablet allergen bioavailability depends highly on the tablet formulation. Only the fast-dissolving freeze-dried tablets provide maximal delivery of soluble allergens and achieve allergen concentrations that reflect the nominal tablet strengths within the recommended sublingual holding time.

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Introduction

Allergy immunotherapy (AIT) is an established treatment option for allergic respiratory diseases. Unlike pharmacotherapy, AIT addresses the basic immunological mechanisms that are responsible for the development

and persistence of allergic conditions. By actively modulating protective allergen-reactive pathways of the immune system, AIT can provide relief from clinical symptoms of allergic rhinitis (AR) [1–6] and allergic asthma [7, 8], and it has the potential to provide long-term post-treatment symptom relief and alter the natural course of the disease [2, 9, 10]. Two main routes of AIT administration are commonly available, i.e., subcutaneous injections (subcutaneous immunotherapy; SCIT) and sublingual administration (sublingual immunotherapy; SLIT), the latter of which is available as a liquid formulation (SLIT drops) or in the form of tablets (SLIT tablets). For both SLIT drops and SLIT tablets, the allergen is administered under the tongue, where it is held until swallowed or spit out.

A number of registered and clinically efficacious SLIT tablet products are currently available, but between individual products of a given allergen species, or a mixture of species, there may be considerable differences in the overall allergen content and the relative ratios of major allergens of the clinically effective doses [11]. This strongly indicates that factors other than just the allergen dose of a given HDM SLIT tablet influence its clinical effectiveness. It furthermore emphasizes that the clinical effects obtained with one HDM SLIT tablet are exclusive to the product of the trial, and that a class effect cannot be claimed for the existing HDM SLIT tablet products.

An appropriate metric for SLIT tablet potency would be allergen bioavailability as a measure of the rate and extent with which the allergens contained in the tablets reach the target organ. Since there is no systemic absorption through the oral mucosa during SLIT [12–14] and gastrointestinal delivery of allergens to the immune system is unlikely to be efficacious due to breakdown or the dose [15, 16], the target organ in SLIT is the local sublingual immune system. This is in line with a mechanism whereby allergen is captured by mucosal antigen-presenting cells that subsequently migrate to the lymph nodes to induce an antiallergic immune response [13, 17]. Two parameters that determine the extent of interactions between allergen and immunocompetent cells of the sublingual mucosa are the allergen concentration and the mucosal contact time [18, 19], and in combination they comprise a relevant measure of SLIT allergen bioavailability.

A particular challenge of SLIT tablets is that the allergen content of each tablet needs to be dissolved in saliva before it becomes available to the oral mucosal immune system. As the mucosal exposure of sublingually administered allergens is short and transient due to inevitable

swallowing or spitting, tablet disintegration and allergen dissolution must be completed within a short period of time. The available time window for tablet disintegration and dissolution is defined by the SLIT tablet manufacturers' recommendation for the sublingual holding time, which is from 1 to a few minutes [14]. Consequently, the ability of a SLIT tablet to deliver the maximal allergen concentration at the fastest rate, and subsequently maintain the highest allergen concentration for the duration of the recommended sublingual holding time, is essential for providing the highest level of SLIT tablet allergen bioavailability.

Here, the disintegration properties and house dust mite (HDM) major allergen release profiles of 3 commercially available HDM SLIT tablets from 2 different manufacturers are examined. Two of the tablets are based on a fast-dissolving freeze-dried formulation [20, 21] and they are available as Acarizax (ACZX) in Europe (ALK A/S, Hørsholm, Denmark) and Miticure (MTC) in Japan (Torii Pharmaceuticals Inc., Tokyo, Japan), respectively. ACZX and MTC are identical with regard to formulation and drug substance and differ only with regard to the amount of HDM allergen extract. The drug substance contained in ACZX and MTC consists of an equal ratio of extracts of the 2 HDM species *Dermaphagoides pteronyssinus* and *D. farinae*, with a balanced content of the group 1 and 2 HDM major allergens [22]. The third tablet is based on a compressed tablet formulation and it is available as Actair (ACT) in Japan (Shionogi & Co. Ltd., Osaka, Japan) and Australia (Stallergenes Greer, France). The drug substance of ACT is also a mixture of *D. pteronyssinus* and *D. farinae* extracts, but it is dominated by Der f 1 [1]. The nominal strengths of the 3 tablets were determined in SQ-HDM (ACZX and MTC) and IR units (ACT), respectively. These are proprietary units based on the manufacturers' in-house references and assays and are therefore not comparable. Recently, however, independently of the 2 manufacturers, the Japanese Society of Allergology (JSA) assigned nominal strengths in Japanese allergen units (JAU) to the HDM SLIT tablets, with the Japanese HDM standard as the common reference [23]. Consequently, the nominal strengths of the tablets will be referred to as follows: ACZX (12 SQ-HDM/20,000 JAU), MTC (6 SQ-HDM/10,000 JAU), ACT (300 IR/57,000 JAU), and ACT (100 IR/19,000 JAU). The purpose of this study was to characterize the dissolution properties of the 2 tablet formulations and to compare tablet nominal strengths with allergen bioavailability.

Table 1. Disintegration times for the freeze-dried and compressed tablets

Sample	Time, s	<i>p</i>
MTC (6 SQ-HDM/10,000 JAU)	1	ns
ACZX (12 SQ-HDM/20,000 JAU)	1	
ACT (100 IR/19,000 JAU)	27±1.5	<0.0001
ACT (300 IR/57,000 JAU)	45±2.2	

Values are presented as means ± SD. *p* values were <0.0001 for all comparisons between MTC and ACT and ACZX and ACT. JAU, Japanese allergen units; MTC, Miticure; ACZX, Acarizax; ACT, Actair.

Materials and Methods

Test Samples

ACZX (batch No. 1464423, expiration date: March 2017; ALK, Hørsholm, Denmark) and MTC (batch No. 1524576, expiration date: January 2018; Torii Pharmaceutical Co. Ltd.) were obtained from the manufacturer (ALK A/S). ACT (Shionogi & Co. Ltd.) at 100 IR (batch No. 1001-2, expiration date: May 2018) and 300 IR (batch No. 3002-1, expiration date: May 2018) was obtained through a Japanese pharmacy. Experiments were completed prior to the tablet expiration dates.

Assay Buffer

A common assay buffer (100 mM phosphate, pH 6.8, 0.125% casein) that is similar in composition to human saliva with regard to pH, ionic strength, and total protein content [24] was used for disintegration and dissolution tests.

Disintegration Test

Tablet disintegration in the assay buffer was measured following the Japanese pharmacopoeia [25]. Briefly, tablets were deposited into a submersible mesh basket (1.8- to 2.2-mm mesh size) and agitated vertically in assay buffer at 37°C until they disintegrated. Disintegration was considered complete when all of the tablet residue had passed through the mesh.

Major Allergen Content

Tablets were dissolved in assay buffer (10 mL) in a conical tube (15 mL). ACT was dissolved by vortexing until it completely disintegrated. Undissolved ACT tablet residue was separated from the supernatant by centrifugation (10 min, 11,000 rpm, room temperature). The HDM major content of dissolved tablets (ACZX and MTC) or supernatant (ACT) was measured by ELISA (Der p 1, Der f 1, and Der 2) essentially as previously described [22, 26].

Tablet Dissolution/Allergen Release Kinetics

A dissolution test was performed according to the paddle method of the Japanese pharmacopoeia [25] using a mini vessel (200 mL) Distek Model 2500 instrument (Distek Inc., USA). Tablets were deposited into the assay buffer (100 mL, 37°C, 50-rpm paddle speed). Test solutions were collected at 15, 30, 45, 60, 90,

120, 180, 300, and 600 s, and the amounts of solubilized HDM major allergens were determined by ELISA, essentially as previously described [22, 26]. The area under the curve (AUC) was calculated using GraphPad Prism 7.02.

Statistical Analyses

All statistical comparisons were performed using GraphPad Prism version 7.02 or SAS version 9.3.

The means of triplicate (independent) AUC determinations were compared via 1-way ANOVA using the Tukey HSD method to correct for multiple comparisons. Normality was assumed for all data and homogeneity of variance was evaluated by a Brown-Forsythe test. Each reported time point was evaluated separately. All 2-sided probabilities less than 0.05 were considered statistically significant.

Results

Disintegration Test

Before the allergens contained in a SLIT tablet can become solubilized and made available to the sublingual mucosal immune system, the tablet must disintegrate to allow access to saliva. In this experiment, tablets were deposited in assay buffer with continuous agitation, and the time to disintegration was recorded. Table 1 shows the average of 6 independent experiments. The 2 freeze-dried tablets (ACZX and MTC) disintegrated within 1 s, with no measurable difference between the 2 strengths. The disintegration times of the compressed tablets (ACT) were considerably longer, and increased with increasing allergen content, as the disintegration times were 27 s for the 100 IR/19,000 JAU tablet and 45 s for the 300 IR/57,000 JAU tablet (*p* < 0.0001). While the 2 freeze-dried tablets disintegrated completely, a seemingly insoluble residue remained even after prolonged incubation of the compressed tablets (data not shown).

Major Allergen Content

It was not possible to obtain the drug substance from all of the tablets, so the total content of HDM major allergen was assessed from solubilized tablets. Each of the 4 tablets was deposited in 10 mL assay buffer and after 10 min of intermittent vigorous shaking the major allergen content was measured by Der f 1-, Der p 1-, and Der 2-specific ELISA [22, 26]. For the compressed tablets (ACT), the insoluble residue was removed by centrifugation before the major allergen content of the supernatant was measured. The results are shown in Table 2. Since the same assays and standards were used, the major allergen contents measured here for the 4 tablets can be compared. The amounts of major allergen that were released from

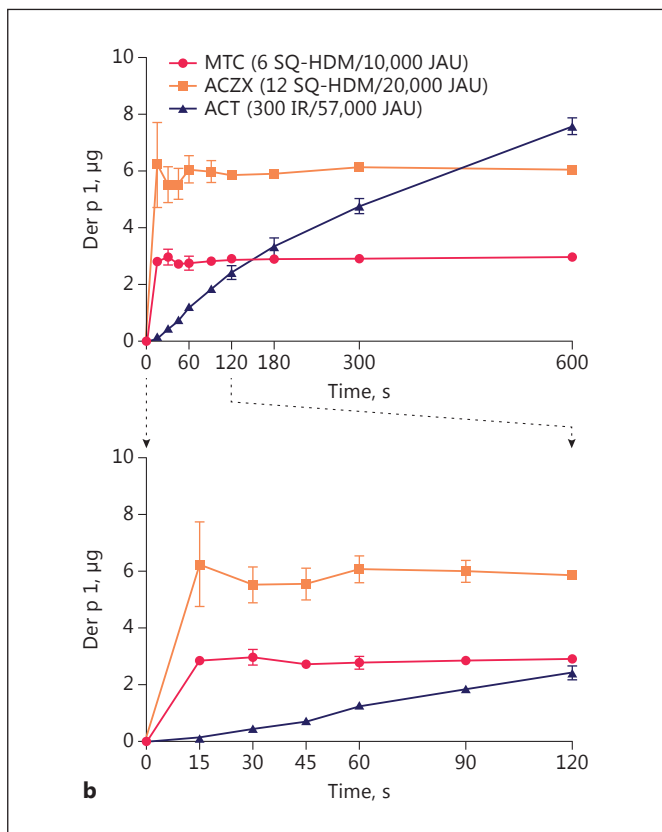
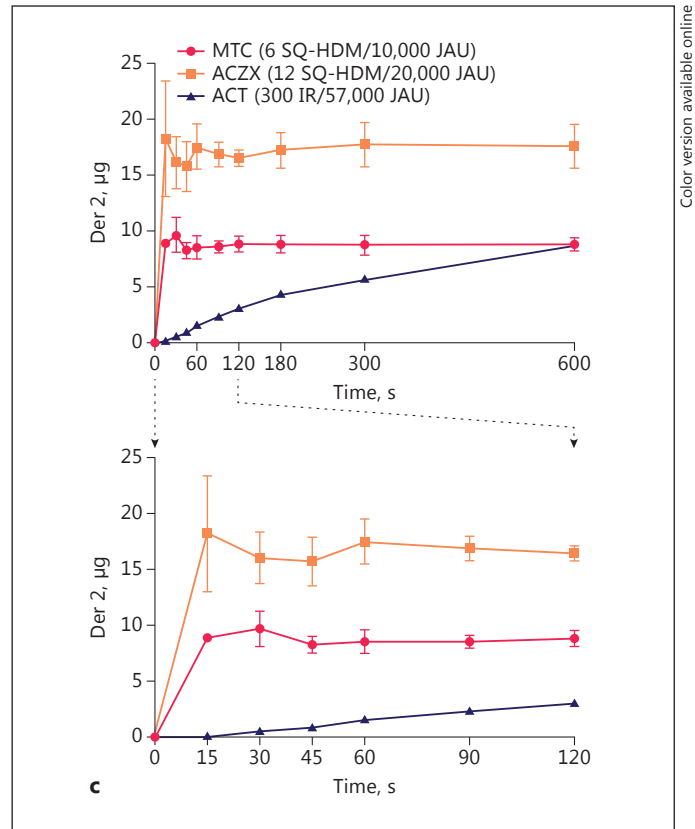
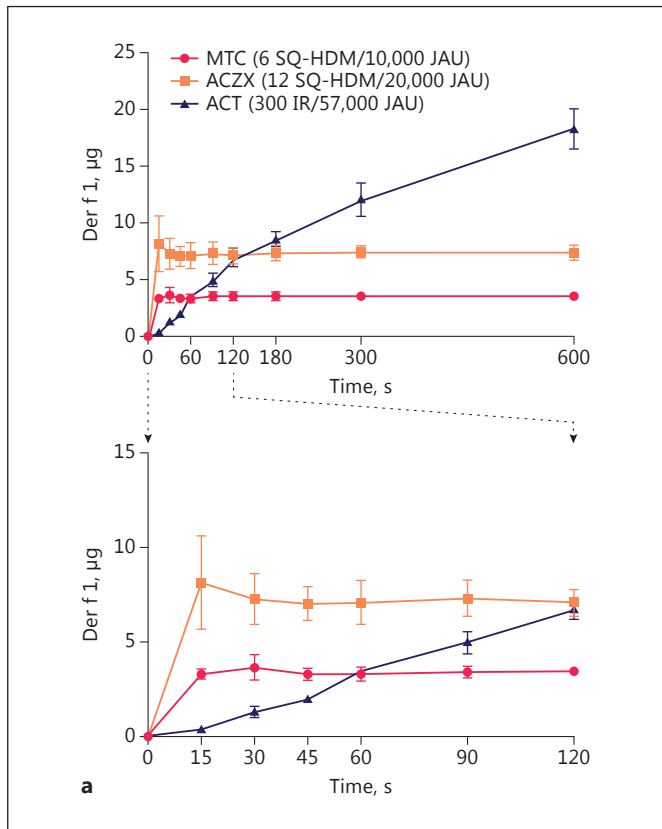


Fig. 1. Cumulated HDM major allergen release from the start of dissolution. Der f 1 (**a**), Der p 1 (**b**), and Der 2 (**c**). Top: cumulated allergen release from $t = 0$ to $t = 10$ min. Bottom: cumulated allergen release from $t = 0$ to $t = 120$ s. Allergen amounts are expressed as means $\pm$ SD. JAU, Japanese allergen units; MTC, Miticure; ACZX, Acarizax; ACT, Actair.

Table 2. HDM SLIT tablet major allergen contents

Sample	Der f 1, µg	Der p 1, µg	Der 2, µg
MTC 6 SQ-HDM/10,000 JAU	3.59±0.23	3.64±0.15	9.30±0.13
ACZX 12 SQ-HDM/20,000 JAU	7.51±0.27	7.25±0.12	18.91±0.09
ACT 100 IR/19,000 JAU	12.80±0.10	5.15±0.53	5.74±0.13
ACT 300 IR/57,000 JAU	38.56±0.73	16.16±0.13	16.79±0.41

Values are presented as means ± SD. JAU, Japanese allergen units; MTC, Miticure; ACZX, Acarizax; ACT, Actair; HDM, house dust mite; SLIT, sublingual immunotherapy.

Table 3. AUC for the different HDM major allergens at the indicated time points relative to MTC

Time, min	Der f 1					Der p 1					Der 2				
	ACZX	<i>p</i>	ACT	<i>p</i>	MTC	ACZX	<i>p</i>	ACT	<i>p</i>	MTC	ACZX	<i>p</i>	ACT	<i>p</i>	MTC
1	2.18	0.0005	0.46	0.0981	1.00	2.05	<0.0001	0.19	0.0023	1.00	1.89	<0.0001	0.08	0.0029	1.00
2	2.14	0.0016	1.00	0.9999	1.00	2.08	<0.0001	0.43	0.0022	1.00	1.92	<0.0001	0.18	0.0010	1.00
5	2.11	0.8770	2.04	0.0012	1.00	2.06	<0.0001	0.95	0.6447	1.00	1.94	<0.0001	0.39	0.0022	1.00
10	2.11	0.0021	3.19	<0.0001	1.00	2.06	<0.0001	1.54	<0.0001	1.00	1.98	<0.0001	0.60	0.0218	1.00

p values indicate the level of statistical significance between the absolute AUC at the indicated time points. All ACZX and MTC AUC were statistically significantly different at all time points (data not shown). HDM, house dust mite; AUC, area under the curve; MTC, Miticure; ACZX, Acarizax; ACT, Actair.

tablets of the same formulation were proportional to their respective nominal strengths. However, the absolute HDM major allergen contents differed considerably between ACZX/MTC and ACT (Table 2). In general, the HDM major allergen content was higher in ACT compared to ACZX and MTC. In the maintenance dose tablets the Der f 1 content was approximately 5-fold higher in ACT (300 IR/57,000 JAU) than in ACZX (12 SQ-HDM/20,000 JAU), and it was almost 11-fold higher than in MTC (6 SQ-HDM/10,000 JAU). The Der p 1 content of ACT (300 IR/57,000 JAU) was approximately 2-fold higher than that of ACZX (12 SQ-HDM/10,000 JAU) and 4-fold higher than that for MTC (6 SQ-HDM/10,000 JAU). The Der 2 content was approximately 2-fold higher in ACT (300 IR/57,000 JAU) than in MTC (6 SQ-HDM/10,000 JAU), but slightly lower (*p* = 0.0009) than that for ACZX (12 SQ-HDM/20,000 JAU) (Table 2). All comparisons of HDM major allergen content (MTC and ACZX vs. ACT [both strengths] and MTC vs. ACZX) were statistically significantly different (*p* ≤ 0.0026; data not shown).

Tablet Dissolution and Allergen Release Kinetics

Figure 1 shows the cumulated release of HDM major allergens at different time points during tablet dissolution. Only data from MTC (6 SQ-HDM/10,000 JAU), ACZX (12 SQ-HDM/20,000 JAU), and ACT (300 IR/57,000 JAU) are shown, since the amounts of released HDM major allergens from ACT (100 IR/19,000 JAU) at early time points were too low to be measured.

All experiments were done in parallel under identical experimental conditions. Tablets were deposited into the assay buffer with continuous agitation. Samples were collected after 15, 30, 45, 60, and 90 s and 2, 3, 5, and 10 min, and the concentrations of HDM major allergen were measured by ELISA. Complete release of Der f 1, Der p 1, and Der 2 from both ACZX (12 SQ-HDM/20,000 JAU) and MTC (6 SQ-HDM/10,000 JAU) was obtained within 30 s and the major allergen concentrations remained stable for the remainder of the experiment (10 min). In contrast, the release of HDM major allergens from ACT (300 IR/57,000 JAU) was much slower and the concentration of soluble HDM major allergens continued to increase for the duration of the experiment, without reaching a plateau.

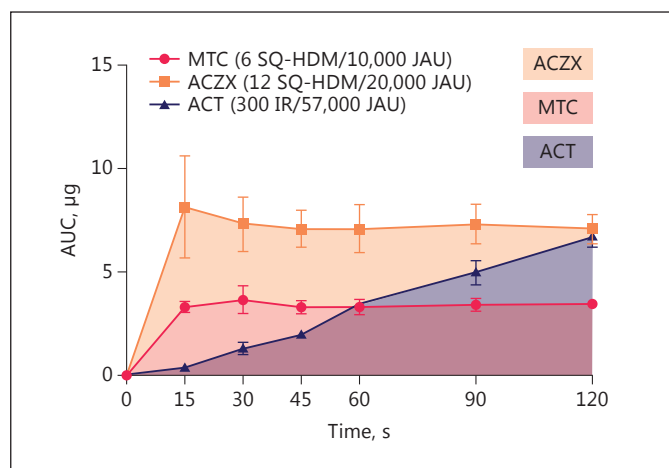


Fig. 2. Area under the curve (AUC) of solubilized Der f 1 for Acarizax (ACZX), Miticure (MTC), and Actair (ACT), respectively, at 0–120 s.

As a measure of the cumulated major allergen availability in solution at different time points, the AUC, as illustrated in Figure 2, was calculated at 1, 2, 5, and 10 min, and AUC were expressed relative to MTC (Table 3).

As could be predicted for tablets that differ 2-fold with regard to allergen amount but share the same allergen composition and tablet disintegration and dissolution profiles, the AUC of ACZX for all HDM major allergens were at all time points approximately 2-fold larger compared to that of MTC (Table 3). Although the difference between the Der f 1 AUC of ACT (300 IR/57,000 JAU) and MTC (6 SQ-HDM/10,000 JAU) did not quite reach statistical significance (ratio = 0.46, $p = 0.0981$; Table 3), the overall HDM major allergen bioavailability achieved after 1 min of dissolution was higher for MTC (6 SQ-HDM/10,000 JAU) than for ACT (300 IR/57,000 JAU) as the AUC of ACT (300 IR/57,000 JAU) was 5.3-fold smaller (19%, $p = 0.0023$) for Der p 1 and 12.5-fold smaller (8%, $p = 0.0029$) for Der 2 compared to MTC (6 SQ-HDM/10,000 JAU) (Table 3). Compared to ACZX (12 SQ-HDM/20,000 JAU), the AUC obtained with ACT (300 IR/57,000 JAU) after 1 min of dissolution was 4.7-fold smaller ($0.46/2.18 = 21\%$, $p = 0.0005$) for Der f 1, 10.8-fold smaller ($0.19/2.05 = 9\%$, $p < 0.0001$) for Der p 1, and 23.6-fold smaller ($0.08/1.89 = 4\%$, $p < 0.0001$) for Der 2. After 2 min of dissolution, the AUC of ACT (300 IR/57,000 JAU) relative to MTC (6 SQ-HDM/10,000 JAU) was equal (100%, $p = 0.9999$) (Der f 1), 2.3-fold smaller (43%, $p = 0.0022$) (Der p 1), and 5.6-fold smaller (18%, $p = 0.0010$) (Der 2), respectively. Compared to

ACZX (12 SQ-HDM/20,000 JAU), the HDM major allergen AUC of ACT (300 IR/57,000 JAU) was 2.1-fold smaller ($1/2.14 = 46\%$, $p = 0.0016$) (Der f 1), 4.8-fold smaller ($0.43/2.08 = 21\%$, $p < 0.0001$) (Der p 1), and 10.7-fold smaller ($0.18/1.92 = 9\%$, $p < 0.0001$) (Der 2), respectively, after 2 min. After a 5-min dissolution time, the combined HDM major allergen AUC was still larger for ACZX (12 SQ-HDM/20,000 JAU) compared to ACT (300 IR/57,000 JAU), while the combined AUC of MTC (6 SQ-HDM/10,000 JAU) and ACT (300 IR/57,000 JAU) were similar.

Discussion

In successful SLIT, the optimal allergen dose is that which achieves the highest therapeutic effect without compromising safety and without exceeding acceptable levels of allergen-mediated discomfort that some allergic individuals may experience during the treatment. The optimal allergen dose must always be determined in clinical trials, as each AIT product has unique properties such as allergen content (dose and composition), formulation, and treatment regimen [27]. Clinically efficacious and non-efficacious doses were identified for 2 different HDM SLIT tablets in phase 2 HDM SLIT tablet dose-finding trials [8, 28, 29], and the clinical efficacy of the selected treatment doses on HDM-induced AR were later confirmed for both products in large phase 3 trials [3, 5, 6]. The freeze-dried SQ HDM SLIT tablet also reduced the risk of moderate or severe asthma exacerbations compared to placebo in a phase 3 trial in HDM allergic asthma [7]. The 12 SQ-HDM tablet (ACZX) is registered in Europe with an indication of house dust mite AR and asthma, while MTC and ACT are indicated for treatment of HDM AR.

In Japan, the nominal strengths of ACT and MTC/ACZX were assigned in JAU according to a common HDM reference standard selected by the JSA. The in vivo allergen potency of the Japanese reference HDM allergen extract was established via intradermal testing. Because of the observed correlation between in vitro IgE reactivity and the concentration of HDM major allergens, tablet strengths were subsequently assigned based on the group 1 HDM allergen contents of tablet drug substances using a JSA-developed Der 1 ELISA as a surrogate in vitro assay [23]. The drug substance HDM allergen content can be used as a representative measure of product strength for SLIT tablets of identical formulations and identical extract compositions, but it cannot be used to compare the

actual, effective strengths of SLIT tablets that differ with regard to allergen composition, disintegration, and dissolution properties. This is supported by the finding that, while MTC (6 SQ-HDM/10,000 JAU) and ACZX (12 SQ-HDM/20,000 JAU) have shown consistent clinical effects [3, 5, 7], 100 IR/19,000 JAU ACT failed to meet statistically significant clinical endpoints in a phase 2 dose-finding trial [28], despite the higher or near-equal nominal strength (in JAU) and Der 1 content compared to MTC and ACZX, respectively. This strongly indicates that additional properties other than allergen content influence the clinical efficacy of SLIT tablets.

In SLIT, allergens are administered as premade solutions, as in SLIT drops, or they become solubilized in saliva following sublingual administration of SLIT tablets. Consequently, SLIT doses must be considered, and eventually compared, based on the achieved sublingual allergen concentrations rather than based on the products' allergen content. A particular challenge of SLIT tablets is that the formulation must permit dissolution of the entire allergen content in saliva before the achieved allergen concentration reflects the nominal strength. In addition, the time permitted for tablet disintegration and allergen dissolution is limited by the recommended sublingual holding time. The manufacturer's recommendations for the freeze-dried ACZX and MTC are to avoid swallowing for 1 min after administration [30]. For the compressed ACT, the manufacturer recommends holding the tablet under the tongue until completely disintegrated [31, 32]. Both manufacturers furthermore recommend avoiding eating or drinking for 5 min after swallowing [30, 32], which for this reason must be considered the absolute maximal sublingual holding time. Under the conditions employed here, ACT disintegrated after between ca. 26 s (100 IR/19,000 JAU) and ca. 45 s (300 IR/57,000 JAU), while disintegration of both ACZX and MTC occurred almost instantly. In the dissolution experiments, close to 100% of the allergen content of ACZX and MTC was solubilized after 30 s, and the concentration of solubilized allergen remained at a stable plateau for 10 min, for the remainder of the experiment. For ACT (300 IR/57,000 JAU), only a minor fraction of the assessed total allergen content was dissolved after 30 s, and the concentration of solubilized allergen increased slowly without reaching a plateau within 10 min. All of the experiments were done in parallel under identical experimental conditions, which warrants comparability of dissolution properties and allergen solubility. If similar relative release kinetics applies to sublingual dissolution, a smaller fraction of the allergen content of ACT (300

IR/57,000 JAU) compared to MTC (6 SQ-HDM/10,000 JAU) and ACZX (12 SQ-HDM/20,000 JAU) can be expected to have been in contact with the sublingual mucosa in soluble form if swallowed after, for example, 1 min.

Allergen concentration and mucosal contact time are essential and inseparable elements of SLIT, and in combination they comprise a measure of SLIT allergen bioavailability. The importance of the allergen concentration for SLIT tablet efficacy has been noted previously [33], and preclinical and human ex vivo biopsy data indicate that the allergen uptake is dose dependent and correlates with the oral mucosal contact time, up to a point of saturation [15, 16, 19], which may be reached at between 1 and 5 min of mucosal contact time [19]. The allergen release data presented here address both the allergen concentration and the time in solution, and we used the AUC from $t = 0$ to a given time point as a surrogate parameter for allergen bioavailability.

Although a direct comparison is hampered by the different major allergen compositions of the 2 products, these data indicate that, despite the higher allergen content, ATC (300 IR/57,000 JAU) did not reach levels of HDM major allergen bioavailability comparable to those of MTC (6 SQ-HDM/10,000 JAU) or ACZX (12 SQ-HDM/20,000 JAU) until after several minutes of dissolution. Whether an extended mucosal contact time can compensate fully for the slow allergen release and the lower allergen concentration achieved with the compressed tablet formulation remains to be established. Factors that oppose a beneficial effect of extended sublingual holding times in SLIT are saturation of the allergen uptake [19] and dilution of the allergen over time by continuous saliva production [34] following, and possibly induced by, tablet administration. From a patient convenience point of view, shorter sublingual holding times can be expected to be preferable, and for treatment regimens that include daily administrations shorter sublingual holding times are very likely to increase the robustness of the treatment throughout the treatment period compared to regimens that on a daily basis rely on longer sublingual holding times.

We have demonstrated that the freeze-dried tablet formulation (ACZX and MTC) is the more efficient formulation for delivering soluble HDM allergens for SLIT compared to compressed tablets (ACT). A more efficient SLIT tablet formulation has the inherent advantage that the allergen load can be minimized without compromising the clinical effect. Even though only a fraction of the compressed tablet's allergen content may be released dur-

ing the recommended sublingual holding time, the entire allergen content of the tablet will eventually be swallowed, and the higher amounts of ingested allergen may potentially increase the risk of gastrointestinal side effects compared to tablets with lower allergen contents.

In conclusion, because of the slower and incomplete release of HDM major allergens provided by the compressed tablet formulation (ACT), a higher allergen content and possibly longer sublingual holding times are likely to be required to provide allergen bioavailability at the same levels that can be obtained with the freeze-dried formulation (ACZX and MTC). This, in turn, has the important implication for patients and prescribing physicians that the nominal strengths of SLIT tablets are not comparable unless the tablets are identical in formulation and allergen composition. SLIT tablets that differ with regard to allergen composition and/or formulation are not interchangeable, and clinical efficacy must be established for each individual product.

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Henrik Ipsen, Pernille Gudmann, and Kaare Lund are employees and shareholders of ALK A/S.

Author Contributions

Katsuyo Ohashi-Doi, Hirokazu Kito, Weibin Du, Hiroshi Nakazawa, Henrik Ipsen, Pernille Gudmann, and Kaare Lund substantially contributed to the conception and design of this study, analysis and interpretation of the data, drafting and critical revision of this article, and approval of the submitted version of this paper. Hirokazu Kito, Weibin Du, Hiroshi Nakazawa, and Pernille Gudmann furthermore contributed with acquisition of data.

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