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Similar biological activity in skin prick test for Oralair® (8.200BAU) and Grazax®(6.200BAU) reinforces effective SLIT dosing level

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Author's contributions

Design of the study (DLL, JS, NR, RE, JJM, RM), Blinding (JS, RE, AM) patient recruitment and data recollection (DLL, NR, JJM, JM), data analysis (DLL, JS, RE, AM, RM), manuscript writing (DLL), manuscript content review (JS, NR, RE, JJM, JM, AM, RM).

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Conflict of interest:

Author and co-authors declare they have no conflict of interest with relation to the content of this manuscript.

Abstract (150 words –max 150)

In Europe, allergen extracts are standardized based on skin-prick wheal-size in 20-30 allergic subjects. To understand the biologic activity of clinically effective SLIT, we used this method to determine the biologic activity of solution and tablet Timothy grass pollen (TIM) extracts, compared to an FDA approved extract (Reference) of 10.000BAU/mL.

Blinded, quadruplicate SPTs with concentrate and three serial half-log dilutions, allowed construction of a semi-logarithmic regression line per extract. BAU values were obtained from comparison with Reference.

Extracts and dilutions showed a neat linear dose-response (all: $R^2 > 0.98$) in 33 rhinitis patients. Relative potencies: Staloral® 12.000BAU/mL, Soluprick® 10.300BAU/mL, Oralair® 8.200BAU, Grazax® 6.200BAU. Even though all extract concentrates differed in wheal-size ($p = 0.01-0.001$), Grazax® producing a 25% smaller wheal-size than Oralair®, the biological activity of these clinically effective TIM tablets led in the same range (6.200-8.200BAU; $0.92-1.23\text{cm}^2$). SLIT dose-finding studies for other pollens might start with allergen extracts producing 1.1cm^2 wheal surface.

Keywords (max 5): allergen extract, allergen immunotherapy, sublingual immunotherapy, timothy grass pollen, allergic rhinitis

Introduction

Different investigators have consistently found major differences in the *in vitro* potency of liquid sublingual allergen immunotherapy (SL-AIT) extracts available on the European market: manufacturer A dosing between 12-45 times more diluted than manufacturer B over the whole range of allergens tested.^{1 2 3} To overcome the variability of SL-AIT liquid dosing and to avoid the need for refrigeration, SLIT tablets were developed: the fast-dissolving timothy grass pollen (TIM) tablet, Grazax/Grastek®, and the 5-grass pollen tablet, Oralair®. An extensive drug development program, in Europe and US, confirmed their value in the treatment of allergic rhinitis, allergic conjunctivitis and in mild allergic asthma. Also, data suggest both tablets have long-term efficacy, which lasts at least for two years after a 3-year continuous (Grazax®)⁴ or pre-co-seasonal (Oralair®)⁵ course. For both tablets the package insert in the US states their dose equivalence in bioequivalent allergy units (BAU), based on *in vitro* testing: 2.800BAU for Grazax® and 9.000BAU for Oralair®: a seemingly 3-fold difference. However, allergen extract standardization for subcutaneous immunotherapy (SC-AIT) extracts is not based on *in vitro* testing, but on skin testing.

Typically, European manufacturers standardize their SC-AIT reference extracts with quadruplicate, titrated, skin prick tests in 15-30 (highly) allergic subjects.⁶

Thus, to further enhance our understanding of SL-AIT it would be of interest to additionally explore the exact dosing of these successful TIM tablets, evaluating their biologic activity in skin prick testing (SPT). Simultaneous testing of a US approved TIM extract, further allowed us to express the TIM tablets' biological activity in Biological Allergy Units (BAU).

Methods

Trial design

In a prospective, multicenter, triple blinded, randomized study we explored the biological activity of TIM solutions and tablets of two main European allergen manufacturers in titrated SPT, with concentrates and three serial dilutions.

Patient selection

Subjects (18-55 years) with rhinitis (and mild asthma) exacerbating during possible grass-pollen exposure were preselected as candidates from the outpatient clinics of three community hospitals. Skin test sensitivity to TIM was documented by a positive SPT (major wheal diameter ≥ 3 mm above the diluent wheal diameter) during a routine SPT with a basic panel. Patients could have sensitivity to other allergens; in such cases care was taken to perform this protocol's SPT outside the respective pollen seasons. A major wheal diameter of ≥ 8 mm with a 10.000 BAU/mL (sic) TIM extract (Greer Laboratories Inc., Lenoir, US) selected only the highly sensitive subjects from among the candidates. Moreover, a vehicle reaction (50% glycerin) of < 3 mm major wheal diameter and the signing of the informed consent document led to recruitment into this trial. Exclusion criteria were the intake of medication that interferes with skin reactivity and other issues that might reduce the safety of a SPT or interfere with its results.⁷ A vehicle reaction during protocol's SPT > 5 mm² (diameter > 2.5 mm) was an elimination criterion. The institutional review boards of the study centers approved the study protocol and informed consent forms.

Allergen extracts and the titrated skin prick testing

The extracts investigated were TIM Soluprick® SQ 30HEP/mL (ALK-Abelló, Madrid, Spain), TIM Staloral® 300IR/mL (Stallergènes, Antony, France), Grazax® 75.000 SQ-T and Oralair® 300IR, each dissolved in 50% glycerin to a total volume of 1mL per tablet (under good-manufacturing-practices (GMP)). The reference was a TIM 10.000 BAU/mL extract (Greer Laboratories Inc., Lenoir, US), as its biological activity would be more in the range of the SLIT extracts, as opposed to the commercial 100.000BAU/mL.

The titrated SPTs were carried out in quadruplicate with the concentrate extracts and three serial half-log dilutions (Concentrate, 1:3, 1:10 and 1:30 v/v) and positive//negative controls (histamine 1mg/mL//vehicle). The study took place at study sites with different climatologic conditions. SPT sessions were planned outside the pollen season. A total of two sessions with duplicate test-extract application were scheduled one to four weeks apart. The extracts were placed in two opposite directions on the forearms of the allergic subjects – as skin sensitivity may vary from rostral to caudal and lateral to medial. SPT were carried out by one and the same technician in each center who had passed the proficiency test (coefficient of variation $\leq 20\%$).⁸). Intra-individual variability was minimum, making kappa testing unnecessary.

Data transfer

Wheal contours were marked with a felt tip black pen at 20min. Putting Transpore® tape on top of the marked wheals, the marker ink transferred to the tape, which was then stuck into the CRF and sent to the IMSIE, Cologne. There, the results were digitalized and wheal surfaces calculated electronically with Adobe Writer, Scion Image.

Statistical analysis

The sample size needed to determine the biologic activity in SPT of an allergen extract, in a properly conducted study, with few technicians that all passed the proficiency testing, is between 20 to 30 subjects (testing in quadruplicate). Allergen manufacturers use the same number of subjects in the standardization process of their products (SPT: Allergy

Therapeutics 25 subjects, ALK and Stallergènes 30 subjects; Intradermal: HAL-Allergy and FDA: 15 subjects)⁶.

Moreover, the investigators conducted three preceding studies comparing the biological activity of other allergen extracts, using the same methodology of quadruplicate, titrated skin-prick testing⁹⁻¹¹ recruiting 20-30 patients per study and showed highly significant wheal-size differences between extracts. Given the same experimental setting of this trial a complete data-set of 30 subjects would allow us to detect a 20% potency difference between extracts.

The geometric mean for all wheals per extract-concentration were calculated and for each extract the four values –concentrate and three 3-fold dilutions- were plotted on a semi-logarithmic chart. Subsequently, the regression line for wheal-concentration was drawn using the model $\text{Area} = a + b \log \text{Concentration}$. We used R^2 values as a measure of goodness-of-fit for the linear regression. Using the trend line equation (see figure 2) the regression-line-corrected wheal surface for the concentrates was calculated.

Each concentrate was assigned a BAU/mL value, depending on its wheal surface in relation to the reference's wheal surface. Statistically significant ($p < 0.05$) differences between the potency of the TIM extracts was determined with the Wilcoxon test (2-tailed, asymptotic significance).

Results

Quadruplicate SPT results per extract and its dilutions were obtained in 33 subjects after excluding data from three patients who only underwent one SPT session. No further data were eliminated. The geometric mean of the wheal surfaces for concentrate and dilutions of all tested extracts showed a very neat dose-response relationship with R^2 values above 0.98, meaning that 98% of the variation in wheal surface could be explained by extract concentration (figs 1-2). The difference in wheal size between concentrated extracts reached statistical significance for most.

The regression-line-corrected wheal sizes were 0.92cm² for Grazax® and 1.23cm² for Oralair®, with a Grazax®-Oralair® mean wheal-sizes of 1.1cm² (12mm diameter). These hardly varied from the measured wheal-size. As such, the real and regression-line-corrected BAU/mL were similar, see table 1.

Table 1

BAU/mL as based on geometric mean of the wheal surfaces (Real surface versus regression line extrapolated surface calculation*)			
	Extract wheal (cm ²)	Reference Wheal (cm ²)	BAU/mL**
Solution A	1.5447	1.4925	10 350
Sol A extrapol	1.5399	1.4925	10 318
Tablet A	0.9153	1.4925	6 133
Tabl A extrapol	0.9214	1.4925	6 174
Solution B	1.8039	1.4925	12 086
Sol B extrapol	1.7842	1.4925	11 954
Tablet B	1.2474	1.4925	8 358
Tabl B extrapol	1.2254	1.4925	8 210
Manufacturers reported Tablet A 2800BAU/mL and B 9000BAU/mL (<i>in vitro</i> data)			

BAU = bioequivalent allergy units, cm = centimeter, extrapol = extrapolated, mL = mililitre, Sol = solution, tabl = tablet

* Regression line extrapolated surface calculation = a trend line was drawn based on the mean wheal surfaces of the concentrated extract and its three half-log dilutions. Using the trend line-equation the extrapolated surface of the concentrated extract was calculated.

** Calculated, based on wheal surface of the *Phleum pratense* 10,000BAU/mL reference extract.

Conclusion

In the present study we determined the biological activity in titrated SPT of TIM SLIT preparations, including Grazax® and Oralair®, relative to an FDA approved, US reference extract. The thus established biological activity of Grazax®, 6.200BAU, is more than twice as high as the reported value in the package insert.¹² The 8.200BAU for Oralair® is close to the package insert value.¹³

Grazax®⁴ and Oralair®⁵ have extensive clinical documentation of short and long-term efficacy. However, biological activity in skin testing had not been documented, even though historically this has been the golden standard for extract potency measurement and standardization^{14 15}; BAU values of the package inserts of both tablets are based on *in vitro* results of competition enzyme-linked immunosorbent assays (ELISAs). These yielded a 3-fold difference for Grazax® and Oralair®: 2.800BAU versus 9.000BAU, respectively. The major allergen content also varied by 66% (15 and 25mcg Phl p 5, respectively). However, the here reported biological activity in SPT of Grazax® and Oralair® differed by only 25%, which could be interpreted as reassuring with regards to the correct TIM tablet dosing. Our results show both are in the same range.

We did quadruplicate SPT with a standardized method and used geometric means, making our test results solid. This was confirmed by the neat dose-responses with R^2 close to unity and the minimum differences between actual measured and regression-line-corrected wheal surfaces of the dose-response lines (table 1).

To estimate the allergen content in a tablet by skin testing, these had to be dissolved to become a 50% glycerin solution. We are aware that in doing so some of the allergenic potency might be lost. The different slopes of solutions versus tablets in figure 2 might indicate that. Even so the biologic SPT activity of Grazax® was more than twice the so far reported potency. Additionally, we see that the geometric mean of the wheal size produced by TIM Staloral® 300IR/mL, is about 30% higher than that of Oralair® 300IR, a 5-grass pollen tablet, containing apart from TIM, pollen from four other grasses. These are highly

cross-reactive, but not completely.¹⁶ Our SPT subjects were selected solely on the basis of SPT sensitivity to TIM. This might further have rested some of the power to detect the total allergenic potency of Oralair®. Even so, the biologic SPT activity of Oralair® was significantly higher than that of Grazax® ($p < 0.001$).

Two important conclusions can be drawn from these findings. Firstly, the effective SLIT, given with the tablets, is really high-dose SLIT. By our measurements, the biologic activity of both tablets is above 6000 BAU, while the US Practice Parameters on Immunotherapy, 3rd update⁷ recommended monthly SCIT dose for pollen is 1000-4000 BAU. That's why mild-moderate local adverse events are seen in over half of the patients with both tablets the first day(s) of treatment. In the future depot formulations linking the allergen with poly-lactic-co-glycolic-acid particles might be an option.¹⁷

Secondly, both tablets do differ somewhat in their allergen extract content. Even so, both are clinically effective, be it that the 2-year post-treatment efficacy was demonstrated for Grazax® with a perennial and for Oralair® with a pre-co-seasonal dosing schedule. It could very well be that the fast-dissolving mechanism of the Grazax® tablet compensates for the slightly lower allergen content, by producing a high trans-mucosal concentration gradient. This is an interesting finding, as historically the medical community has always focused on the allergen content as being the sole variable that confers efficacy to immunotherapy, as is the case for SCIT.¹⁸⁻²⁰ As such, for SCIT probable effective doses per allergen are suggested.⁷ For SLIT to be effective, it is probable that an optimal combination of several parameters should be sought: e.g. adequate biologic activity of the extract, an optimal trans-mucosal diffusion gradient,²¹ for liquid SLIT directly related to the allergen volume and for tablet-SLIT to the vehicle (fast or slow dissolving tablets), dosing frequency²² and duration of dosing (daily all year long vs pre-co seasonally²³). Even so, our data show that also for SLIT the most important variable for efficacy still seems to be the biological activity of the product, as both tablets have similar biological activity.

For SCIT, biological standardization has always been based on SPT results: e.g. in Stallergènes an extract producing a wheal diameter similar to the positive control of 9% codeine phosphate (7mm) is given the 100IR/mL potency. SCIT has shown to be effective with 80IR TIM monthly. Now, SCIT for the other pollens is also recommended with 80IR, based on SPT wheal size per pollen, without having further clinical trials.

Thus, now we have a more precise idea of the biological activity of the clinically effective TIM tablets, this might help to pick the correct dose-range for the development of SLIT tablets of other pollen allergens. SLIT maintenance dosing could be investigated with that concentration of pollen extract that causes a 1.1cm² (12mm diameter) wheal in highly sensitized subjects. In SCIT for animal dander more, and for house dust mite less concentrated extracts are effective, thus probably SLIT dosing is different for these allergens.

Finally, each allergen extract has to show its own efficacy-safety profile in clinical trials, as has lately be emphasized by the European Medicinal Agency and a recent World Allergy Organization task force report²⁴ seconds this line of recommendations. Our data can give a hint as to which doses might probably be effective as starting doses for dose-finding clinical trials.

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Legends to figures

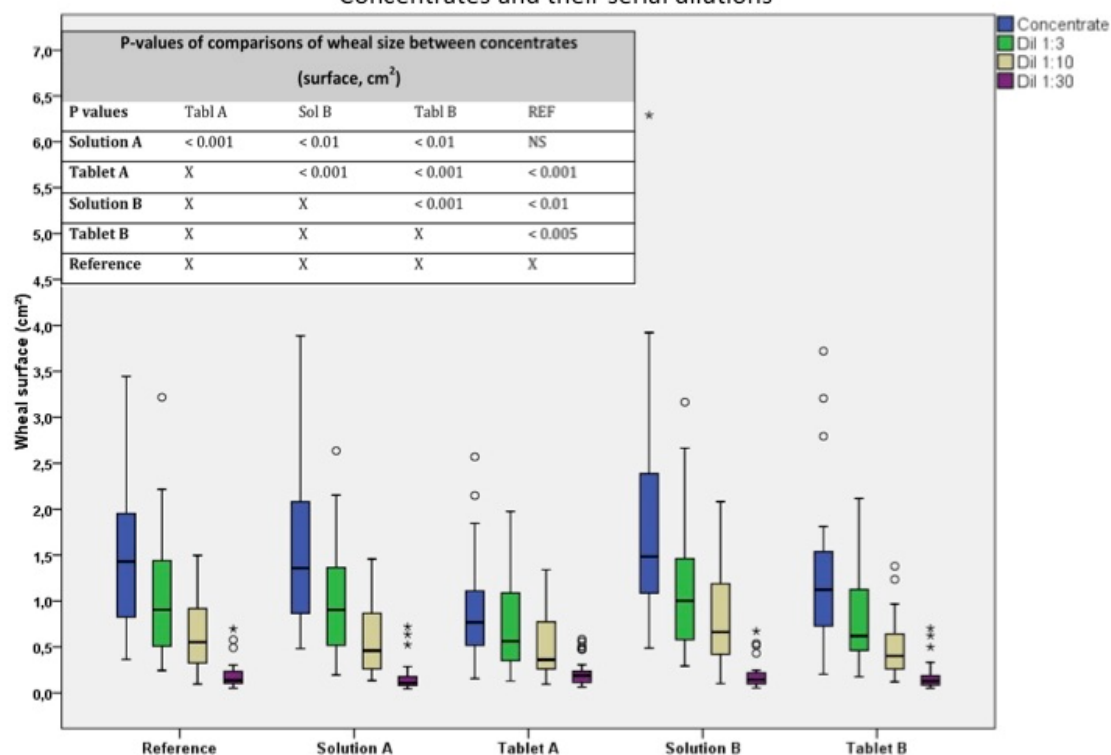
Figure 1:

Box plots of the geometric mean of the wheal surface areas (cm²) for the four grass pollen test extracts and the *Phleum pratense* grass pollen 10.000BAU/mL reference. Concentrates and three serial 3-fold dilutions. Solution A = Soluprick 30HEP/mL, Tablet A = Grazax®-in-1mL-glycerin50%, Solution B = Staloral® 300IR/mL, Tablet B = Oralair®-in-1mL-glycerin50%. Median and 25-75th percentiles. Open circles (o) are suspected outliers, asterisk (*) are outliers. In the box: p-values for differences between allergen concentrates (Wilcoxon asymptotic significance, 2-tailed).

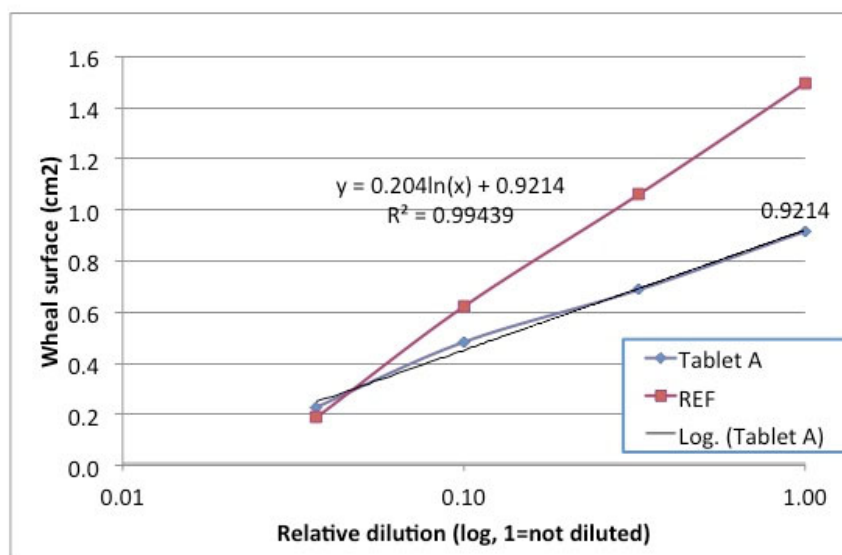
Figure 2a and b: Geometric mean of wheal surfaces for concentrate and three serial half-log dilutions of Grazax®-in-1mL-glycerin50% (upper panel) and Oralair®-in-1mL-glycerin50% (lower panel) against the 10.000BAU/mL (sic) reference extract, of *Phleum pratense* grass pollen. Trend lines, line equations and R² values are included for both tablets, as are the trend line extrapolated wheal surfaces (0.9214cm² for tablet A and 1.2254cm² for tablet B).

SPT Wheal surface areas

Concentrates and their serial dilutions



Geometric mean of wheal surface Tablet A vs. REF



Geometric mean of wheal surface Tablet B + trendline vs. REF

