

Comparison of the Biological Activity of the Most Common Sublingual Allergen Solutions Made by Two European Manufacturers

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Key Words

Rhinitis, allergic · Hyposensitization · Immunotherapy, sublingual

Abstract

Background: The clinical efficacy of sublingual immunotherapy has been documented in numerous studies and meta-analyses and is argued to be a favored alternative to the subcutaneous route if allergic symptoms are treated with a high-dose therapy. What is still lacking is a 'conversion rate' between the biological activities of different allergen solutions to determine the one with the higher concentration. **Methods:** A randomized, double-blind, parallel-group study was done with three groups sensitized to the allergens birch, grass or house dust mite via skin prick test. Staloral® in the concentrations 10, 100 and 300 IR and SLITone™ in the concentration of 1,000 STU per milliliter were used for a computer-based comparison of the geometric mean wheal sizes of the different solutions. For each patient, individual regression curves led to the calculation of a mean corresponding IR value for the SLITone solution. **Results:** A total of 47 patients took part in this clinical trial, most of whom were sensitized to more than one allergen. Values for

birch (30 patients) showed that 1,000 STU corresponded to 77 IR, for grass (29 patients) 1,000 STU matched 78 IR and for house dust mite (30 patients) 1,000 STU matched 27 IR based on dose-response relationships. The Wilcoxon test showed that for all allergens the allergenic activity of the SLITone solution was significantly higher than the 10 IR solution and significantly lower than the 100- and 300-IR solutions. **Conclusion:** We established a conversion rate between the two leading sublingual allergen solutions on the European market to compare different units of measurement.

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Introduction

Hyposensitization via the sublingual route has gained in importance during recent years [1, 2]. This treatment procedure is thus recommended in the WHO status paper for the treatment for more pronounced forms of allergic rhinitis [3]. However, many practicing allergologists are skeptical about this treatment alternative and some even blatantly refuse to accept it, because their own experience with the administered allergen solutions has been everything but convincing. Not only have

there been reports of considerable local side effects, but also of lacking efficacy [4].

The current study situation reflects this picture. Besides trials demonstrating a significant reduction of symptoms, others have shown practically no effect [5]. After a more critical look and as indicated by current meta-analyses [6, 7], one gets the impression that treatment success is closely dependent upon the biological potency contained in the solution [8–11]. Accordingly, the WHO has released the recommendation for treatment with high-dose medicinal products [12].

Yet, at the same time it is difficult for the practicing allergologist to identify the solutions with high concentration because each company represented on the market has chosen its own allergen standardization with its own unit of measure. The direct conversion from one unit to another is not possible. What is missing is the 'gold standard' upon which the various 'currencies' orient themselves and which allows clear comparability. In order to solve this dilemma, we compared the most important sublingual allergen solutions made by the two leading manufacturers on the European market on the basis of their biological activity in the allergic patient. We used the biological activity because the major allergen content is not a reliable guide for the relative biological potency of extracts with widely differing composition [13]. Also the total allergen activity is still regarded as relevant for therapeutic allergen extracts as already described in 1993 [14]. Thus, for the first time it has been possible to establish a 'conversion rate' between the most common sublingual allergen concentrations.

Patients and Methods

The study was performed as a randomized, double-blind, parallel-group trial with three groups sensitized to the allergens birch, grass or house dust mite (HDM). For birch 17 (56.7%) female and 13 (43.3%) male patients with a mean age of 27.75 years ($SD \pm 8.51$) took part in this trial. For grass, 11 (37.9%) females and 18 (62.1%) males with a mean age of 27.91 years ($SD \pm 7.87$) were sensitized, and for HDM there were 14 (46.7%) females and 16 (53.3%) males with a mean age of 29.05 years ($SD \pm 8.39$).

All patients had symptoms of allergic rhinitis during the respective pollen season or in the presence of HDM. A positive RAST $\geq$ class II using the CAP method carried out within the previous year led to inclusion into the trial. Mean values for birch were 15.22 kU/l ($SD \pm 17.45$), for grass 30.94 kU/l ($SD \pm 33.01$) and for HDM 11.27 kU/l ($SD \pm 13.52$).

Sensitization was documented by a positive skin prick test. A positive histamine reaction with a circumference ≥ 3.5 mm and a negative vehicle reaction were inclusion criteria on the day of prick testing. The study was conducted in October and November 2004,

which was after the local pollen season for the seasonal allergens. The study was approved by the regional Ethics Committee of North Rhine-Westphalia, and all patients that volunteered to participate gave their informed consent before entering the trial.

The methodology of the skin prick tests with quadruplicate testing in clinically sensitized patients was in accordance with the Nordic guidelines [15] as described in the EAACI position paper on allergen standardization and skin tests [13]. Bottles of Staloral® (Stallergenes, Kamp-Lintfort, Germany) and SLITone™ (Alk-Scherax, Hamburg, Germany) were purchased from the manufacturers and packed in blind canisters by an independent institute (Profil, Neuss, Germany). Individual bottles for each patient of Staloral solution in the concentrations of 10, 100 and 300 IR and SLITone in the concentration of 1,000 STU were used for the skin prick tests together with histamine (100 μ g/ml) and the control vehicle.

The skin prick tests were carried out on the volar surfaces of the forearm with four test areas per solution tested plus one area for histamine and control vehicle each per arm. Two of the test areas for each solution were on the right forearm, the other two on the left forearm. Altogether, there were 10 test areas per arm. The parameter used to determine the biological activity of the allergen solution was the wheal induced by the allergen injection. This was traced at 20 min after injection onto an acetate sheet. The wheal area was then digitized and analyzed using the software Adobe Photoshop 6.0 and Scion Image 4.02. The geometric mean of the patient's four individual wheal areas for each concentration was then determined and the log of the mean value was plotted against the log of the IR value. An individual regression curve for the three Staloral concentrations of 10, 100 and 300 IR per allergen was calculated based on the variables $\log_{10}$ wheal area and $\log_{10}$ allergen concentration (in IR) for each patient. This permitted the calculation of an individual corresponding IR value for the SLITone solution, per allergen and per patient.

The mean diameters of SLITone allergen solution were then calculated per patient and per allergen, and the corresponding IR values for each were determined. Also, the nonparametric Wilcoxon test was used to compare the SLITone mean values for each allergen with the three mean values of the corresponding Staloral solutions.

Results

A total of 47 patients, most of whom were sensitized to more than one allergen, took part in this clinical trial. Valid values, defined as a positive RAST $\geq$ class II plus a positive histamine reaction with a circumference ≥ 3.5 mm and negative control vehicle reaction, were obtained for 30 patients with birch sensitization, 29 patients with grass sensitization, and 30 patients with HDM sensitization (to explain the analysis process, see the flow chart in fig. 1).

The individually and cumulatively determined regression curves are depicted in figure 2 for grass (fig. 2a), birch (fig. 2b), and HDM (fig. 2c). Based on these intraindi-

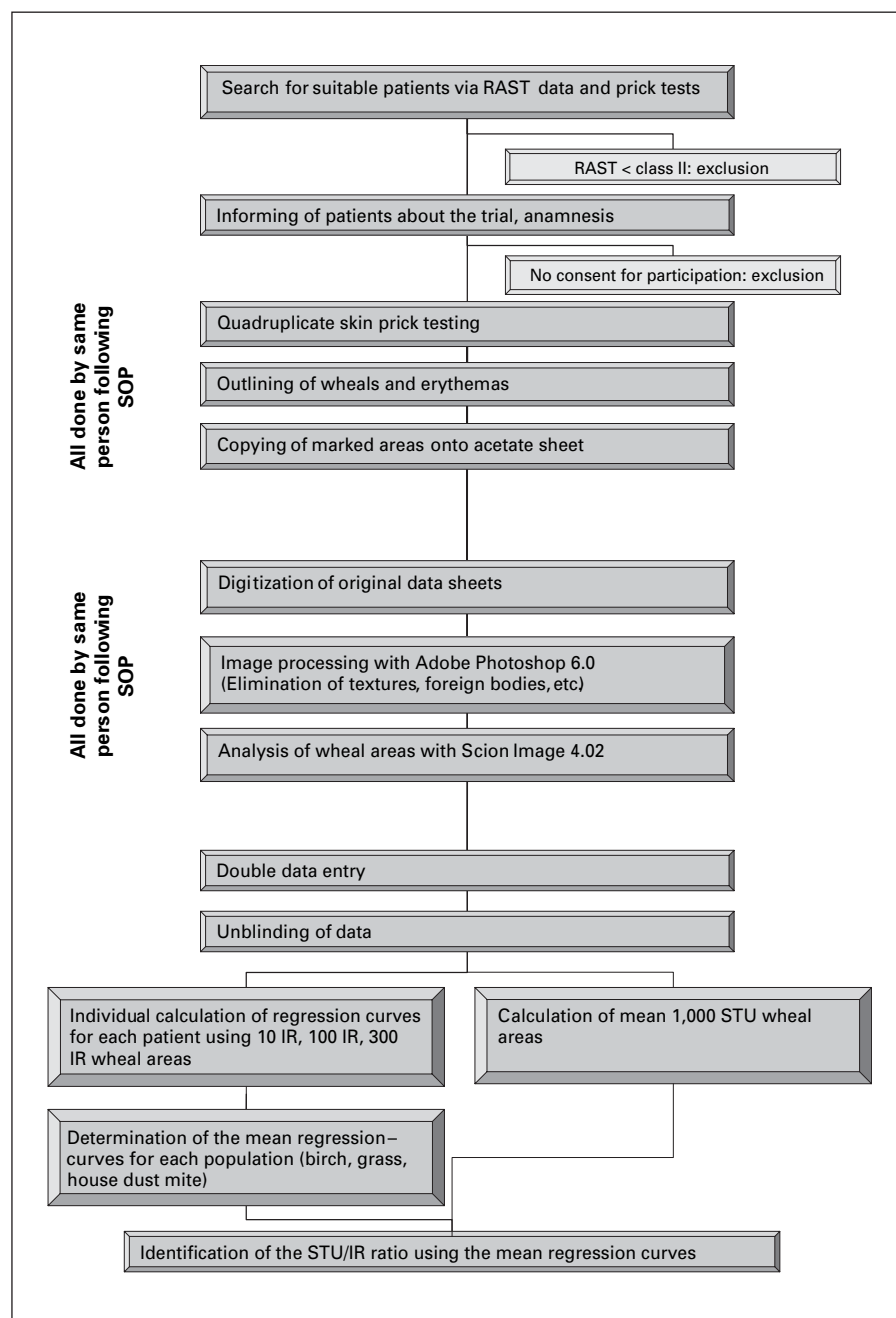


Fig. 1. Process of obtaining results. SOP = Standard operating procedure.

vidual dose-response relationships, a mean regression curve was calculated for the relationship between the mean wheal area and the corresponding Staloral allergen concentration in IR (bold curves). Based on the mean wheal area values calculated for the SLITone allergen solutions, the corresponding IR values can be determined. These were: 77 IR for 1,000 STU SLITone of the birch pollen allergen solution, 78 IR for 1,000 STU SLITone of

the grass pollen allergen solution and 27 IR for 1,000 STU SLITone of the HDM solution.

The statistical tests demonstrated that the SLITone concentrations for all allergens were significantly higher than the 10-IR solutions of the corresponding Staloral allergens and significantly lower than the 100- and the 300-IR solutions as shown in table 1.

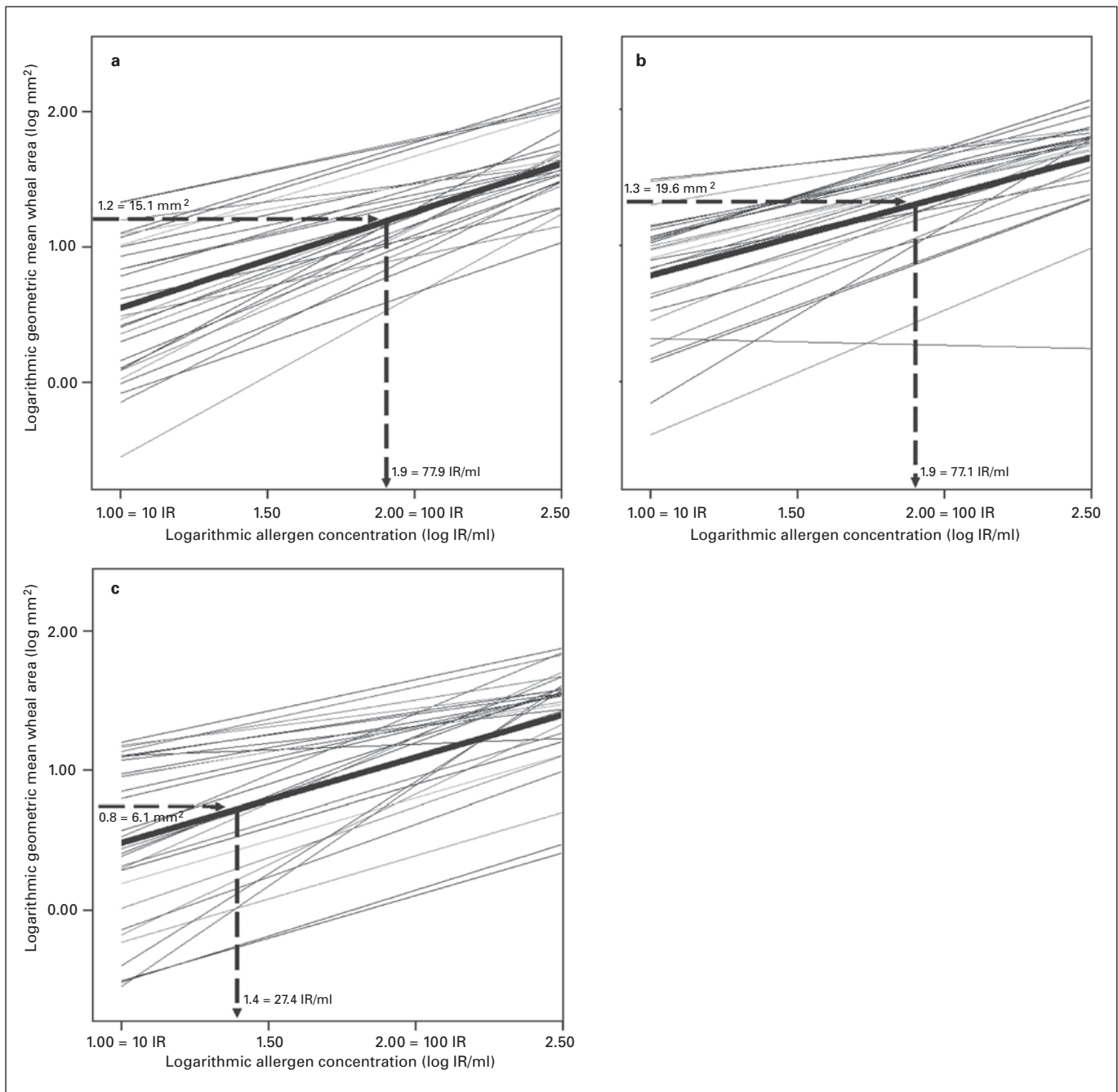


Fig. 2. Regression curves as a result of allergen sensitization. (Individual curves are represented by thin lines, cumulative curves by bold ones.) **a** Grass. **b** Birch. **c** HDM.

Safety. All skin prick tests were well tolerated by the patients. In one case of persistent erythema, a corrective antiallergic treatment with cetirizine tablets and 100 mg prednisolone intravenously was sufficient to control the adverse event.

Discussion

We established the 'exchange rate' for the most widely used allergen solutions of typical allergens for sublingual vaccination in Europe. Regarding the daily maintenance

Table 1. Parameters of the geometric mean of the wheal area per allergen and concentration and results of the Wilcoxon test

Allergen	Concentration	Area, mm ²			Results, Wilcoxon test	p value
		mean	95% CI	min/max		
Birch	10 IR/ml	5.89	3.94–8.81	0.36/31.62	1,000 STU > 10 IR	0.0000
	100 IR/ml	24.27	18.11–32.52	1.66/72.44	100 IR > 1,000 STU	0.0175
	300 IR/ml	41.25	30.46–55.86	1.86/123.03	300 IR > 1,000 STU	0.0000
	1,000 STU/ml	19.59	14.62–26.25	2.00/51.29		
Grass	10 IR/ml	3.34	2.12–5.26	0.21/20.89	1,000 STU > 10 IR	0.0000
	100 IR/ml	21.63	16.78–27.89	5.75/67.61	100 IR > 1,000 STU	0.0052
	300 IR/ml	34.95	27.02–45.22	8.71/114.82	300 IR > 1,000 STU	0.0000
	1,000 STU/ml	15.13	10.36–22.10	0.51/70.79		
Mite	10 IR/ml	3.12	1.92–5.09	0.26/16.60	1,000 STU > 10 IR	0.0002
	100 IR/ml	11.63	7.62–17.73	0.44/44.67	100 IR > 1,000 STU	0.0001
	300 IR/ml	25.31	19.00–33.71	3.39/74.43	300 IR > 1,000 STU	0.0000
	1,000 STU/ml	6.12	3.62–10.35	0.38/34.67		

dose typically prescribed (0.2 ml for SLITone vs. 0.4 ml for Staloral), the biological activity as defined by the skin prick test wheal reaction of the Staloral solutions is about 8 times higher for birch and grass pollen and 22 times higher for HDM. This is in line with a previous investigation [16] which demonstrated a ratio of 2.5:1 between the biological activity of birch and grass compared with mite for the 1,000-STU solutions.

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