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# The biological potency of different extracts for sublingual immunotherapy assessed by skin prick tests

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## KEY WORDS

Sublingual immunotherapy;  
biological potency; skin prick test

## SUMMARY

*The standardization of allergen extracts is of primary relevance to the clinical efficacy. Biological standardization procedures are widely used in the commercial production of vaccines. We tested, in grass-allergic patients, the potency of three different grass extracts for sublingual immunotherapy by means of skin prick tests. Specific IgE against Phl p 1, 2, 4, 5, 7, 11 and 12 were also assayed. Allerslit® and Sublivac® were directly applied as skin test. Grazax®, was prepared by dissolving two tablets in 2mL saline. Thirty-three subjects (mean age 38.8) were studied. The skin response was significantly different among extracts, decreasing from Allerslit to Grazax ( $t$  test  $<0.01$ ), but all the extract produced a skin response greater than histamine. All the subjects had specific IgE to Phl p 1 and Phl p 4 but 24% did not have specific IgE to Phl p 5. In those subjects the skin response to the three extracts did not differ from that of Phl p 5-positive subjects. Our findings confirm that there is a variability in the biological potency among different extracts. In addition, the standardization of grass extracts based on Phl p 5 only, may be insufficient in some cases.*

## Introduction

Sublingual Immunotherapy (SLIT) is now recognized as an effective treatment for respiratory allergy (1). It is widely used in most European countries, where numerous different products are commercially available. Due to the large heterogeneity of the commercial preparations, in term of doses and allergen content, one of the important aspects of SLIT still remains the standardization of products. The standardization, which is the reproducibility of the extracts, is mainly related to the concentration or content of the major allergen(s). This problem is of great clinical relevance for two main reason. First, the clinical efficacy and the safety of an extract is at a certain extent dose-dependent, as clearly shown in the recent dose-finding tri-

als with grass extracts (2, 3). Second, the knowledge of the exact allergenic content of each product would allow comparisons among products, a better definition of the dose-response aspects and would also provide a support for investigating the mechanisms (4). As usually done in the biological standardization procedures, the overall potency of an allergenic extract can be roughly evaluated by means of skin prick test. In fact, the biological standardization, which is based on skin reactions is still largely used among manufacturers, although for many extracts the content of allergen(s) in micrograms is currently available. We performed an evaluation of the biological potency of three commercial extracts for SLIT, by means of skin prick tests (SPT), in subjects sensitised to grasses who were also evaluated by component resolved diagnosis.

## Methods

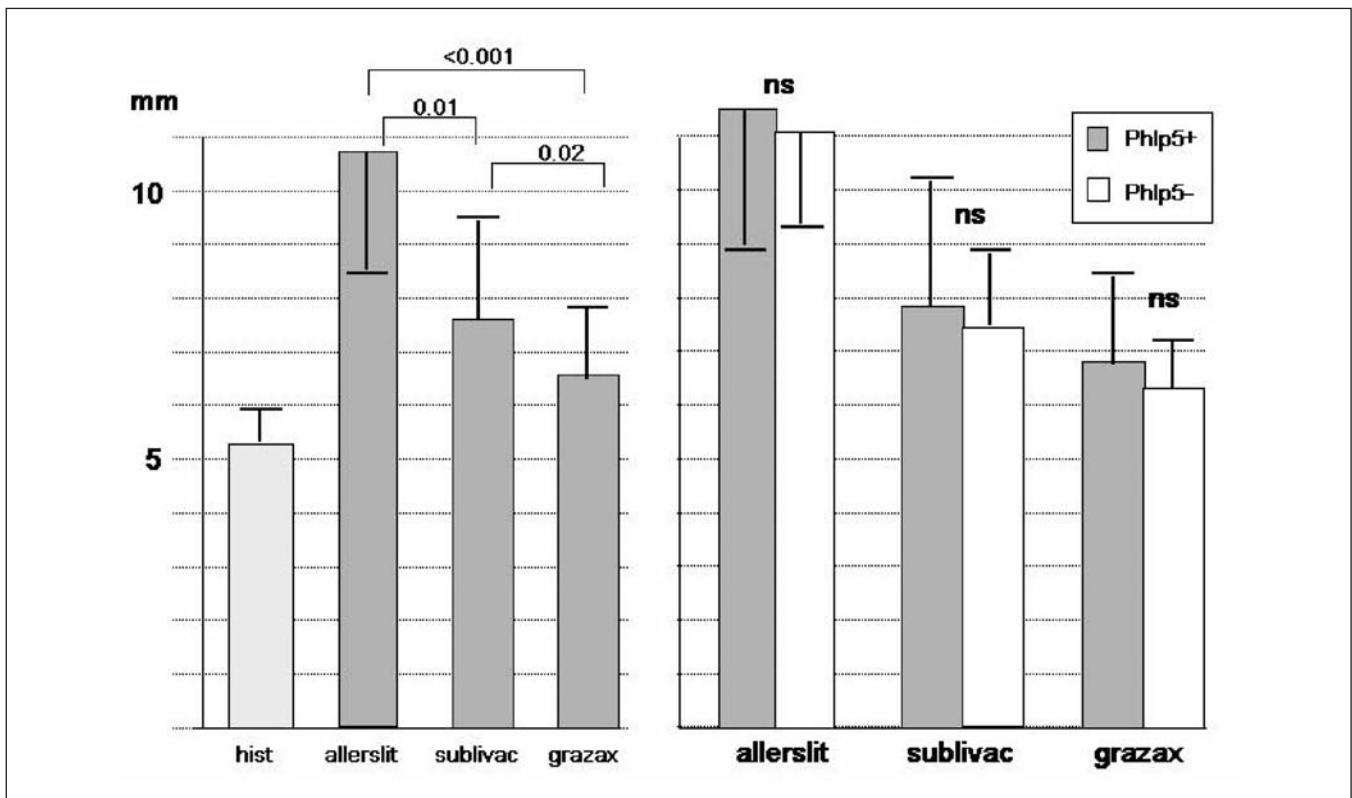
Adult patients with seasonal asthma and/or rhinoconjunctivitis underwent the standard diagnostic procedure including clinical history, examination, skin prick tests with commercial extracts (Stallergens Italy, Lainate, Milan), and total IgE assay. Those patients with sensitisation to grass pollen ad eligible for grass-specific immunotherapy underwent further investigations, as follows. An ImmunoCAP assay (Phadia SrL) was performed according to the manufacturer's instruction, to detect the presence of specific IgE to Phl p 1, 2, 4, 5, 7, 11 and 12. The results of the assay were expressed in kUA/l. Additional skin tests were performed with three commercial extracts: Allerslit (Allergopharma, D), Grazax (Alk-Abellò, DK) and Sublivac (Hal Allergy, NL). Allerslit (40 µg/mL Phl p 5) and Sublivac (1,000 AU/mL) are prepared as solution, thus they were used as in a standard skin test. Grazax, that is a tablet formulation, was prepared by dissolving two 75000 SQ-T tablets in 2 ml of saline. This produced a solution containing 15 µg/mL Phl p 5. The fresh solution was

then used within 12 hours. The results were expressed as the mean of the major diameter of the wheal plus its orthogonal. Histamine HCl 0.1% and saline were used as positive and negative controls.

## Results

Thirty-three subjects (17 male, age range 9-62 years, mean age 38.8) were studied. Of them, 22 had rhinoconjunctivitis alone and 11 had also asthma. Their mean total IgE level was  $271 \pm 32$  kU/L. The results of the skin prick with the three SLIT extracts are reported in figure 1 (left). It could be noticed that the skin response was significantly different among extracts, in decreasing order from Allerslit to Grazax (t test  $<0.01$ ). On the other hand, the skin response was significantly greater than with histamine for each of the three extract ( $p <0.05$ ). The percentages of patients with positive specific IgE to each grass pollen allergen, as assessed by the component resolved diagnosis, was as follows: Phl p 1 =100%, Phl p 2

**Figure 1** - Left: mean and SD wheal diameter with the three extracts in the whole population (n=33). Right: mean and SD wheal diameter with the three extracts in patients with (n=25) or without (n=8) specific IgE to Phl p 5



= 66%, Phl p 4 =100%, Phl p 5 =76%, Phl p 6 =66%, Phl p 7 =3%, Phl p 11 = 50%, Phl p 12= 45%. Of note, 8/33 (24%) patients had undetectable specific IgE to Phl p 5, but their skin response to the three extracts did not differ from that of Phl p 5 positive subjects, as shown in figure 1 (right).

## Discussion

Overall, our results confirm that some differences in the biological potency exist among commercial extracts. This probably reflects the largely variable content of proteins among products, as recently shown in comparison studies (5, 6). This should be true also for sublingual vaccines under investigation and their content in relevant grass pollen allergens. This fact may have consequences in terms of immunological and clinical response, as well as in terms of possible adverse reactions (4). It is true that the preparation of the solution from the Grazax tablets may have introduced an artefact, since the resulting solution does not exactly reproduce the concentration of allergens in the tablets. Nevertheless, since the volume of a tablet is approximately one mL, the error cannot be expected to be great. The IgE sensitisation to the major allergens largely contribute to patients' skin reactivity since all the patients had specific IgE to Phl p 1. More interestingly, 24% of the patients did not have specific IgE to Phl p 5, that is considered a major allergen (7, 8), but their skin reactivity was not decreased. The concomitant presence of other antigenically relevant proteins in the extracts may reasonably explain the observation. Nevertheless, almost all the commercial products are standard-

ized according to their content in Phl p 5. As a consequence, the standardization based on only one allergenic protein may be considered imprecise or incomplete, since several proteins are involved in the sensitisation and, therefore, intervene in the mechanism of action of immunotherapy.

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