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Quality and Clinical Efficacy of Allergen Mixtures

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Quality of allergen products

The quality of allergen products for treatment (allergen-specific immunotherapy) depends on:

- The allergen raw material sources
- The standardization of the final allergen product
- The documentation in clinical studies

The allergen raw material should be obtained from relevant allergen source material, and detailed information on harvesting, storage, extraction and purification should be provided [1]. Specific guidelines for standardization of the intermittent and the final product in relation to an in-house reference are available. The quality assessment supplied by the manufacturer is important in optimizing the clinical efficacy and safety of allergen-specific immunotherapy. For comparative studies, information on major allergen content labelled in mass units ($\mu\text{g}/\text{ml}$) is useful, although comparison between different manufacturers, labelling may not be possible due to differences in assays and methodologies for measuring major allergens. Finally, allergen products have to pass the ultimate quality test related to the documentation of a clinically relevant efficacy and safety in controlled studies [2].

Allergen mixtures

The debate on allergen mixtures has for several decades divided allergists in highly emotional groups arguing for and against the use of mixtures. It seems, however, like the debate on mixtures is based rather on philosophy than on scientific evidence founded on controlled studies. Several arguments for mixing allergens in a treatment product exist, but the major issues are with respect to available documentation of efficacy and safety.

The scientific community is left with a paradox. There is a generally consent that for diagnostic purposes, the precision of determining the exact etiologic agents elic-

iting symptoms is of paramount importance. On the other hand, it has been widely accepted, that mixing allergens with unknown properties for treatment, are consistent with Good Clinical Practice. This paradox is based on the concept, that all allergens to which a patient demonstrates IgE-reactivity (skin test and determination of specific IgE-antibodies) represent clinically relevant sensitization. However, sensitization to an allergen does not implicitly mean that the IgE-response results in clinical symptoms. For most patients a careful evaluation will make it possible to define the clinically relevant allergens inducing clinical symptoms and those allergen-sensitizations being only a bystander. From a practical point of view, only clinically relevant allergens should be used routinely for immunotherapy [1,3].

In the discussion on allergen mixtures, we have to differentiate between mixtures of related, cross-reacting allergens, such as a mixture of individual grasses, tree pollens and the mixture of *D. pteronyssinus* and *D. farinae*, and mixtures of unrelated allergens. Several studies have documented a relevant clinical efficacy of mixing related, cross-reacting allergens due to their high level of homology between individual major allergens [4-6]. In contrast, the evidence of efficacy using mixtures based on the sensitization pattern is based on aged studies using unstandardized allergens and factually, the only recent study applying extracts quantized in mass units of major allergens failed to document efficacy above the placebo level [7].

European attitude

The approach to allergen mixtures in the latest position paper from European Academy of Allergology and Clinical Immunology [8] is clear. Mixing of unrelated allergens is technically feasible and with the available methodologies, individual allergens can be controlled in the mixture. In view of the potential interactions between enzymatic components, detailed quality control and stability assessments are required. A major problem in mixing unrelated allergens is whether optimal doses of individual allergens can be achieved due to the inevitable dilution of individual components. Licensed allergen products with proven safety and efficacy should be used whenever possible. The overall recommendation is not to use mixtures of unrelated allergens.

US attitude

The Practice parameters from the American Academy of Allergy, Asthma & Immunology and the American College of Allergy, Asthma & Immunology [9] state that whenever possible, standardized extracts should be used to prepare vaccine treatment sets, indicating that mixtures are the standards. Some requirements are, however, essential like in choosing the components for a clinically relevant vaccine; the physician should be familiar with local and regional aerobiology and indoor and outdoor allergens, paying special attention to potential allergens in the patient's own environment. Likewise, knowledge of allergen cross-reactivity is important in the selection of allergens for immunotherapy because limiting the numbers of allergens in a treatment vial is necessary to obtain optimal therapeutic doses for the individual patients.

Advantages of allergen mixtures

By mixing multiple allergens in a single treatment vial, the physician obtains a convenient administration by reducing the number of injections and saving time. In Europe it is recommended to inject non-related extracts separately and spaced by 30 minutes [8]. As the average number of allergens used for individual treatment in US is about five, the European way of working implies a waiting period of $> 2\frac{1}{2}$ hours. However, Europeans recommend limiting the number of allergens to two or at most three different allergens. Although many patients do demonstrate sensitization to more allergens, the thinking is based on the concept that patients with multiple allergic sensitivities may be effectively treated with a limited number of individual allergen products according to their individual exposure. In relation to the risk of eliciting side effects the human factor making mistakes of allergens, doses and patients, having altogether in one vial the different allergens might reduce this risk. Finally, the concept of “treating all allergic symptoms by one shot” might be attempting.

Problems with allergen mixtures

Problems related to the use of allergen mixtures imply: standard dosing for each allergen; a risk of inadequate dosing for individual allergens; a potential deleterious effect of mixing, and finally, the use of mixtures is not comparable with modern pharmaceutical essential.

Standard dosing for each allergen

Being a biologic treatment, immunotherapy in terms of efficacy and safety has to be individualized according to the sensitivity and response of the patient. As the top dose is often defined by side effects, a major problem with mixtures is that the allergen eliciting side effects define the top dose. That allergen might be administered in an optimal dose, but all other allergens included in the mixture will be delivered in sub-optimal doses, and might very well result in ineffective treatment. Using single allergens, the possibility to reach the optimal dose resulting in a high clinical efficacy is greater by individualizing the dose according to the patient's response. Another problem is that when side effects are elicited, the responsible allergen cannot be identified, and as a consequence, all allergen doses have to be reduced (and again with the risk of low-dose treatment).

Inadequate dosing of individual allergens

The clinical effect of immunotherapy is related to delivering sufficient single doses of allergen extracts regularly (the optimal dose). Immunotherapy studies with ragweed, grass, mite, cat, and venom allergens have documented a significant improvement in disease severity, using maintenance doses of 5-20 μ g major allergen per injection [4]. This amount of allergen can only be obtained with standardized extracts with known optimal doses. For the majority of weak extracts, effective doses cannot be attained due to dilution of the individual components in the mixture. In clinical practice injection of volumes > 1 ml is inconvenient, and consequently, it is unlikely that truly effective doses can be attained in mixtures containing weak extracts [10].

Deleterious effect of mixing

Some pollen extracts contain enzymes that may cause autodigestion and loss of potency of a vaccine. Due to the enzymatic activity of proteases in some allergens (especially mould and cockroach allergens) the biologic activity of other allergens in a mixture may be reduced significantly. The capacity of degrading allergens is varying depending on which allergens included in the mixture and from one production lot to another, and consequently, even a detailed knowledge of interactions might be insufficient to ensure consistency in future mixtures [11]. Furthermore, the degrading capacity shows inconsistent effect on different major allergens from an allergen material. On mixing with *Fusarium*, the major birch and grass allergens Bet v 6 and Phl p 5 degraded almost completely, while Bet v 1 and Phl p 1 remained relatively stable [12] making the potential hazard of mixing unpredictable and exposing the patient at risk when treated with a freshly prepared vaccine containing allergens not present in the experienced mixture used previously.

Mixing not comparable with modern pharmaceutical standard

An important issue in relation to the safety of injection immunotherapy is the composition and stability of the allergenic components in a vial. Degradation of allergens during long-term use of a vial may not represent safety aspects (as the potency of the injected allergens simply decline). The problems arise when changing to a new vial. The higher potency of a new vial might induce anaphylactic systemic reactions. Mixed standardized products delivered by a pharmaceutical company applying "Good Manufacturing Practice" may not represent a problem, but the possibility of making mistakes when mixing is done by practising allergist should not be neglected. Using "kitchen-made" mixtures shift the medico-legal responsibility of allergen content and stability of the vaccine from the allergen manufacturer to the treating physician. In view of modern accreditation standards, homemade mixtures are at best problematic and at worst incompatible with Good Clinical Practice.

Conclusion

Mixtures of cross-reacting allergens like grasses, mites, etc. might be used in immunotherapy, but have limited advantages over single allergens due to the high degree of homology for major epitopes. Allergen mixtures of unrelated allergens are not recommended in routine immunotherapy due to insufficient scientific documentation of efficacy. This is based on a potential shortage in administering the optimal dose of each individual allergen (leading to low-dose immunotherapy for some allergens in a mixture). The top dose will be defined by the individual allergen in a mixture eliciting side effects and all other allergens included will be administered in sub-optimal doses. Furthermore, due to varying degree of enzymatic degradation in some mixtures, the risk of low-dose treatment exists beside an increased risk of eliciting anaphylactic reactions, when a freshly prepared vial is used. Lastly, home-made mixtures shift the medico-legal responsibility for the stability and composition from the allergen manufacturer to the mixing allergist.

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Discussion

Drachenberg: I agree with almost all your comments, but one thing, I think, is not true. Your fear of mutual degradation, I think, is only true in aqueous extracts and not in adsorbed preparations.

Malling: Yes, you might be right. The American way of solving that problem is to use glycerol, but in my opinion it is not very pleasant to have injections of high concentration of glycerol. You might be right, but I have never seen any data on it.

Bousquet: In fact, the Americans mix their extracts, but these are aqueous extracts - these are not depot preparations. So, a depot preparation might be eventually done by a company. But, e.g. in France, it is not allowed to mix more than 5 extracts in a vial, the mixtures are sold in this way. So the way Hans-Jørgen Malling said it is the glycerol added, but glycerol does not prevent the degradation.

Malling: It does for some allergens but not completely.

Dreborg: I would like to add to your slide about the study by Nelson who added *Cladosporium* to other allergens and checked the degradation. But we found ten years earlier that *Cladosporium* degraded itself in one week in aqueous solution. So what he tested was probably nothing. *Cladosporium* is very dangerous to mix with anything and should certainly not be used in mixtures.