

A Double-Blind Randomized Placebo-Controlled Trial With Short-Term β -Glucuronidase Therapy in Children With Chronic Rhinoconjunctivitis and/or Asthma Due to Dust Mite Allergy

E Galli,¹ MS Bassi,² E Mora,³ M Martelli,⁴ S Gianni,¹ G Auricchio,¹
E Arabito,⁵ P Rossi⁵

¹Research Center San Pietro, Fatebenefratelli Hospital, AfaR, Rome, Italy

²AOG Salvini, PO Rho, Italy

³San Martino Hospital, Genova, Italy

⁴Santa Maria Bianca Hospital, Mirandola, Italy

⁵Department of Pediatrics, Tor Vergata University, Rome, Italy

Abstract. *Background:* Enzyme potentiated desensitization, in which β -glucuronidase (BG) is administered with low doses of mixed allergens, was proposed in the 1970s for specific immunotherapy. The BG currently commercially available in a purified and standardized preparation devoid of any allergen has been suggested as a regulator in the allergic immune response, acting on the cytokine-network of type 2 helper T cells. A double-blind trial with a single-dose of BG proved effective in preventing symptoms in adult patients with rhinoconjunctivitis due to grass pollens.

Objective: The aim of this randomized double-blind placebo-controlled trial was to confirm the safety and effectiveness of double-dose intradermal BG immunotherapy in preventing symptoms in children suffering from chronic rhinoconjunctivitis and/or asthma due to dust mite.

Method: We randomized 125 children with dust-mite related chronic rhinoconjunctivitis and/or asthma to the BG treated group (67) or the placebo group (58). All patients were screened before treatment (T0), at BG or placebo administration (T1 and T3), and at 3 and 9 months after T1 (T2 and T4). Drug intake and bronchial, nasal and ocular symptoms were recorded in a diary.

Results: Patients in both groups completed the study and BG treatment was well tolerated without side effects. Significant differences in symptoms were observed, in particular for conjunctivitis ($P = .008$). The total drug intake for allergic symptoms was significantly lower in the treated group than in the placebo group ($P < .01$).

Conclusions: BG immunotherapy is efficacious, safe, and well tolerated in allergic children. Moreover, good compliance with the administration of 2 doses per year and the lack of significant side effects makes the benefit/risk ratio of this treatment particularly favorable.

Key words: β -glucuronidase. Immunotherapy. Children. Allergic diseases.

Resumen. *Antecedentes:* La desensibilización potenciada con enzimas, en que se administra β -glucuronidasa (BG) con dosis bajas de alérgenos mixtos, se propuso en 1970 como inmunoterapia específica. Se ha sugerido que la BG disponible comercialmente en una preparación estandarizada y purificada, sin ningún alérgeno, funciona como reguladora de la respuesta inmunitaria alérgica, actuando sobre la red de citocinas de los linfocitos T cooperadores del tipo 2. En un estudio de doble ciego con una sola dosis de BG, se demostró la efectividad de esta enzima para prevenir los síntomas de rinoconjuntivitis debida a pólenes de gramíneas en pacientes adultos.

Objetivo: El objetivo de este estudio aleatorizado controlado con placebo y doble ciego fue confirmar la seguridad y eficacia de una doble dosis de inmunoterapia con la administración de BG intradérmica para la prevención de los síntomas en niños que padecían rinoconjuntivitis crónica o asma debido al ácaro del polvo.

Métodos: Aleatorizamos a 125 niños con rinoconjuntivitis o asma relacionadas con el ácaro del polvo en un grupo tratado con BG (67) o en un grupo con placebo (58). Se realizó el cribado de todos los pacientes antes del tratamiento (T0), al administrar la BG o el placebo (T1 y T3), y tres y nueve meses después de la fase T1 (T2 y T4). Se registró en un diario la toma de fármacos, y los síntomas bronquiales, nasales y oculares.

Resultados: Los pacientes de ambos grupos completaron el estudio y el tratamiento con BG se toleró bien y sin efectos secundarios. Se observaron diferencias significativas en los síntomas, en particular de la conjuntivitis ($P=0,008$). La toma total de fármacos para controlar los síntomas alérgicos fue significativamente menor entre los sujetos del grupo al que se administró tratamiento activo comparado con el grupo al que se administró un placebo ($P<0,01$).

Conclusiones: La inmunoterapia con BG es eficaz, segura y bien tolerada por los niños alérgicos. Lo que es más, el cumplimiento de la administración de dos dosis al año y la ausencia de efectos secundarios importantes hace que la relación beneficio-riesgo de este tratamiento sea especialmente favorable.

Palabras clave: β -glucuronidasa. Inmunoterapia. Niños. Enfermedades alérgicas.

Introduction

In the last two decades, allergen specific immunotherapy by subcutaneous administration of the allergen has gradually fallen out of favor, particularly because of limited compliance and risk of rare but life-threatening anaphylactic reactions [1-4]. For this reason, local (noninjected) means for administering immunotherapy have been proposed and developed. The main one is sublingual immunotherapy [5-8]. Several double-blind controlled trials have indicated that such therapy is safe and clinically effective for some allergens in both pediatric and adult patients [9, 10]. In particular, children's compliance with sublingual immunotherapy is an important positive aspect of this therapy. However, it is expensive and needs to be continued for several years with daily administration.

Immunotherapy with enzyme-potentiated desensitization, in which β -glucuronidase (BG) is injected with low doses of a mixture of allergens, was proposed in the 1970s and has been demonstrated to be safe and effective in rinoconjunctivitis, asthma, and food hyperkinetic syndrome [11-14], although some authors have obtained opposite results [15]. Nowadays BG is commercially available for intradermal administration in purified and standardized preparations devoid of any allergen. It has been suggested that BG might play a role in the immune response to allergy by redirecting the cytokine network of type 2 helper T cells; specifically, its immunological effects involve the differentiation and maturation of dendritic cells, thus modifying the response of the immune system to subsequent allergen exposure [16]. A double-blind trial in which a single-dose of BG was used demonstrated the effectiveness of the treatment to prevent symptoms in adults with rinoconjunctivitis due to grass pollen [17].

The aim of this double-blind randomized trial was to confirm the safety and the effectiveness of immunotherapy with a double dose of BG in preventing symptoms in children suffering from chronic rinoconjunctivitis and/or asthma due to dust mite.

Patients and Methods

Study Design

The protocol for this randomized controlled trial was approved by the ethics committee of San Pietro Hospital Fatebenefratelli. It was carried out in accordance with good clinical practice, and informed consent was obtained from all patients' parents. The children were enrolled in September and October 2004 and randomly assigned to either the BG group or the placebo group. All patients were seen before treatment (T0), when BG immunotherapy or placebo were injected (T1, October, and T3, February, before the peak of the dust mite season), and at 3 and 9 months following T1 (T2, January and T4, June). During clinical follow-up patients were allowed to use oral antihistamines (cetirizine), topical steroids (fluticasone) and β_2 -agonists for symptoms control. Each day, patients filled in a diary recording symptoms and drug intake throughout the study (September 2004 – June 2005). At T1 and T4 peripheral blood was collected from patients for the immunological study and prick tests were performed.

Patients

One hundred twenty-five children with dust-mite

allergy (to *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*), chronic rhinoconjunctivitis, and/or asthma were randomly assigned to the BG treated group (67 children: 37 male and 30 female; mean ± SD age, 10.5 ± 3.9 years), or to the placebo group (58 children: 31 male and 27 female; age, 11.2 ± 2.7 years) (table). The inclusion criteria were based on a) personal history of allergic rhinoconjunctivitis and/or asthma lasting at least 2 years; b) positive prick tests to one of the dust mite extracts prepared as hydroglycerol solutions, titrated at 20000 Bodansky units per milliliter (SARM Allergeni, Guidonia, Rome, Italy), assessed according to usual practice [18] (allergens eliciting a wheal diameter at least 3 mm greater than the negative control were considered positive); and c) positive ADVIA Centaur immunoassay system (Bayer Diagnostic, Tarrytown, New York, USA) to one of the dust mite extracts (0.35–100 kU/L).

Exclusion criteria were a) a previous report of clinical allergy to shellfish, b) specific immunotherapy treatment within the previous 3 years, and c) presence of other immune disorders.

Immunotherapy

The BG preparation (kindly supplied by SARM) consisted of 100 µg of BG derived from the shellfish *Haliotis* species and purified by column chromatography (Seravac Ltd, Johannesburg, South Africa), in a total volume of 0.05 mL of buffered saline solution. Placebo consisted of 0.05 mL of buffered saline solution packed identically to the BG preparations. BG and placebo were twice administered intradermally, the first time in October (T1) and the second time in February (T3), before the peak of dust mite season.

Diary Card

At enrollment (September–October) and during the follow-up (until June 2005), patients recorded their bronchial, nasal and ocular symptoms every day. Nasal symptoms included rhinorrhea, nasal itching, nasal obstruction, and sneezing. Ocular symptoms were tearing, itching, and redness. Bronchial symptoms were cough, wheezing, and shortness of breath. Patients' diary data were used to calculate a mean score (0, none; 1, mild; 2, moderate; 3, severe) for each symptom for each month of follow-up. Drug intake was recorded daily and a mean score for each month was calculated on the basis of the assumption or not of allowed drugs, whether oral antihistamines (cetirizine), topical preparations (fluticasone) or β₂-agonists. Patients' subjective evaluation of the disease was assessed on a visual analogue scale.

Adverse Event Recording

Any local or systemic symptom occurring within 4 hours after BG treatment was recorded under supervision and documented at the study site. Small areas of temporary local erythema and a brief stinging sensation after injection were considered normal. Patients were asked to report any delayed (within 48 hours) local or generalized symptoms.

Statistical Analysis

Intragroup evolution was analyzed by using the Wilcoxon or Friedman tests. The intergroup comparison was performed with the Mann–Whitney test. Two-sided tests were used and *P* values of less than .05 were considered significant.

Clinical and Allergy Assessment*

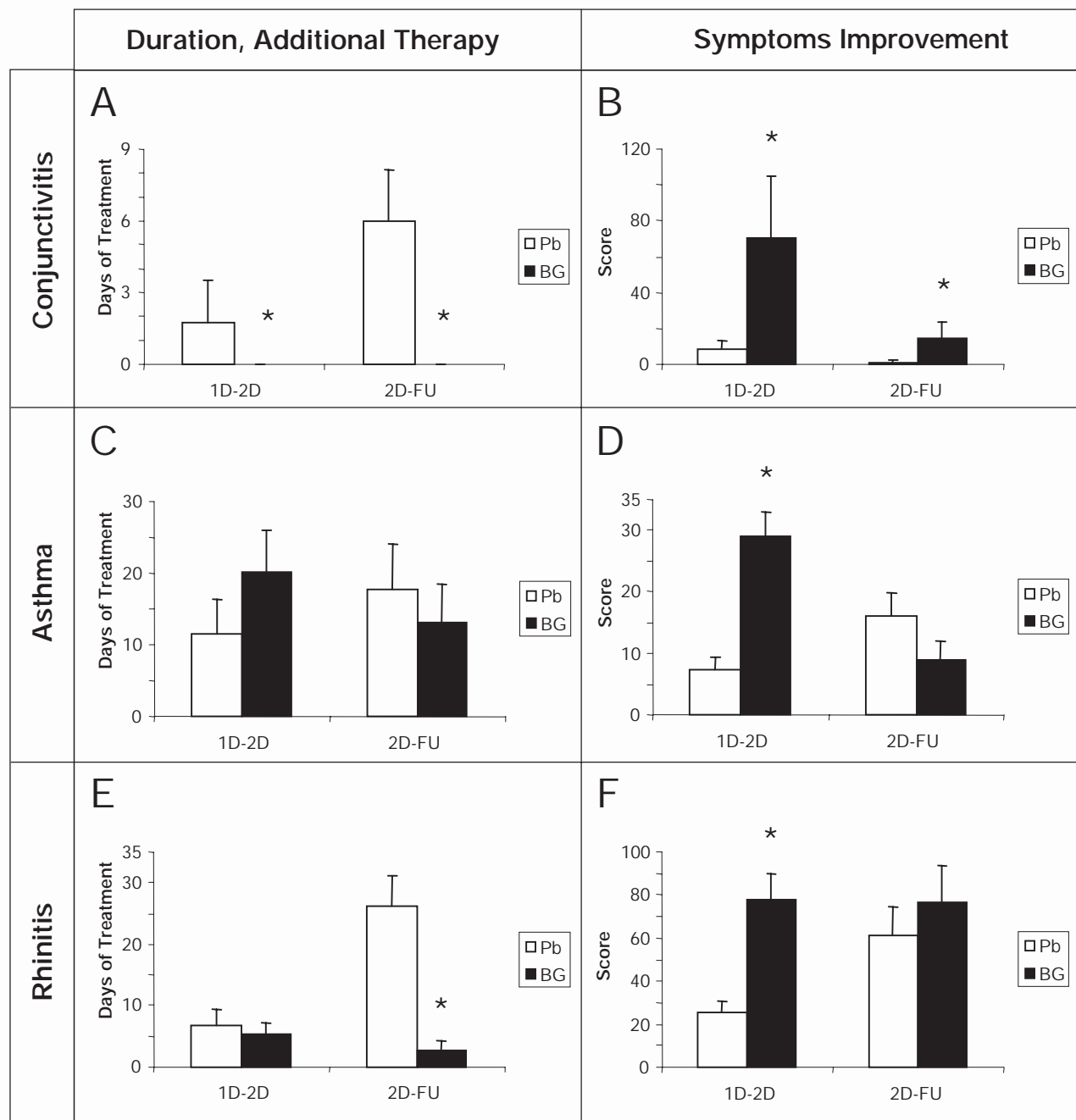
| | BG Group (n=67) | Placebo Group (n=58) |
|-------------------------------|--------------------|-------------------------|
| Age | 10.5 ± 3.9 years | 11.2 ± 2.7 years |
| Sex | 37 M; 30F | 31 M; 27 F |
| Family history | 28/67 (42%) | 21/58 (37%) |
| Asthma | 32/67 (47.2%) | 26/58 (44.8%) |
| Conjunctivitis | 7/67 (10.4%) | 8/58 (13.8%) |
| Rhinitis | 44/67 (65.7%) | 41/58 (70.7%) |
| SPT Dust Mites, mm | 4.5 ± 0.5 | 4.5 ± 0.5 |
| IgE Specific Dust Mites, kU/L | 42.69 ± 41.07 | 40.12 ± 38.92 |
| Total IgE, IU/L | 284.2 ± 276.7 | 312.1 ± 299.6 |
| Other Sensitization: | | |
| – Grass pollen | 41% | 42% |
| – Cat dander | 9% | 10% |
| – Mold | 36% | 32% |

* Data are presented as mean ± SD for specific IgE and total IgE. M indicates male; F, female, SPT, skin prick test.

Results

Patients in both treated and placebo groups completed the study. Demographic features were comparable between the groups and no significant differences were found in age, sex, or sensitization to other allergens (table). BG treatment was well tolerated overall and there were no important side effects.

Results of the comparison of duration of therapy and symptom scores in the BG- and placebo-treated groups are depicted in the figure. We found that in patients with conjunctivitis, the BG treatment was significantly associated with both a shorter period of pharmacological treatment (part A of the figure) and a better symptom score (part B of the figure) ($P=.032$ and $P=.008$, respectively).



Analysis of additional therapy duration and symptoms improvement in children with conjunctivitis, asthma and/or rhinitis and intradermally treated twice with a 100 μg of β -Glucuronidase (BG) preparation or placebo (Pb). Administrations were performed in October and March, and analysis was carried out by comparing the improvements during the periods between the first and second dose injections (1D-2D) and between the second dose injection and the follow-up analysis (2D-FU).

Moreover, such effects were evident from the time of the first injection and stable after the second one. In particular, we observed clinically significant results of pharmacological treatment, since no additional therapy was needed by conjunctivitis-affected BG-treated children ($P=.802$). On the other hand, none of the analyzed parameters improved for children with asthma ($P=.174$) at the end of the therapy (parts C and D of the figure), except for a transient symptom improvement observed after the first BG injection. We observed an intermediate situation in patients with rhinitis. The transient improvement with BG treatment (part F of the figure) vanished after the end of treatment ($P=.156$). On the other hand, pharmacological treatment duration was greatly decreased by the therapy ($P=.036$), although a second administration was required to achieve that effect (part E of the figure).

On the whole, total drug consumption during the entire follow-up period was significantly lower in the treated group than in the placebo group ($P<.01$), and the holistic evaluation of the treatment by parents of BG group children revealed clear improvement in 74.6% of cases. Only 4.55% reported worsening.

Discussion

Allergen avoidance and specific immunotherapy are currently the only means of changing the course of allergic diseases by affecting their natural history or progression. A variety of approaches are being used in the attempt to subvert immune deregulation in allergic disease: immunotherapy with conventional antigens, designed peptides, oligonucleotides, anti-immunoglobulin E specific antibodies, pharmacotherapy with immune modulating drugs, and cytokine and chemokine antagonists [19-22]. All these treatments require repeated administrations to reduce symptoms, sometimes over a long period of time. Conversely, a single or double dose of BG administered before the beginning of pollination might be able to induce a favorable modification of the immune response to subsequent allergen exposure, particularly in terms of interleukin 10 inhibition as we have previously described [16, 17].

Here, we report the results of the first double-blind, randomized placebo-controlled trial in children affected by chronic allergic rhinoconjunctivitis and/or asthma. Our data show a reduction of several symptoms in patients treated with BG. In fact, most of the patients treated with BG needed smaller amounts of drugs to control their symptoms during the 9-month follow-up period. This was particularly evident in conjunctivitis-affected children, although the number of children within this specific group was small. The conjunctivitis patients were the most improved by the treatment, since they needed no additional therapy at all.

The situation observed in rhinitis-affected children is difficult to interpret. Nevertheless, the fact that symptoms improved but a reduction in additional therapy in rhinitis-affected children did not accompany the improvement is

only apparently a contradiction. In fact, during the first period (after the first injection) children were taking additional drugs, and that is probably why the first symptoms report was positive. Therefore, children stopped taking more drugs, but this caused symptoms to exacerbate as observed at the second time-point. Thus, it appears that the transient symptoms improvement observed in rhinitis-affected children was due to additional drug intake.

The good compliance with treatment with a single double-dose administration, the lack of significant side effects and the lower cost in comparison with subcutaneous and sublingual immunotherapy makes the benefit/risk ratio of this therapy particularly favorable.

In conclusion, this study shows that double-dose BG immunotherapy is safe, well tolerated, and efficacious in the treatment of allergic children, especially those affected by conjunctivitis. Future studies should address the long-term effects of this new modality of immunotherapy and compare it with conventional immunotherapy.

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References

1. Bousquet J, Lockey RF, Malling HJ. WHO Position Paper. Allergen Immunotherapy: therapeutic vaccines for allergic diseases. *Allergy*. 1998;53:S44.
2. Karaayvaz M, Erel F, Caliskaner Z, Ozanguc N. Systemic reactions due to allergen immunotherapy. *J Invest Allergol Clin Immunol*. 1999;9:39-44.
3. Bernstein DI, Wanner M, Borish L, Liss GM and ICAAASI. Twelve-year survey of fatal reactions to allergen injections and skin testing: 1990-2001. *J Allergy Clin Immunol*. 2004;113:1129-36.
4. Amin HS, Liss GM, Bernstein DI. Evaluation of near-fatal reactions to allergen immunotherapy injections. *J Allergy Clin Immunol*. 2006;117:169-75.
5. Canonica GW, Passalacqua G. Noninjection routes for immunotherapy. *J Allergy Clin Immunol*. 2003;111:437-48.
6. Casale TB. Status of immunotherapy: current and future. *J Allergy Clin Immunol*. 2004;113:1036-9.
7. Norman PS. Immunotherapy :1999-2004. *J Allergy Clin Immunol*. 2004;113:1013-23.
8. Bousquet J, van Cauwenberge P. (eds). WHO initiative. Allergic rhinitis and its impact on asthma (ARIA) *J Allergy Clin Immunol*. 2001;108 (Supp 5):147-334.
9. Gidaro G, Marcucci F, Sensi L, Incorvaia C, Frati F, Ciprandi G. The safety of sublingual-swallow immunotherapy: an analysis of published studies. *Clin Exp Allergy*. 2005;35:565-71.
10. Wilson DR, Torres-Lima M, Durham S. Sublingual immunotherapy for allergic rhinitis: systematic review and meta-analysis. *Allergy*. 2005;60:4-12.
11. McEwen LM, Ganderton MA, Wilson CWM. Hyaluronidase in the treatment of allergy. *Br Med J*. 1967;11:509-30.
12. Cantani A, Ragno V, Monteleone A, Businco L. Enzyme-

- potentiated desensitization in children with asthma and mite allergy: a double-blind study. *J Invest Allergol Clin Immunol*. 1996; 6(4):270-6.
13. Di Stanislao C, Di Berardino L, Bianchi I. A double-blind placebo-controlled study of preventive immunotherapy with EPD in the treatment of seasonal allergic disease. *Allergy et Immunologie*. 1997;2:39-42.
 14. Egger J, Stolla A, McEwen LM. Controlled trial of hyposensitization in children with food-induced hyperkinetic syndrome. *Lancet*. 1992;2339:1150-3.
 15. Radcliffe MJ, Lewith GT, Turner RG, Prescott P, Church MK, Holgate ST. Enzyme potentiated desensitisation in treatment of seasonal allergic rhinitis: double blind randomised controlled study. *BMJ*. 2003;327:1-7.
 16. Di Stanislao C, Angelini F, Gagliardi MC, Di Bernardino L, Galli E, Rossi P. Beta glucuronidase short-term immunotherapy. *Allergy*. 2003;58:459.
 17. Di Stanislao C, Angelini F, Gagliardi MC, Di Berardino L, Galli E, Rossi P. Beta glucuronidase short-term immunotherapy to prevent seasonal rhinoconjunctivitis in grass pollen allergic patients. Possible effect on differentiation and maturation of dendritic cells. In: Marone G editor. *Clinical Immunology and Allergy in Medicine*. Naples, Italy: JGC Editions; 2003. p. 741-9.
 18. Di Berardino L, Agrisano A, Comments on BU. *Allergy*. 1997;52:775.
 19. Lewis DB. Allergy immunotherapy and inhibition of Th2 immune responses: a sufficient strategy? *Curr Opin Immunol*. 2002;14:644-51.
 20. Smitt JJ, Van Loveren H, Hoekstra MO, Schijf MA, Folkerts G and Nijcamp FP. Mycobacterium vaccae administration during allergen sensitization or challenge suppresses asthma features. *Clin Exp Allergy*. 2003;33:1083-9.
 21. Ali FR, Larche M. Peptide-based immunotherapy: a novel strategy for allergic disease. *Expert Rev Vaccines*. 2005;4:881-9.
 22. Tsalik EL. DNA-based immunotherapy to treat atopic disease. *Ann Allergy Asthma Immunol*. 2005;95:403-10.

Elena Galli

Research Center San Pietro
Fatebenefratelli Hospital
Via Cassia 600
00189, Rome, Italy
E-mail: galli.elena@fbfrm.it