



# Effect of inhaled inactivated *Mycobacterium phlei* in children with moderate asthma

Bacillus Calmette–Guérin and other mycobacterial vaccines are important therapeutic methods in a series of chronic inflammatory disorders characterized by Th1/Th2 imbalance in which Th2 type cells and cytokines often increase. However, few studies have investigated whether it can reduce or prevent the symptoms and attacks in children with asthma. This study evaluated the effect of inactivated *Mycobacterium phlei* inhaled by an atomizing device placed on asthmatic children. In this randomized, single-center, Seretide-controlled study, children aged 4–12 years with newly diagnosed, moderate, persistent asthma were treated with either inhaled inactivated *M. phlei* or inhaled Seretide patch. The efficacy of inhaled inactivated *M. phlei* was related with the alleviation of asthma symptoms, improvement of lung function and reduction of bronchial hyper-responsiveness and total serum IgE, which was similar with Seretide. These findings may have important clinical value in confirming inhaled inactivated *M. phlei* as a new therapeutic method in moderately asthmatic children.

**KEYWORDS:** bronchial asthma ■ C-ACT ■ children ■ FEV1 ■ FEV1-PD2 ■ IgE ■ immunotherapy ■ inactivated *Mycobacterium phlei* ■ inhalation ■ PEF

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Asthma and other allergic diseases represent the largest group of chronic pediatric diseases in many western countries. The long-term uncontrolled asthma symptoms are often associated with deterioration of lung function [1]. The burden of childhood asthma varies worldwide, but is substantial everywhere [2]. Nevertheless, a considerable proportion of patients with asthma who use inhaled steroids and long-acting  $\beta$ -agonist regularly have poorly controlled symptoms and are at increased risk of exacerbations [3]. Asthma causes children to miss school. There is a need for additional or new therapies [4]. Shirakawa and his colleagues observed a phenomenon where there was a negative correlation between tuberculin reaction and atopic diseases among Japanese children [5]. To date, several studies have reported the immunomodulatory functions of vaccines administered via the mucosa in animal models. For example, Levine *et al.* demonstrated not only neutralizing serum antibodies and cell-mediated immune responses, but also that mucosal secretory IgA antibodies can cause irritation [6]. Thus, it is reasonable to believe that the vaccine administered via mucosal surfaces may trigger a dramatic cell-mediated immune response, thereby effectively preventing not only tuberculosis, but also atopic diseases in humans [6]. Zhang *et al.* have shown that inactivated *Mycobacterium phlei* in nebulized therapy is effective in adult patients with moderate persistent asthma [7]; however, its efficacy in progressive or persistent asthma in children has

not been evaluated. In this article, we describe the strategy used and the outcomes achieved in treating children with moderate persistent asthma.

## Methods

### ■ Subjects & study design

Details of the design and methods of the study were explained to all parents/guardians of patients, were allowed by Guangxi Medical Ethics Committee and have been registered in the WHO clinical trial registry (ChiCTR-TRC-10000765). Children aged 4–12 years of either sex who were diagnosed with moderate persistent asthma in our outpatient department between January 2010 and May 2012 were recruited in the study. The judgment of asthma was determined on the basis of history and pulmonary function tests (PFTs) according to the Global Initiative for Asthma 2009 guidelines [101]. Eligibility for potential enrollment was as follows:

- Newly diagnosed, uncontrolled, moderate persistent bronchial asthma
- Bodyweight <90 kg

Patients were excluded from consideration in the study if they:

- Had previously received any other medications;
- Were already allergic to any study drug at the start of the study;

- Had acute respiratory tract infection or active lung disease other than allergic asthma within 1 month, or any other significant concomitant disease within 3 months, for example, diabetes mellitus, liver disease or tuberculosis;
- Were unable to follow therapy instructions.

### ■ Treatments

There were four scheduled visits to the clinic: at the start of treatment (day 0) and at 6, 30, and 90 days of treatment (day 6, 30 and 90). The patients were randomly allocated into two groups:

- Inactivated *M. phlei* treatment group;
- Salmeterol xinafoate and fluticasone treatment control group;

Since a placebo control may not be feasible and ethically acceptable, a placebo group was not included in the study. The patients in the inactivated *M. phlei* treatment group were administered two ampoules solution of inactivated *M. phlei* (Jinxing Health Pharmaceutical Co. Ltd, Chengdu, China) dissolved in 3 ml of saline, by inhalation, once daily, on 5 consecutive days. The program was repeated once again on visit three, according to a modification of the methods provided by Zhang *et al.* [7]. The patients in the salmeterol xinafoate and fluticasone treatment control groups inhaled salmeterol xinafoate and fluticasone through propionate powder (Seretide; 50/100 µg; GlaxoSmithKline, PA, USA), twice, on a daily basis. During the study period, the use of  $\beta_2$ -adrenergic agonists was to be permitted. The guardian could administer the salbutamol sulphate to the child by inhalation as a means of relief.

### ■ Measurements

Asthma symptom outcomes were assessed and the pulmonary function and bronchial challenge testing with methacholine was carried out during every visit, which was measured using a validated, electronic spirometer (MasterScope, Jaeger, Würzburg, Germany), permitting the collection of pre- and post-bronchodilator forced expiratory volume in 1 s, forced expiratory volume in 1 s:forced vital capacity ratio and peak expiratory flow, analyzed as quantitative traits. At the same time, asthma control situation was assessed with the childhood-asthma control test (C-ACT), which ranges from 0 to 27 points and estimates the severity of asthma symptoms. Scores of 20

or higher indicate satisfactory control [8]. Total serum IgE was assessed on day 0 and day 90.

Blood samples were collected from the two groups. Total serum IgE was determined using a commercial IgE ELISA kit (Mabtech Inc., Stockholm, Sweden) according to the manufacturer's instructions. IgE levels were expressed as ng/ml. Each sample was assayed three times. The average IgE concentration was used for statistical analysis.

### ■ Safety assessments

Safety and tolerability were assessed in terms of frequency, duration and severity of adverse events and the registered relationship to the test drug; causing death, carcinogenic, teratogenic and permanent damage to the organ; life-threatening resulting in hospitalization; special treatment with adverse reactions; clinically significant changes in laboratory measure and vital signs; and local reactions at the inhalation site.

### ■ Statistical analysis

We used SPSS 17.0 statistics software (SPSS Inc., IL, USA). Dichotomous variables were summarized as a number (percentage) and analyzed with a  $\chi^2$ -test, whereas continuous values were expressed as means and standard deviations, and were performed by means of Student two-sample t-tests. Two-sided tests were performed for all statistical tests and the significance level was defined as  $\alpha = 0.05$ .

## Results

### ■ Patients

The 22 children recruited into the single-center, randomized, controlled study were randomized to receive inactivated *M. phlei* (Group A;  $n = 12$ ) or salmeterol xinafoate and fluticasone (Group B;  $n = 10$ ). Baseline demographics and clinical characteristics of the two study populations that were well matched within each part are summarized in TABLE 1.

### ■ Asthma control status

All the patients in the two groups were characterized by markedly increasing C-ACT scores on days 30 and 90 ( $p < 0.05$ ), but no significant difference was noted between Group A and B ( $p > 0.05$ ). On day 30, the rates of satisfactorily controlled asthma (C-ACT score  $\geq 20$ ) in Group A and B were 75.0 and 60%, respectively, and the median C-ACT score in Group A was lower than the other group, although it did not achieve statistical significance. On day 90, the proportion of children with a

**Table 1. Summary of demographic and baseline characteristics by treatment (all randomized).**

| Variable                                                       | Group A (n = 12) | Group B (n = 10) |
|----------------------------------------------------------------|------------------|------------------|
| Age (years) <sup>†</sup>                                       | 7.23 ± 2.17      | 6.89 ± 2.56      |
| Sex (male/female)                                              | 7/5              | 5/5              |
| BMI                                                            | 19.21 ± 1.85     | 18.72 ± 2.05     |
| Doctor-diagnosed atopic dermatitis, n (%)                      | 7 (58.3)         | 5 (50.0)         |
| Doctor-diagnosed allergic rhinitis, n (%)                      | 8 (66.7)         | 7 (70.0)         |
| Parental smoking, n (%)                                        | 4 (33.3)         | 4 (40)           |
| Immediate family history of asthma, n (%)                      | 3 (25.0)         | 2 (20.0)         |
| Allergy skin prick test (dust mites, cockroaches, pets), n (%) | 8 (66.7)         | 6 (60.0)         |

Group A: inactivated *Mycobacterium phlei* treatment group; Group B: salmeterol xinafoate and fluticasone treatment control group.  
<sup>†</sup>Data presented as mean ± standard deviation.

C-ACT score of  $\geq 20$  in Group A rose to 83.3%, which was lower than 90% in Group B with no statistical significance and the median C-ACT score in Group B was not significantly different from Group A (FIGURE 1).

#### ■ PFT & bronchial challenge testing with methacholine

Higher negative conversion rate in the two groups was achieved after treatment (FIGURE 2). On day 6, 30 and 90, the negative conversion rates of the bronchial challenge testing with methacholine in Group A were not statistically lower than that of Group B (33.3 vs 50.0% on day 6, 75.0 vs 60.0% on day 30 and 83.3 vs 90% on day 90;  $p = 0.666$  by  $\chi^2$  test). After treatment, PFTs were improved compared with pretreatment (TABLE 2).

#### ■ Total serum IgE

Lower total serum IgE levels were achieved after treatment in the two groups. On day 0 and 90, total serum IgE levels in Group A were  $352.4 \pm 107.2$  and  $106.3 \pm 57.8$  ng/ml, respectively ( $p < 0.01$ ). In Group B, they were  $367.5 \pm 97.6$  and  $98.8 \pm 67.2$  ng/ml, respectively ( $p < 0.01$ ). There was no significant difference between the two groups ( $p = 0.725$ ). This demonstrated that inhalation of inactivated *M. phlei* had a similar effect on decreasing total serum IgE.

#### ■ Safety & tolerability

Although side effects were mentioned by two patients, none were classified as severe. The main side effect was coughing, which occurred during the first contact with inhaled inactivated *M. phlei* and was minor, and never a reason to stop study medication. The occurrences of sinusitis, upper respiratory tract infection and

bronchitis were zero in the two groups and there were no significant changes in height, weight and laboratory measures in the two groups during the study.

### Discussion

In the past three decades the incidence of asthma and allergic disease has increased dramatically

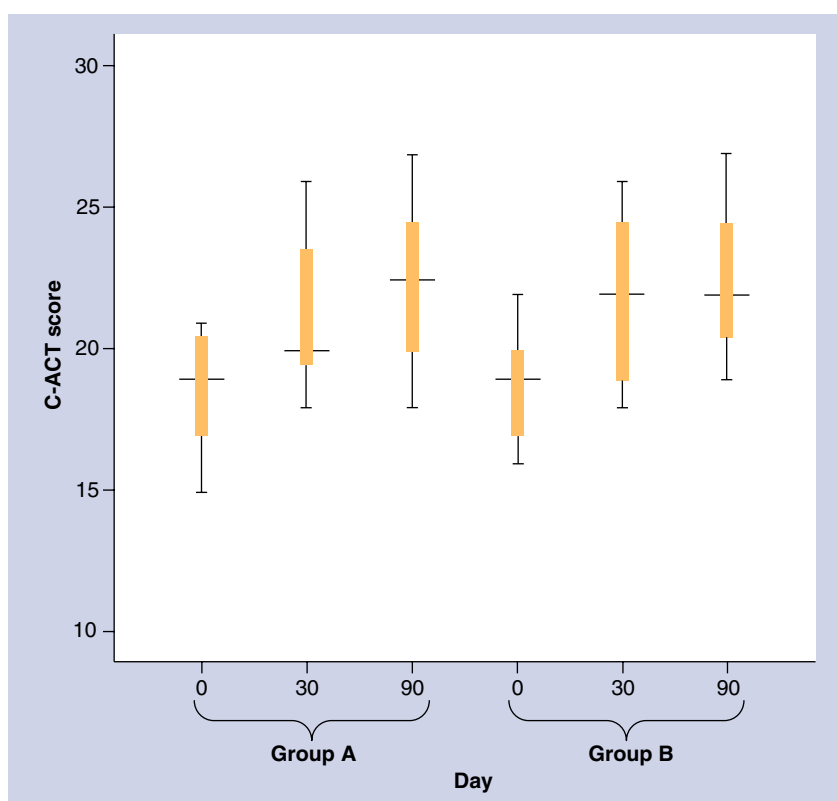


**Figure 1. Childhood-asthma control test scores of the two groups at different times.** Group A: inactivated *Mycobacterium phlei* treatment group; Group B: salmeterol xinafoate and fluticasone treatment control group. C-ACT: Childhood-asthma control test.

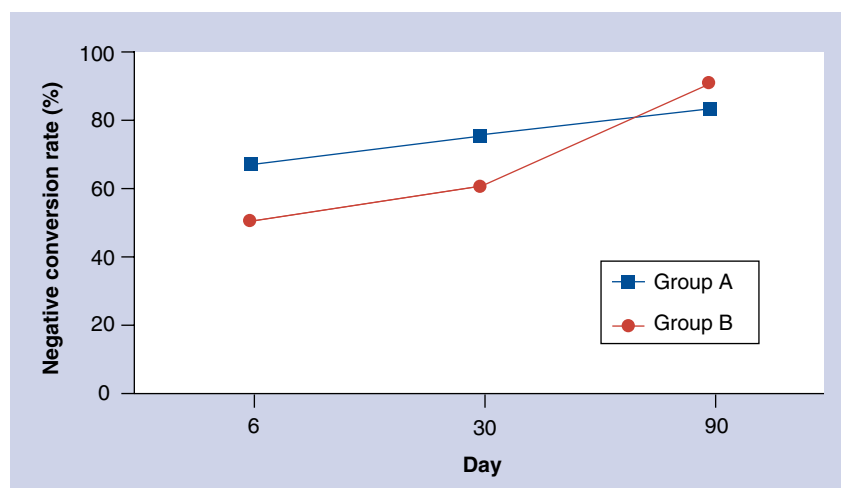


**Figure 2. Negative conversion rate (%) of bronchial provocation test after treatment in the two groups.** Group A: inactivated *Mycobacterium phlei* treatment group; Group B: salmeterol xinafoate and fluticasone treatment control group.

worldwide, especially in countries characterized by a western lifestyle [9]. Asthma, a common pediatric disorder, afflicts approximately 9% of the general population of children in the USA (and a much higher percentage among inner-city African-American and Hispanic children) [10]. Most cases of asthma first appear in childhood, with 80–90% of patients diagnosed by 6 years of age [11]. Gene–environment interactions in the pathogenesis of asthma occupy a very important position [12]. Children suffering from atopic dermatitis and allergic rhinitis are more likely to develop bronchial asthma [13–15]. In our study, 54.5% of the total patients were diagnosed with atopic dermatitis and 68.2% of the total number suffered from allergic rhinitis. In contrast to our study, Al-Biltagi *et al.* reported a lower proportion of atopic dermatitis and allergic rhinitis [16]. This discrepancy may be due to differences in race, genetics and environment.

We examined the hypothesis that the efficacy of inactivated *M. phlei* inhalation was similar to salmeterol xinafoate and fluticasone in children whose asthma was newly diagnosed. This study shows that inhaled inactivated *M. phlei* can increase C-ACT score significantly on day 30 and 90. After a 5-day therapy, we have already observed improvement in pulmonary function and the negative conversion of the bronchial challenge testing with methacholine in 33.3% of patients. Until day 90, the total negative conversion rates increased to 83.3%. Bronchial provocation test conversion was higher in the study patients who were in Group A as compared with Group B on day 6, yet the improvement of pulmonary function was not noted as significantly different between the two groups

of patients. Inhaled inactivated *M. phlei* was also associated with rapid improvements in negative conversion rate of bronchial provocation test. It is noteworthy that inhaled inactivated *M. phlei* was well tolerated throughout the study. There was no evidence of serious drug toxicity and nobody withdrew owing to treatment-related adverse events.

Recently, Zhang *et al.* investigated the therapeutic effect of inhaled inactivated *M. phlei* on remission rate in adults [7], using a study protocol similar to ours with the exception of the study population. Our results differ in some respects [7]. We found that there were no marked differences in spirometry between the two groups; this might be explained by the difference in study population.

Several principles of management were suggested for asthmatic patients, which included inhaled corticosteroids (ICS) and short- and long-acting  $\beta$ -agonists. Both ICS and long-acting  $\beta$ -agonists – inhaled controller medications – being taken on a long-term daily basis were used for maximized clinical therapeutic benefit. Animal models have confirmed that exposure to *Mycobacterium tuberculosis* can restore the immune balance; therefore further granting *Mycobacterium* an important position in the hygiene hypothesis [17–19]. No previous data have proven the effect of inhaled inactivated *M. phlei* for asthma treatment in children. Inactivated *M. phlei* was administered via inhalation to mouse models of asthma by Zhang *et al.* [20] It showed that IL-10 and IFN- $\gamma$  in bronchoalveolar lavage fluid were significantly increased and airway inflammation was attenuated. This ability may be correlated with its immunomodulatory effect on regulating IL-4, IL-10 and IFN- $\gamma$ , as well as secretion and upregulation of TLR2 [21]. This was the first study to assess the benefit of novel inhaled inactivated *M. phlei* therapy in children with persistent asthma. In the present study, PFT and C-ACT had statistically improved and total serum IgE had obviously decreased in Group A patients, which was similar to Group B patients.

We investigated the effect of inhaled inactivated *M. phlei* on decreasing serum IgE levels in asthma and found that the serum total IgE level on day 90 was  $106.3 \pm 57.8$  ng/ml, which was lower than  $352.0 \pm 107.2$  ng/ml on baseline, which was a significant difference. Using unique strains of probiotics can decrease the level of IgE [22], IgG1 [23] and reduce the ratio of IgE and IgG2 [24]. It is suggested that the mechanisms behind inhaled inactivated *M. phlei* may include the production of decreasing

serum IgE levels. In the present study, we did not evaluate the allergen-specific IgE. As far as we know, inactivated *M. phlei* is not just for a particular allergen, which is dissimilar to allergen immunotherapy. Allergen immunotherapy can also reduce IgE levels, but its efficacy is limited [25]. Moreover, the main shortcomings of immunotherapy include efficacy, timing of treatment and patient selection, all of which need to be taken into serious consideration [26]. In our study, children were enrolled as long as they were not allergic to the study drug or specially selected. Inactivated *M. phlei* could be used in a wider range of people.

ICS do not seem to alter the course of the disease, let alone cure asthma. Once asthma patients stop ICS, asthma symptoms and inflammation usually quickly reappear [27]. In our study the salmeterol xinafoate and fluticasone was delivered for 90 consecutive days, which was protracted as compared with 10 days in group A (daily for 5 consecutive days at the beginning of the study and once again after 1 month). Th2 immune responses play a key role in the pathogenesis of asthma [28]. Immunotherapy may be the only management that can change the natural history and delay the occurrence of asthma [29]. Mucosal immunotherapy is a favorable treatment method due to its noninjection routes of management and less adverse reactions [27]. Many methods for mucosal immunotherapy have been applied involving straight swallow, nasal or trachea, and sublingual. Inhalation management can reach the respiratory tract and act on the mucosal immune system directly. Due to the ease of operation and convenience, this method of administration has a higher compliance with children.

There is a subset of the asthmatic population who continue to have persistent airway inflammation despite treatment with conventional therapy with inhaled or oral corticosteroids; they are referred to as having 'steroid-resistant asthma'. Perhaps inhaled inactivated *M. phlei* therapy is a new and convenient option for those asthmatic children whose symptoms are not adequately controlled on corticosteroid therapy, require unacceptably high doses of ICS or systemic corticosteroids, or additional treatment, or suffer unacceptable side effects of corticosteroid therapy.

We have not applied the drug in asthmatic children under the age of 4 years. Further research is still necessary to identify beneficial yet safe alternative treatments for this subgroup of children. Furthermore, more targeted therapeutic

Table 2. Spirometry in the two groups compared before and after treatment.

| Tests            | Group A <sup>†</sup> |               |               |                |               | Group B <sup>‡</sup> |                |                |  |  |
|------------------|----------------------|---------------|---------------|----------------|---------------|----------------------|----------------|----------------|--|--|
|                  | Day 0                | Day 6         | Day 30        | Day 90         | Day 0         | Day 6                | Day 30         | Day 90         |  |  |
| FEV1 (l)         | 0.82 ± 0.13          | 0.84 ± 0.12   | 0.86 ± 0.12   | 0.97 ± 0.16    | 0.79 ± 0.14   | 0.91 ± 0.15          | 0.93 ± 0.11    | 1.01 ± 0.17*   |  |  |
| FEV1 (% of pred) | 72.32 ± 12.15        | 75.52 ± 11.10 | 88.53 ± 14.83 | 92.66 ± 16.65  | 72.85 ± 13.60 | 84.42 ± 10.53        | 90.05 ± 12.28* | 94.87 ± 14.66* |  |  |
| PEF (l/s)        | 2.14 ± 0.76          | 3.04 ± 0.65*  | 3.35 ± 0.58*  | 4.26 ± 1.08*   | 2.22 ± 0.63   | 3.82 ± 0.45*         | 3.54 ± 0.86*   | 4.43 ± 0.92*   |  |  |
| PEF (% of pred)  | 64.62 ± 12.45        | 71.82 ± 9.05  | 73.15 ± 15.13 | 90.30 ± 18.22* | 66.37 ± 23.13 | 88.25 ± 10.86*       | 86.65 ± 13.12* | 96.32 ± 21.85* |  |  |
| FEV1-PD20 (mg)   | 0.86 ± 0.32          | 2.32 ± 0.25*  | 2.35 ± 0.32*  | 2.25 ± 0.75*   | 0.76 ± 0.28   | 2.06 ± 0.45*         | 2.28 ± 0.48*   | 2.46 ± 0.23*   |  |  |

Data presented as mean ± standard deviation.

\* $p < 0.05$  as compared with pretreatment in each group.

<sup>†</sup>Inactivated *Mycobacterium phlei* treatment group.

<sup>‡</sup>Salmeterol xinafoate and fluticasone treatment control group.

FEV1: Forced expiratory volume in 1 s; PD20: Provocative dose producing a 20% fall in FEV1; PEF: Peak expiratory flow; Pred: Predicted.

approaches in terms of the appropriate patient population should be explored.

### Future perspective

An important feature of asthma is an abnormal Th2 reaction, with strong response to various stimuli that are usually harmless to nonatopic individuals. Inhalation of inactivated *M. phlei* is safe, able to induce Th1 response and stimulate immunological memory in the host effectively. We hope that inhalation of inactivated *M. phlei* could prove effective in severe asthma, especially steroid-resistant asthma in further research. Perhaps, in the next decade, it can occupy the same position as ICS. However, further research is needed to determine whether the

therapy is consistent in its effectiveness. We hope that constantly updated knowledge of the immunotherapeutic scheme for asthmatic children will provide safer and more effective and reasonable dose utility in the medical field.

### Financial & competing interests disclosure

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### Executive summary

- Although inhaled inactivated *Mycobacterium phlei* treatment was administrated only twice, which was less than the daily inhalation of salmeterol xinafoate and fluticasone, it was associated with improvement in Asthma Control Test score and an increase in peak expiratory flow, percentage of forced expiratory volume in 1 s (FEV1), and FEV1 with a provocative dose, producing a 20% fall in FEV1 in patients with moderate persistent asthma.
- Immunotherapy with *M. phlei* is well tolerated, with efficacy profiles similar to salmeterol xinafoate and fluticasone.
- Taken together, our results suggest that inactivated *M. phlei* therapy delivered through inhalation presents new and convenient options for the treatment of persistent asthma in children.

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