Efficacy and Safety of a New Immunostimulating Bacterial Lysate in the Prophylaxis of Acute Lower Respiratory Tract Infections

A randomised, open, controlled clinical trial

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Summary

The aim of the present trial was the assessment of the efficacy and safety of a treatment with Ismigen®, a new immunostimulating lysate prepared by bacteria mechanical lysis (MLBL), in the prophylaxis of lower respiratory tract infections (LRTIs) in adults, in comparison to a treatment with a lysate obtained by bacteria chemical lysis (CLBL) and to a "no immunostimulant treatment" (NT). Sixty-nine patients, 36 females (52 %) and 33 males (48 %), age range 18–82 years (mean ± SD: 63.8 ± 3.1), with recurrent respiratory tract infections were enrolled and allocated at random to the three groups. The patients of the first two groups (MLBL and CLBL) were treated, in open conditions, with the preparation assigned (one tablet and one capsule daily, respectively), for ten days a month for three consecutive months, thereafter the patients of the three groups underwent to a three month follow up. LRTIs during the treatment period: in the NT group 22 (96 %) patients had, at least, one acute episode (41 in total); in the MLBL group 5 patients (22 %) had atotal of 7 episodes and in the CLBL group 16 (70 %) had a total of 16 episodes. The difference among the three groups was statistically significant and in favour of MLBL. No LRTIs in this last group were observed in the first month of treatment. The mean number of acute episodes was significantly lower ($p < 0.05$) in the MLBL group than in the CLBL and in the NT groups (0.3 ± 0.63 vs 0.9 ± 0.7 vs 1.7 ± 0.9). During the three months of treatment a significantly lower number of patients in the MLBL group needed an antibiotic therapy and complained of dyspnoea in comparison to the other two groups.

No adverse events were reported by the patients of the three groups.

Key words

- Bacterial lysate
- Immunostimulant
- Ismigen®, efficacy, safety,
- Lower respiratory tract infections

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Zusammenfassung

Wirksamkeit und Verträglichkeit eines neuen immunstimulierenden bakteriellen Lysates in der Prophylaxe von Infektionen der unteren Atemwege / Eine randomisierte, offene, kontrollierte klinische Studie

Das Ziel der vorliegenden Untersuchung war die Bewertung der Wirksamkeit und Verträglichkeit einer Behandlung mit Ismigen®, einem neuen, durch bakteriell-mechanische Lyse (MLBL) erzeugten immunstimulierenden Lysat, in der Prophylaxe von Entzündungen der unteren Atemwege (LRTI) Erwachsener im Vergleich zu einer Behandlung mit einem durch bakteriell-chemische Lyse (CLBL) erzeugten Lysat und einer "nicht-immunstimulierenden Behandlung" (NT). Neunundsechzig Patienten, darunter 36 Frauen (52 %) und 33 Männer (48 %) im Alter von 18–82 Jahren (Mittelwert ± SD: 63,8 ± 3,1) mit rezidivierenden Infektionen der unteren Atemwege wurden in die Studie aufgenommen und den drei Gruppen randomisiert zugeteilt. Die Patienten der beiden ersten Gruppen (MLBL und CLBL) wurden unter offenen Bedingungen mit dem zugewiesenen Präparat (eine Tablette bzw. eine Kapsel täglich) zehn Tage pro Monat während dreier aufeinanderfolgender Monate behandelt, danach wurden die Patienten der drei Gruppen einem dreimonatigen Follow-up unterzogen. LRTI während der Behandlungsperiode: in der NT-Gruppe hatten 22 (96 %) Patienten mindestens eine akute Episode (insgesamt 41); in der MLBL-Gruppe hatten 5 Patienten (22 %) insgesamt 7 Episoden, und in der CLBL-Gruppe hatten 16 (70 %) Patienten insgesamt 16 Episoden. Der Unterschied zwischen den drei Gruppen war statistisch signifikant und günstig für MLBL. Im ersten Behandlungsmonat wurden in der letzten Gruppe keine LRTI beobachtet. Die durchschnittliche Zahl akuter Episoden war in der MLBL-Gruppe signifikant niedriger ($p < 0,05$) als in der CLBL- und in der NT-Gruppe (0,3 ± 0,63 vs 0,9 ± 0,7 vs 1,7 ± 0,9). Während der drei Behandlungsmonate benötigte eine signifikant geringere Zahl von Patienten in der MLBL-Gruppe eine Antibiotikatherapie bzw. klagte über Dyspnoe als in den zwei anderen Gruppen.

Die Patienten der drei Gruppen berichteten keine unerwünschten Nebenwirkungen.

1. Introduction

Lower respiratory tract infections (LRTIs) seem to play an important role in increasing the risk of chronic obstructive pulmonary diseases (COPDs) and in the deterioration of the patient's Quality of Life (QoL). LRTIs, moreover, have a heavy impact on National Health Service costs, being an important cause of patients' hospitalisation and pharmaceutical spending.

Early childhood and old age are more susceptible to lower respiratory tract infections than other ages. High exposure to infectious agents due to precocious socialization and a slow down shift of TH1 – TH2 lymphocytes are the main causes of LRTIs in early childhood [2, 4]. In the elderly, progressive mucus clearance reduction, immunoglobulin A (IgA) deficit and prolonged exposure to risk factors increase the susceptibility to LRTIs, especially in COPD patients [5]. It is generally accepted that LRTIs are a major cause of morbidity and mortality in COPD patients, particularly in elderly [6]. In Italy about six millions/year of COPD patients have one or more acute exacerbations of chronic bronchitis (AECB); in early childhood LRTIs are responsible of 28 % of deaths world-wide [7].

Therefore to slow down or better to stop this trend by prophylaxis of LRTIs is an important goal in clinical practice and in health costs control.

The use of immunostimulanting agents to restore the immunological protection seems to be an interesting approach in these patients and positive results were obtained with an oral immunostimulanting agent composed of 36 billions of bacteria, in acute exacerbations of chronic obstructive pulmonary disease [11]; this agent modulates cytokines synthesis and mast-cells degranulation, it enhances the expression of adhesion molecules on normal human blood monocytes and granulocytes.

A new sublingual immunostimulating agent, Ismigen®[1], a vaccine composed of a lysate of 48 billions of bacteria belonging to eight species and obtained by bacteria mechanical lysis (MLBL) has been recently introduced into the market.

The aim of the present trial is to assess the efficacy and safety of a treatment with this new immunostimulating vaccine (MLBL), in the prophylaxis of respiratory tract infections, in comparison to: a treatment with a lysate of 36 billions of bacteria obtained by bacteria chemical lysis (CLBL) and to a "no immunostimulating treatment" (NT).

2. Patients and methods

2.1. Study design

This open, randomized, controlled, parallel group clinical study was carried out from October 2001 to July 2002 in the Pneumology Operative Unit of the Azienda Sanitaria Locale N° 4 of Terni (Italy).

[1] Manufacturer: Zambon Italia SRL, Bresso/Milan (Italy).

2.2. Study population

The following inclusion/exclusion criteria led the enrollment of the patients:

Inclusion criteria:

Patients of either gender older than 18 years with recurrent respiratory tract infections (3 or more acute episodes in the last 12 months).

Exclusion criteria:

- Patients' age ≤ 18 years
- Patients with less than 3 acute LRTIs in the last 12 months
- Patients with bronchiectasis
- Patients with cancer
- Patients with autoimmune diseases
- Patients with tuberculosis in active phase
- Patients with immunologic deficiency
- Patients treated with antibiotics during the 3 days preceding this study
- Patients with psychiatric disorders
- Patients with primary interstitial pneumopathy
- Patients enrolled in clinical trials in the previous 3 months.

Sixty-nine eligible patients, 36 females (52 %) and 33 males (48 %), aged 18–82 years (mean ± SD: 63.8 ± 3.1) were enrolled and allocated at random by a SAS program (SAS® Institute Inc., Cary, USA) to the three experimental groups (MLBL, CLBL, NT) which, at baseline visit, resulted to be well matched for age, sex, smoking habits, with no statistically significant differences among them (Table 1). The 3 groups were homogeneous also for the number of infectious respiratory episodes suffered in the 12 months preceding the study, their duration and the number of days of antibiotic administration (Table 2).

Concomitant diseases are reported in Table 3, COPD and asthma were the most frequent affections in the enrolled populations.

Only 8 patients out of 69 (11.5 %) had been treated with anti-influenza vaccine.

2.3. Immunostimulating agents tested in this study

MLBL is an immunostimulating vaccine obtained by mechanical lysis through sonication of 48 billions of bacteria belonging to the following species: *S. aureus, S. pyogenes, S. viridans, S. pneumoniae, K. pneumoniae, K. ozaenae, H. influenzae, N. catharralis.* The product is formulated in tablets containing 50 mg of the freeze-dried lysate of which 7 mg correspond to 48 billions of bacteria and 43 to excipients.

MLBL stimulates the natural defences of the body. It has shown a protecting activity against experimental infections by inducing a specific antibody response.

MLBL immunostimulating properties seem ascribable to:

– The restoration of the properties of T lymphocytes cell membrane in case of selective IgA deficiency.

– A definite enhancement of non specific response to polyclonal mitogens in both healthy subjects and patients.

– A mild increase of circulating immunocomplexes.

The specific response in subjects treated with MLBL has been shown by the in vitro stimulation of their lymphocytes, in presence of the bacterial lysate which has an adjuvant effect on the macrophages – monocytes system.

CLBL is an immunostimulating compound obtained by chemical lysis of 36 billions of bacteria belonging to the following species: *H. influenzae, D. pneumoniae, K. pneumoniae, S. pyogenes, K. ozaenae, S. aureus, S. viridans, N. catharralis.*

Table 1: Patient characteristics.

	MLBL	CLBL	NT
No. of patients	23	23	23
Sex: Female	11 (47.8 %)	16 (69.6 %)	9 (39.1 %)
Male	12 (52.2 %)	7 (30.4 %)	14 (60.9 %)
Age (years) Mean±SD	62.6 ± 13.06	61.5 ± 16.88	67.4 ± 7.59
Smokers	7 (30.4 %)	5 (21.7 %)	4 (17.4 %)
Non smokers	12 (52.2 %)	11 (47.8 %)	9 (39.2 %)
Ex smokers	3 (13.0 %)	7 (30.4 %)	10 (43.4 %)
Missing data	1 (4.4 %)	–	

Comparison among the three groups: $p > 0.05$.

Table 2: LTRIs over 12 months preceding the study.

	MLBL (n = 23)	CLBL (n = 23)	N.T. (n = 23)
No. of infective episodes	80	83	85
Mean ± SD	3.5 ± 0.59	3.6 ± 0.58	3.7 ±0.70
Min : max	3 : 5	3 : 5	3: 5
Duration of infective episodes (days)			
Mean ± SD	9.4 ± 3.24	10.0 ± 4.20	10.5 ± 4.83
Min : max	4 : 21	5 : 30	5 : 40
Days of antibiotic treatment			
Mean ± SD	7.6 ± 2.85	7.0 ± 2.11	7.6 ± 2.65
Min : max	3 : 17	3 : 15	3 : 20
No. of treatments	72	82	79
Admission to hospital for infective episodes	1	–	2

$p > 0.05$ for all variables by ANOVA test.

Table 3: Concomitant diseases.

	MLBL (n = 23)	CLBL (n = 23)	NT (n = 23)	Total
Ischemic heart disease	11	16	18	45 (65.8 %)
Renal failure	1	1	1	3 (4.3 %)
Prostate diseases	1	–	–	1 (1.4 %)
Hepatic cirrhosis	–	–	1	1 (1.4 %)
Gastroenteric disease	5	2	6	13 (18.8 %)
Depression	–	4	1	5 (7.2 %)
Transient ischemic attack	–	–	1	1 (1.4 %)
Diabetes	1	–	2	3 (4.3 %)
Thyroid diseases	4	–	1	5 (7.2 %)
COPD	7	8	8	23 (33.3 %)
Asthma	6	9	8	23 (33.3 %)
Allergy	3	5	–	8 (11.6 %)
Other diseases	3	3	4	10 (14.5 %)

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The product is formulated in capsules containing 7 mg of the freeze dried lysate (corresponding to 36 billions of bacteria).

CLBL is claimed to stimulate the natural defences of the body and to increase the resistance to respiratory tract infections. This activity has been shown by active protection tests, of macrophages stimulation and by the increase of circulating T lymphocytes and of the immunoglobulins secreted at the level of respiratory tract mucous membrane.

2.4. Treatment

Patients of the first group (MLBL) were treated by sublingual route with one tablet of the assigned compound every morning for 10 days of each month of the 3 months period; to the patients of the second group (CLBL) one capsule of the assigned compound was administered by oral route every morning with the same therapeutic schedule as above. The patients of the third group (NT) did not get any immunostimulating treatment.

Primary variable for efficacy was the number of infective acute exacerbations recorded during three months of therapy. in the 3 groups.

Secondary variables were:

- Duration of acute episodes
- Antibiotic need in case of an infectious episode
- Reported adverse events

Patients were submitted to a baseline examination (T0) including the record of demographic data, of the patients' medical history with particular reference to previous respiratory infections (number of episodes in the previous year), of the physical examination and of the concomitant treatment.

Further assessments were made at days 30 (T1), 60 (T2), 90 (T3) of the treatment period, and at 180 days (T4) at the end of a 3-month follow-up, and included a clinical examination, a questionnaire collecting the number, duration and type of respiratory events, and the dosage and duration of their treatment. Cough, sputum, dyspnoea, fever and the need of concomitant therapy (antibiotics and other drugs) were recorded by the patients on a daily symptoms chart. Dyspnoea was measured by Visual Analogue Scale (VAS), sputum examination was performed in patients with LRTIs. All other medical events and untoward side effects were also noted.

The results were analysed using descriptive statistics (mean values and standard deviations) and tested by parametric test (ANOVA) and Chi-Square Test (X^2). The main variable (number of infective episodes) was tested using ANOVA and Tukey's test. Type 1 error was set at 0.05.

3. Results

LRTIs during the 3-month treatment: In the NT group 22 (96 %) patients had at least one acute episode (41 in total); in 5 patients (22 %) (with a total of 7 episodes) in the MLBL and in 16 (70 %) with a total of 16 episodes in CLBL group, LRTIs were recorded. The difference among groups is statistically significant and in favour of MLBL (see Table 4). No LRTI was observed in this last group in the first month of treatment.

The mean number of acute episodes (primary variable) was significantly lower ($p < 0.05$) in the MLBL group than in the CLBL and in the NT group (0.3 ± 0.63 vs 0.9 ± 0.7 vs 1.7 ± 0.9); however no significant difference was observed in the mean duration of acute respiratory infective episodes reported during the 3 month treatment (Table 4).

Significant differences were observed in the antibiotic need. Only 4 patients (17.4 %) received an antibiotic treatment in the MLBL group, versus 14 patients (60.9 %) in the CLBL group and 21 patients (91.3 %) in the NT group (Table 5); not all acute infective episodes needed an antibiotic treatment, but only in 5 of the 7 episodes (71.4 %) recorded in MLBL patients and in 17 out of 21 (81 %) in the CLBL group and in 36 out of 41 (87.8 %) in the NT group. There were no differences in the duration of antibiotic treatment between the three groups (Table 5).

Table 4: Number of LRTIs during three months of treatment.

	MLBL (n = 23)	CLBL (n = 23)	NT (n = 23)
No. of episodes	7	21	41
1st Month	0	5	16
2nd Month	4	5	9
3rd Month	3	11	16
N. of patients with at least one LRTI episode[+]	5 (21.7 %)	16 (69.6 %)	22 (95.7 %)
N. of LRTIs *			
Mean ± SD	0.30 ± 0.63	0.91 ± 0.73	1.78 ± 0.90
Min : max	0 : 2	0 : 2	0 : 4
No of patients	23	23	23
Duration of episodes (days)°			
Mean ± SD	8.6 ± 5.88	7.2 ± 3.14	9.1 ± 3.34
Min : max	3 : 21	1 : 15	5 : 20

[+] MLBL vs NT $p < 0.00001$; CLBL vs NT $p = 0.047$; MLBL vs CLBL $p = 0.0027$ (Fisher's exact test). * MLBL vs NT $p < 0.05$; CLBL vs NT $p < 0.05$; MLBL vs CLBL $p < 0.05$ (Tukey's test). ° $p > 0.05$ (ANOVA test).

Table 5: No. of days of antibiotic therapy during the three months of treatment.

	MLBL (n = 23)	CLBL (n = 23)	NT (n = 23)
No. of patients with LRTIs treated with antibiotics[+]	4 (17.4 %)	14 (60.9 %)	21 (91.3 %)
No. of LRTIs episodes treated with antibiotics[+]	5/7 (71.4 %)	17/21 (81.0 %)	36/41 (87.8 %)
No. of days of antibiotic treatment*			
Mean ± SD	6.8 ± 1.3	5.8 ± 1.71	6.6 ± 2.20
Min : max	5 : 8	3 : 8	3 : 13

[+] MLBL vs NT $p < 0.0001$; CLBL vs NT $p = 0.035$; MLBL vs CLBL $p = 0.0058$ (Fisher's exact test. * $p > 0.05$ (ANOVA test).

Table 6: LRTIs and antibiotic treatment during follow-up.

	MLBL (n = 23)	CLBL (n = 23)	NT (n = 23)
No. of episodes	5	6	7
1 st Month	1	5	4
2 nd Month	3	1	3
3 rd Month	1	0	0
No. of patients with at least one episode of LRTI	5 (21.7 %)	6 (26.1 %)	7 (30.4 %)
Duration of episodes (days)+			
Mean ± SD	10.2 ± 5.63	14.2 ± 8.01	9.1 ± 2.91
Min : max	7 : 20	10 : 30	7 : 15
No. of episodes treated with antibiotics	5/5	6/6	7/7
No of days of antibiotic treatment*			
Mean ± SD	6.8 ± 2.49	7.3 ± 2.58	7.3 ± 2.36
Min : max	3 : 10	3 : 10	3 : 10

+ $p > 0.05$ (ANOVA). * $p > 0.05$ (ANOVA).

In the three month follow-up period, infectious episodes were reported in 5 patients (21.7 %) of the MLBL group, in 6 (26.1 %) of the CLBL and in 7 (30.4 %) of the NT group. No significant difference was found among groups in the duration of these episodes and in the length of the antibiotic treatment (Table 6).

Symptomatology: At baseline visit dyspnoea was observed in about 70 % of the patients, cough in about 50 % and auscultatory pathological symptoms in about 80 %, without any significant difference among groups. At the end of the first 3 months dyspnoea improved or disappeared in 86.7 % of the patients (13 out of 15) of MLBL group while improved or disappeared in 40 % (6 out of 15) and 11.8 % (2 out of 17) of CLBL and NT patients respectively (MLBL vs CLBL $p = 0.02$, MLBL vs NT $p < 0.0001$, CLBL vs NT $p = 0.10$). At follow-up 9 out of 23 patients in the MLBL group (39.1 %) and in the CLBL group showed dyspnoea versus 17/23 (73.9 %) in the NT group.

Cough, sputum and thoracic pain disappeared in almost all patients while auscultatory thoracic pathological findings disappeared in 67 %, 56 %, and 32 % in MLBL, CLBL and NT patients, respectively, without any significant difference among groups.

No adverse events were reported during the study

4. Discusssion

In 1997, Collet et al. [11] in a prospective randomised, double-bind, placebo controlled clinical trial (PARI-IS) found that COPD patients treated with an immunostimulating agent had fewer hospitalizations than placebo treated patients. Anthonisen [12] on the basis of these results remarked that it would be very hard to criticize the use of immunostimulating agents in the prophylaxis of LRTIs.

Immunostimulating agents are not new drugs and since 1980 placebo controlled clinical trials have been showing not only the safety of these drugs but also an improvement of clinical and haematological parameters, of IgA and T lymphocytes together with some evidence of a reduction of infectious respiratory episodes and of antibiotic need. Orcel [10] reported a reduction of respiratory re-exacerbations of the order of 28 % in comparison to placebo. However, only the PARI-IS study published in 1997 fulfilled the main criteria of the "evidence based medicine" and was quoted in the GOLD guidelines [11] in the section dedicated to immunostimulators.

The role of bacteria in LRTTs is still debated [13] and prophylactic antibiotic therapy provided no clear-cut evidence for a substantial benefit [13, 14]. On this basis it is evident that immunotherapy with bacterial extracts can play an interesting role in the prophylaxis of low respiratory tract infections.

The theoretical basis for oral immunisation, according to Bienestock, is the recognition of bacterial fractions administered by Gut Associated Lymphoid Tissue (GALT) and Bronchial Associated Lymphoid Tissue (BALT) through co-operation and cells traffic between these two systems [15, 16]. But the mechanisms involved in the immunostimulating properties of bacterial extracts are not fully understood; some experimental evidences are available and show that they act on cells of the immune system and on mediators in man and experimental animals [17–22].

The present study has shown that in an elderly population at risk, the treatment with a lysate obtained by bacteria mechanical lysis is effective in the prevention of respiratory tract infections during the cold months.

Both lysates tested (MLBL and CLBL) were effective in the reduction of the number of infective episodes (primary end point) during the treatment period in comparison to a No Immunostimulating Treatment (NT). Moreover MLBL was significantly better than CLBL on this variable. It should be noted that in the first month of treatment no patient in the MLBL group had respiratory infectious disorders; in contrast, these were present in 5 and 16 patients of the CLBL and NT groups. Associated to respiratory infections is the antibiotic need. Only 4 patients (17 %) in MLBL group needed an antibiotic treatment, proportion significantly lower than that found in the CLBL group (61 %) and in the NT (91 %) group, respectively. This last variable is of significant importance because it is an evidence of the protective activity of MLBL vaccine and because of the impact that a reduction in antibiotic consumption has on the economy side. The importance of this last problem was stressed by Collet et al. [27] who recognized for immunostimulating lysates a key role both in

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the prevention of acute exacerbations in COPD patients and in the control of related costs. There are some other elements worth of discussion: dyspnoea was present at baseline in about 70 % of the study population and was homogeneously represented in the three groups. At the end of the treatment period this symptom was reported by a significantly lower number of patients of the MLBL group "2 out of 15" (13.3 %) than in the CLBL "9 out of 15" (60 %) and NT "15 out of 17" (88.2 %) groups. Even though the perception of dyspnoea may be influenced by several factors such as patient variability in the assessment of this symptom, the activity of concomitant steroid inhalation, the adaptability of subjects to breath on reduced pulmonary volumes, we have to consider that patients with dyspnoea were at baseline in similar conditions and at the end of treatment this symptom was significantly improved only in the group treated with the lysate obtained by bacteria mechanical lysis (MLBL). It may be hypothesized that the improvement of dyspnoea is probably due to a greater reduction of the incidence of respiratory infections and consequently to a greater stability of respiratory performance with patient's perception of an improvement or disappearance of this symptom.

To explain the different activity of the two lysates in the variables explored, it may be speculated that the elevated number of bacteria lyse, (48 billions), the preparation technique and the route of administration have played an important role. As far as the preparation technique of the lysate obtained by bacteria mechanical lysis, should be noted that the breaking of the bacteria cell wall, by sonication, occurs in stable physico-chemical conditions; without the presence of contaminating substances potentially denaturing the antigenic structures, highly purified bacterial fractions are thus obtained with surface antigens potentially stimulating both aspecific and specific immunity. The sublingual administration of the MLBL vaccine may be also important to explain the differences between the two types of lysates tested in this study. According to Czerkinski et al. [23] sublingual administration of antigens can stimulate immune system more than an oral swallowing; this can be attributed to a prolonged contact of the antigen with oral mucous membranes so that antigens are more available for immune cells of Waldeyer's ring. Moreover substances absorbed through oral mucous membrane skip hepatic first-pass effect [23].

In conclusion, the significant reduction of the number of respiratory infections, the lesser need of antibiotics and the greater reduction of dyspnoea observed in patients treated with the MLBL vaccine in comparison to the patients treated with a compound obtained by bacteria chemical lysis, and to those without any immunostimulating agent, provide evidence of the efficacy of this product which seems to be a therapeutic improvement in this field.

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