Title: USE OF A LYOPHILISED BACTERIAL LYSATE FOR THE PREVENTION OF TUBERCULOSIS RELAPSE

Abstract: Lyophilized bacterial extracts obtained by mechanical or alkaline lysis of bacteria responsible for infections of the upper and lower respiratory tract can be effectively used for the preparation of a medicament for the prevention of tuberculosis relapse, to be administered in association with antimicrobial agents.
USE OF A LYOPHILISED BACTERIAL LYSATE FOR THE PREVENTION OF TUBERCULOSIS RELAPSE

Field of the invention

The present invention relates to the treatment of tuberculosis, in particular to the prevention of tuberculosis relapse.

Background of the invention

Tuberculosis is a pulmonary infection caused by Mycobacterium tuberculosis, Micobacterium bovis or Mycobacterium africanum. The pathogenesis comprises three stages, (1) primary or initial infection, (2) latent or dormant infection and (3) rescrudescent or adult-type tuberculosis. Tuberculosis therapy comprises the administration of antimicrobial drugs associations, such as isoniazide, pirazinamide and ethambutol. The association of more chemotherapeutics is necessary to prevent development of resistance, which is the main cause of relapse.

However, in recent years there has been a constant increase of cases of primary infections and relapse; there is therefore the need for a new preventive treatment.

Description of the invention

It has now been found that lyophilized bacterial extracts obtained by mechanical or alkaline lysis of bacteria responsible for infections of the upper and lower respiratory tract can be effectively used for the preparation of a medicament for the prevention of tuberculosis relapse, to be administered in association with antimicrobial agents.

Bacterial extracts suitable for carrying out the invention are known and currently used in therapy for the prevention and treatment of infections of the upper and lower respiratory tract, especially in children and in the elderly. These extracts contain microbial strains selected from the following
microorganisms: Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus viridans, Klebsiella pneumoniae, Klebsiella ozaenae, Klebsiella pneumoniae, Haemophilus influenzae serotype B, Neisseria catarrhalis, Diplococcus pneumoniae and Branhamella catarrhalis. Particularly preferred is a lyophilised extract obtained by mechanical or alkaline lysis of the following bacteria: Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Klebsiella pneumoniae, Klebsiella ozaenae, Haemophilus influenzae serotype B, Neisseria catarrhalis and Diplococcus pneumoniae. The bacterial extract is formulated as a sublingual vaccine, in admixture with pharmaceutically acceptable excipients, for example those reported in Remington’s Pharmaceutical Sciences Handbook, XVII ed. Mack Pub., N.Y., U.S.A.; particularly preferred are the preparations marketed under the name Ismigen®; in the following description, for the sake of conciseness, reference will be made to O-BAC™ (“obtrita bacteria”). According to a preferred embodiment, the vaccine is formulated as an association with isoniazide, pirazinamide and ethambutol or as a combined preparation containing a lyophilised bacterial extract as defined above, isoniazide, pirazinamide and ethambutol for simultaneous, separate, sequential administration. A further object of the present invention is an article of manufacture comprising a sublingual vaccine as defined above, suitably packaged and containing a leaflet with specific indications for the preventive treatment of tuberculosis relapse.

The vaccine will be administrated at the dose of 10 mg/day for 10 days a month for two consecutive months, in association with isoniazide and/or ethambutol and/or pirazinamide, but the dosage and the administration regimen can be adjusted by those skilled in the art of medicine according to the patient’s needs.

The invention will be now illustrated in greater detail in the
experimental section.

**Experimental section**

**Materials and methods**

Our study was carried out on 280 subjects who had contracted the infection and 30 healthy volunteers.

Inclusion criteria were age comprised between 50 and 79, negative analysis to BPCO and previous infection. Exclusion criteria were the concomitance of serious neurological and cardiovascular diseases, kidney and liver insufficiency and significant immune system alterations or previous prophylaxis therapies with bacterial lysates or other drugs interfering with the immune response in the previous 18 months.

The study was carried out over a two months period in autumn (2003), when prophylaxis is usually undertaken.

The treated group was randomly divided in two subgroups of 190 people each. The first subgroup, further to the standard antibiotic treatment against tuberculosis relapse (an association of isoniazide, ethambutol and pirazinamide), received also O-BAC™ by sublingual route, at the dose of 10 mg/day for 10 days a month for two consecutive months.

The second group of patients received the standard antibiotic treatment and a placebo by sublingual route, following the same administration regimen as the first group.

The patients of both groups were submitted to hematochemical tests in order to evaluate the immune system function (RTBL with PhHA, PPD, TIC9) before and after treatments. IgE and specific antibodies for anti-streptococcus, anti-staphylococcus and anti-pneumococcus antibodies have also been counted.

The efficacy of the treatment was also evaluated from the clinical standpoint as number of relapses and concomitant respiratory infections.
Results

Tables 1 and 2 report the results of the clinical study described above.

The differences between the group treated with O-BAC™ and the non-treated one are statistically meaningful. In the treated group concomitant respiratory infections were one-third of those observed in the non-treated group (37 versus 122) and the number of relapses in the treated group was half the number of non-treated one (8 cases versus 36).

28.6% of the treated patients showed an increase in T-lymphocytes activity (RTBL with PhHA) from 21.7±1.3% before treatment to 34.1±1.5% after administration of O-BAC™. Reduction of RBTL PPD, TIC, IgE, ME and increase of antibodies against streptococcus, staphylococcus and pneumococcus was also observed.

Variations of immune function parameters in patients not receiving O-BAC™ were less relevant than in the treated group and in most cases not statistically meaningful (sometimes the variations even had the opposite sign).

These results demonstrate that lyophilised bacterial extracts in association with antibacterial therapy reduce to half the incidence of relapses and can therefore be effectively used for the prevention of tuberculosis relapse.

**Table 1 - Clinical efficacy of the treatment with BMBL**

<table>
<thead>
<tr>
<th>Number of episodes of intercurrent disease</th>
<th>Number of patients</th>
<th>Treatment with O-BAC™</th>
<th>Treatment without O-Bac™</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. of patients</td>
<td>140</td>
<td>37</td>
<td>26.4</td>
</tr>
<tr>
<td>sick patients</td>
<td></td>
<td>%</td>
<td>N. of patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>patients</td>
</tr>
<tr>
<td></td>
<td>280</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Number of tuberculosis relapses          | 280                | 140                   | 18                      |
| N. of patients                           |                    |                       | %                      |
| sick patients                            | 140                | 36                    | 25.7                   |
|                                           |                    |                       |                        |    |      |      |
Table 2 - Clinical Efficacy of the Treatment with O-BAC™ on the main laboratory parameters

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Healthy before</th>
<th>Healthy after</th>
<th>Control group receiving placebo (n = 140) before</th>
<th>Control group receiving placebo (n = 140) after</th>
<th>P</th>
<th>Treated group receiving O-BAC™ before</th>
<th>Treated group receiving O-BAC™ after</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBTL with PhHla</td>
<td>32.5±2.7%</td>
<td>20.0±2.6%</td>
<td>26.1±1.2%</td>
<td>ns</td>
<td></td>
<td>21.7±1.3%</td>
<td>34.1±1.5%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fused Protein Derived PPD %</td>
<td>1.5±0.5</td>
<td>3.3±0.3</td>
<td>2.6±0.3</td>
<td>ns</td>
<td></td>
<td>3.0±0.3</td>
<td>2.3±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Turn immunology complex TIC</td>
<td>78.5±7.0</td>
<td>134.0±4ml</td>
<td>128.1±6.2</td>
<td>ns</td>
<td></td>
<td>210.0±10.1</td>
<td>280.0±21.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ig E MO</td>
<td>175.0±15.0</td>
<td>260.0±20.1</td>
<td>230.2±10.1</td>
<td>ns</td>
<td></td>
<td>210.0±10.1</td>
<td>278.0±21.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-pneumococcus antibodies</td>
<td>4.5±0.5</td>
<td>6.5±0.3</td>
<td>6.0±0.3</td>
<td>ns</td>
<td></td>
<td>12.5±0.4</td>
<td>17.0±0.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-streptococcus antibodies</td>
<td>5.5±0.5</td>
<td>10.2±2.6</td>
<td>6.8±0.3</td>
<td>&lt;0.05</td>
<td></td>
<td>10.8±0.4</td>
<td>16.1±0.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Anti-staphylococcus antibodies</td>
<td>5.5±0.5</td>
<td>8.1±0.4</td>
<td>6.0±0.3</td>
<td>ns</td>
<td></td>
<td>8.8±0.4</td>
<td>15.1±0.3</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
CLAIMS

1. Use of a bacterial lysate of bacteria responsible for infections of the upper and lower respiratory tract for the preparation of a medicament for the prevention of tuberculosis relapse.

2. The use according to claim 1 wherein the bacteria are selected from the group consisting of Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus viridans, Klebsiella pneumoniae, Klebsiella ozaenae, Klebsiella pneumoniae, Haemophilus influenzae serotype B, Neisseria catarrhalis and Diplococcus pneumoniae, Branhamella catarrhalis.

3. The use according to claim 2, wherein the lysate contains the following bacteria: Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Klebsiella pneumoniae, Klebsiella ozaenae, Haemophilus influenzae serotype B, Neisseria catarrhalis and Diplococcus pneumoniae.

4. The use according to any one of claims 1 to 3 wherein the lysate is a lyophilised lysate obtained by mechanical or alkaline lysis.

5. The use according to any one of claims 1 to 4 wherein the medicament further contains isoniazide and/or pirazinamide and/or ethambutol.

6. A combined preparation containing a lyophilised bacterial extract as defined in any one of claims 1 to 4, isoniazide and/or, pirazinamide and/or ethambutol for simultaneous, separate, sequential administration for the prevention of tuberculosis relapse.

pneumoniae and Branhamella catarrhalis.

8. A method according to claim 7 comprising the administration of 10 mg/day of lysate for 10 days a month for two consecutive months.

9. A method according to claims 7 and 8 further comprising the administration of isoniazide and/or pirazinamide and/or ethambutol.
**INTERNATIONAL SEARCH REPORT**

A. CLASSIFICATION OF SUBJECT MATTER
A61K35/74 A61K31/133 A61K31/453 A61K31/495 A61P31/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Date of the actual completion of the international search
23 November 2005

Date of mailing of the international search report
02/12/2005

Name and mailing address of the ISA
European Patent Office, P.B. 5618 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epc nl, Fac. (+31-70) 340-3016

Authorized officer
Ceder, O
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>TRICARICO D ET AL: &quot;PREVENTION OF RECURRENT UPPER RESPIRATORY TRACT INFECTIONS IN A COMMUNITY OF CLOISTERED NUNS USING A NEW IMMUNOSTIMULATING BACTERIAL LYSATE A RANDOMIZED, DOUBLE-BLIND CLINICAL TRIAL&quot; ARZNEIMITTEL FORSCHUNG. DRUG RESEARCH, ECV EDITOR CANTOR VERLAG, AULENDORF, DE, vol. 54, no. 1, 2004, pages 57-63, XP008054882 ISSN: 0004-4172 abstract</td>
<td>1-4</td>
</tr>
</tbody>
</table>
C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>ROMAIN F ET AL: &quot;IDENTIFICATION OF A MYCOBACTERIUM BOVIS BCG 45/47-KILODALTON ANTIGEN COMPLEX, AN IMMUNODOMINANT TARGET FOR ANTIBODY RESPONSE AFTER IMMUNIZATION WITH LIVING BACTERIA&quot; INFECTION AND IMMUNITY, AMERICAN SOCIETY FOR MICROBIOLOGY. WASHINGTON, US, vol. 61, no. 2, 1 February 1993 (1993-02-01), pages 742-750, XP002001490 ISSN: 0019-9567 abstract</td>
<td>1</td>
</tr>
</tbody>
</table>
INTERNATIONAL SEARCH REPORT

Box II  Observations where certain claims were found unsearchable (Continuation of Item 2 of first sheet)

This international Search Report has not been established in respect of certain claims under Article 17(2)(e) for the following reasons:

1. [X] Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

   Although claims 7–9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2.  [ ] Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3.  [ ] Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III  Observations where unity of invention is lacking (Continuation of Item 3 of first sheet)

This international Searching Authority found multiple inventions in this international application, as follows:

1.  [ ] As all required additional search fees were timely paid by the applicant, this international Search Report covers all searchable claims.

2.  [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invoke payment of any additional fee.

3.  [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4.  [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.