Influence of a Bacterial Immunostimulant (Human *Enterococcus faecalis* Bacteria) on the Occurrence of Relapses in Patients with Chronic Recurrent Bronchitis

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Summary

The following double-blind, placebo-controlled, multicenter study investigated the influence of a bacterial immunostimulant (Symbioflor® 1, cells and autolysate of human *Enterococcus faecalis*) on the occurrence of relapses in patients with chronic recurrent bronchitis (n = 136; placebo n = 66, verum n = 70) in a 6 months treatment period and a follow-up period of 8 months, compared to placebo. Under verum 39 incidents of relapses were recorded, which was about 60 % the number observed among the patients treated with placebo (66 incidents). The verum preparation exhibited superior clinical efficacy compared to placebo (p = 0.001) in the Kaplan-Meier test. This superior clinical efficiency of the test preparation was particularly apparent during the treatment period, with 12 vs. 27 relapses (p = 0.013), but less during the follow-up observation period, with 27 vs. 39 relapses (p = 0.127). In addition, the time span until occurrence of the first relapse was clearly longer under verum (699 days) than under placebo (334 days) and after the end of the observation period 91 % of patients under the verum experienced only one relapse compared to 62 % in the placebo group (p = 0.01). The severity of relapses under verum was also reduced significantly (χ^2; p = 0,001. Only 4 patients under verum required antibiotic therapy compared to 13 patients under placebo. The verum preparation was as well tolerated as placebo, with no serious side effects in either. No changes in laboratory tests – haematology and clinical chemistry – were observed. It can be concluded, that previously demonstrated immunomodifying effects of the test preparation have clinical relevance for the treatment of chronic recurrent bronchitis because not only the number but also the severity of acute relapses could be clearly reduced. This is discussed in view of the current literature.

Key words

- Chronic recurrent bronchitis
- Cytokines
- *Enterococcus faecalis*
- Immunostimulation
- Medical probiotic
- Symbioflor® 1

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1. Introduction

Chronic recurrent infections of the upper respiratory tract and in ENT medicine represent a phenomenon frequently observed by general practioners. Although the term of 'chronic obstructive pulmonary diseases' includes different disease manifestations of the lower respiratory tract (e.g. chronic bronchitis, emphysema, asthma and bronchiectasis), they display a common cause of multifactorial origin with regard to the persistent damage of the respiratory airway epithelium [1, 21]. Acute respiratory tract diseases originating from viral infections are common in many patients suffering from chronic bronchitis [3] contributing to an acute exacerbation of the disease in one third of all bronchitis patients [3]. Considering the prognosis and the progression of chronic obstruction of the respiratory tract, the incidence of acute infections is of particular pathogenetic relevance as the structure and functionality of the respiratory epithelium becomes severely affected during each infection [3]. Acute infections of mixed bacterial origin may be mostly controlled by the administration of antibiotics [4] but considering the progression of the disease a reduction of infection-free intervals generally remains an essential therapeutic objective. A prolongation of the infection free period highly facilitates the regeneration of the respiratory epithelium resulting in an incease of the local defense mechanims of the respiratory tract. There is substantial evidence that an impairment of the body's defense mechanisms results in chronic recurrent infections [5, 6]. Accordingly, the use of immunostimulants has been attracting more and more interest in recent years [7, 8, 9]. Therefore, the clinical relevance of an immunostimulation by perorally applied *Enterococcus faecalis* bacteria of human origin was studied compared to placebo with patients suffering from chronic obstructive recurrent bronchitis. The main outcome measure of the study was to examine the relapse incidence during a 6-month treatment with verum or placebo and in an 8-month follow-up phase. Tolerability of the preparation and its effects on clinical chemical and hematological parameters were also assessed. The study was carried out in accordance with the currently accepted guidelines of Good Clinical Practice (EC-GCP) in the European Community and of the German Drug Law (AMG) after being approved by the Ethics Commission of the Regional Medical Council of Hessen in Frankfurt/Main (Germany). A total of 27 general practioners in the greater Frankfurt/Main area, Wiesbaden (Germany) and the Wetterau District (Germany) participated in the study[1].

2. Patients, materials and methods

2.1. Patients

The present study was carried out as a double-blind, randomized comparative design. 136 patients of both genders with a confirmed medical history of chronic obstructive bronchitis, characterized by productive cough [10, 11] which should have been occurred during the preceeding 2 years on most days for at least three months before admission to the study, were enrolled. Purulent sputum for several days, fever, coughing and shortness of breath, and antibiotic therapy, were considered as an acute relapse during the anamnestic phase. All patients had a forced expiratory 1-second volume (FEV_1) > 1. Not enrolled in the study were pregnant or nursing mothers, patients with severe allergic diathesis or other severe systemic diseases, as well as patients with chronic emphysema, a type A bronchitis, Kartageners syndrome or cystic fibrosis.

2.2. Treatment

The test preparation[2] contained cells and autolysate of *Enterococcus faecalis* of human origin at a concentration of 1.5–4.5 × 10^7 bacteria/ml. These are *Enterococcus faecalis* bacteria of the serotype group D being part of the physiologic intestinal flora of the human intestinal tract. Isotonic saline with a starch suspension served as the placebo.

Patients received 30 drops t.i.d. (corresponding to 11.25–33.75 × 10^7 bacteria/day) verum or placebo (in a randomized, double-blind comparative study design).

An additional treatment with immunosuppressants, systemic corticoids, local or systemic antibiotics and mouth and throat disinfectants was not permitted during the study period. If indicated, antibiotics could be administered during a relapse.

2.3. Main objectives

The primary outcome measure of the study was the incidence of relapses during the 6-month treatment with the verum or placebo and in the subsequent 8-month follow-up phase. The number of antibiotic prescriptions necessary was included in the evaluation of the data as a secondary parameter. The severity of relapses was classified according to three criteria. "Mild" indicated the presence of purulent sputum, coughing and expectoration without impairment of the daily living activities.

"Moderate" included impairment of daily living habits and the need of an antibiotic therapy. With a severe relapse, the patient had bed rest for at least 2 days with fever of more than 38.5 °C and antibiotics must be administered. In addition, the symptoms of elevated temperature, coughing, expectoration and dyspnea during a relapse were also documented and classified. On admission to the study and at the end of the 6-month therapy phase, a spirometry was carried out together with a complete blood count and clinico-chemical parameters were measured. The patients were evaluated by the physician one week and four weeks after the beginning of the therapy, and thereafter at monthly intervals.

2.4. Statistical evaluation

The design of a prospective, randomized, double-blind multicenter, parallel-group study was considered to be an adequate tool for the investigation of the verum preparation in comparison with a placebo in chronic bronchitis with regard to the relapse incidence. An estimation of the number of cases – due to the absence of reliable historical data concerning the relapse rate – was based on a success rate of 80 % (verum) and 50 % (placebo) for the alleviation of mucosal damage. With α = 5 %

[1] A list of the investigators is held by the corresponding author.

[2] Manufacturer: SymbioPharm GmbH, Herborn (Germany).

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Table 1: Demographic data in the groups treated with verum or placebo (mean value ± standard deviation).

		Verum	Placebo
Age	(years)	47.1 ± 11.5	47.4 ± 11.1
Body height	(cm)	171.1 ± 8.2	171.8 ± 8.8
Body weight	(kg)	72.0 ± 12.5	73.5 ± 14.7

and β = 20 %, 45 patients per group were enrolled for Fisher's exact χ^2-test, and after an additional compensation by 40 % for drop-outs and the inclusion of an additional patient/center (to analyse center-related effects) finally 70 patients per treatment group resulted. The primary objective – a reduction in the relapse rate during the 6-months' treatment period and the subsequent 8-month follow-up period – was calculated first with Fisher's exact test, however this assumes the same obervation period for both groups. To take into account the different time periods the individual patients had to remain in the study, the proportion of patients being free of symptoms up to a time "t" was additionally estimated by the Kaplan-Meier method and compared between the treatment groups.

3. Results

136 patients were enrolled in the study altogether (age 19–70 years, placebo n = 66, verum n = 70) and all were finally accepted for the evaluation of the efficacy and tolerability. Of these, 67 were women and 67 men; no data were known for 2 patients. The two treatment groups did not differ in demographic or anamnestic data (Table 1).

84 % in the verum group had purulent sputum for several days and 88 % in the placebo group. For a treatment of a preceeding relapse 74 % in the verum group and 82 % in the placebo group had to be treated with antibiotics. Both groups differed only marginally in the time between the last two relapses before the start of the study. The median value for the relapse-free time was about 92 days in both treatment groups. The number of relapses and the number of patients affected by relapses is shown in Fig. 1.

Under verum, the total number of relapses with 39 cases reached only 60 % compared to those under placebo with 66 relapses. This difference was 44 % during the treatment period, (corresponding to 12 vs. 27 relapses; p = 0.013) and was therefore more prominent than during the follow-up period (= 69.2 %, corresponding to 27 vs 39 relapses, p = 0.127). Likewise, the number of patients with relapses was reduced under verum. Under verum, the relative risk for a relapse during the treatment phase was with 0.425 less than half than under placebo. During the follow-up period, the relative risk for a relapse under the verum was 0.68 (corresponding to one-third less than under the placebo). These differences were statistically significant in both phases with p = 0.019 and p = 0.013 (2-way in each case). Fig. 2 shows the relapse incidence in the two

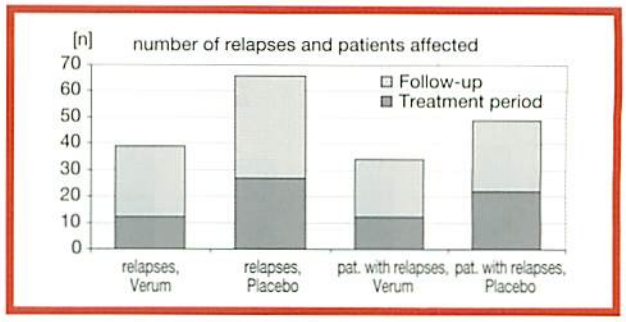

Fig. 1: Absolute incidence of relapses under verum and placebo and number of patients affected in the treatment group under the therapy or follow-up.

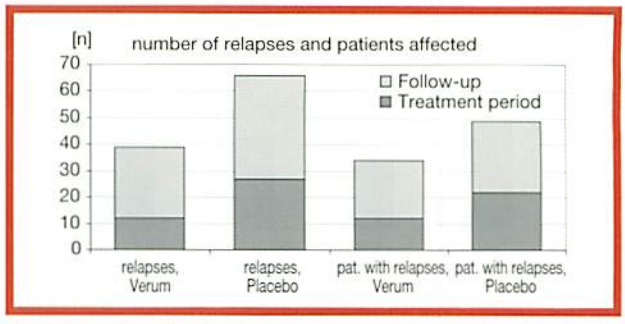

Fig. 2: Number of patients with one or more than one relapse.

treatment groups separately for the two treatment phases.

Under and after verum respectively, no patient had more than 2 relapses in contrast to 3 relapses in the patients of the placebo group. During treatment, 12 patients under verum and 21 patients under placebo experienced at least one relapse. The corresponding data for the follow-up period are likewise in favor of the test preparation, being 21 versus 27. When the relapse rate was analyzed by Kaplan and Meier's method which takes into account the actual observation period for each individual, in contrasts to Fisher's exact Test, the relapse incidence under verum was significantly lower than under placebo (p = 0.001; log-rank test). The time period (median) until the first relapse occurred was 699 days in the verum group in contrast to 344 days in the placebo group. These results are shown in Fig. 3.

The number of relapse-free patients declined much more rapidly under placebo than under verum. At the end of the 14 months observation period 91 % of the patients in the verum group, but only 62 % of the patients under placebo remained without a second relapse (p = 0.01).

The number of months with and without relapse together with the relative risk and the odds ratio calculated thereof is summarized in Table 2.

The relative risk and the odds ratio were less than half of the verum group during the treatment phase and during the follow-up period they rose to only about two-third. The severity of relapses was provided for all 50 relapses (see Fig. 4). An estimation of the severity of

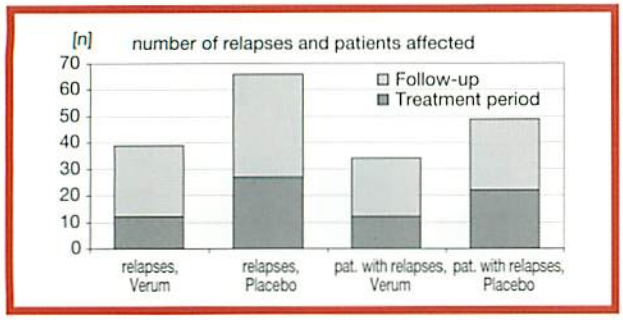

Fig 3: Time span from the start of the study to the occurrence of the first relapse. KM: Kaplan-Meier, LR: log-rank test.

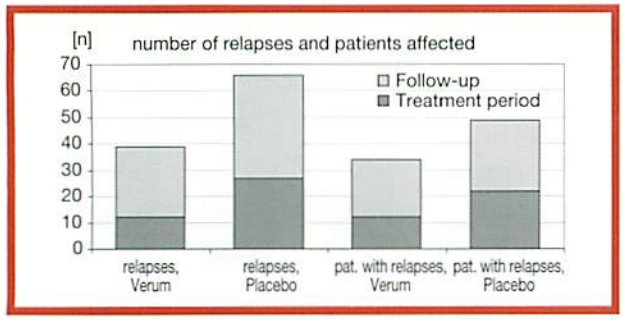

Fig. 4: Severity of the relapses under verum and placebo, respectively.

the relapses by the physician using a score revealed clear differences in favor of the test preparation. A mean score of 1.67 resulted for the verum group and for the placebo a score of 2.14 ($p = 0.001$, multiple-field χ^2-test) was obtained. Under verum, 4 patients required an additional antibiotic therapy (under placebo: 13 patients). The pulmonary function parameters at the start and the end of the 6 month therapy phase remained unchanged in both treatment groups. Only the "peak-flow" clearly rose in patients above 50 years of age in both treatment groups during therapy. Hematological and clinico-chemical laboratory findings did not differ between the two treatment groups. With regard to tolerability the test preparation was well tolerated by most of the patients. Four patients under verum and 3 under placebo reported adverse reactions. Of them mainly gastrointestinal complaints, such as intermittent diarrhea, emesis, flatulence or nauseae were recorded. Serious adverse events were not obserevd in either group.

Table 2: Number of months with and without relapses during the 6-month treatment period and the 8-month follow-up period, and the relative risk and odds ratio calculated thereof for both treatment groups.

	Treatment period		Follow-up	
	Verum	Placebo	Verum	Placebo
Number of months with relapses	12	27	27	39
Number of relapse-free months	383	351	464	446
Total	395	378	491	485
Relative risk	42.5 %		68.3 %	
Odds ratio	40.7 %		66.5 %	

4. Disscussion

The aim of the present study was to investigate the clinical efficacy and tolerability of a preparation known as an immunomodulator in patients with chronic bronchitis. The results revealed a statistically significant reduction of about 43 % in the risk for a relapses during the treatment phase ($p = 0.013$) and of about 68 % during the follow-up period ($p = 0.127$), which can be regarded as medically relevant.

Furthermore, the clinical superiority of the test preparation compared to placebo was also evident by a significant prolongation of the time span until the occurrence of a first relapse together with an alleviation of its severity ($p = 0.01$). From these results it is reasonable to assume, that perorally applied *Enterococcus faecalis* bacteria for the treatment of chronic bronchitis results in clinically relevant, measurable parameters, that is a reduction in relapse rates and, in addition, a reduction of antibiotic use. Peroral immunostimulants of bacterial origin represent a therapeutic measure to counteract an increased risk of infection in children or elderly individuals [12]. Meanwhile, it is therefore quite clear that the therapeutic application of perorally administered killed or live bacteria to increase the body's defense mechanisms has been described repeatedly in placebo-controlled and randomized clinical studies [13–17]. The clinical efficacy of these kind of preparations, apart from a specific vaccination induced by them [16, 17], is believed to be associated, among other mechanisms, with a stimulation of the mucosa-associated immune system [17]. Due to the existence of a common mucosa-associated immune system [18], it is proposed, that perorally administered bacterial preparations resulted in an enhanced antibacterial defense of the respiratory tract (secretory and functional), by the preceeding stimulation of the committed gut-derived lymphocytes and their subsequent migration into the peripheral mucosae of the respiratory tract. Depending on the composition of the bacterial preparation used, both antigen-specific immune mechanisms, [17], and antigen non-specific immunity may be evoked, whereby both parts of the immune system often interact in an overlapping manner. For example, a polyclonal B-cell activation [7, 8] enhances the opsonization of pathogens which in turn positively affects the secretory functions of monocytes and granulocytes and the killing capacity of natural killer cells. Generally this results in an increased resistance to bacterial and viral infections. The maintenance of adequate local mucosal protection also depends on the organism's capacity to produce immunoglobulin A [17, 18, 19]. In particular,

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IgA antibodies are characterized by their ability to agglutinate microorganisms and to suppress their adherence to mucosae. Furthermore, compared to immunoglobulin G, IgA possess a lower opsonization capacity contributing to reduced inflammation of the mucosae [17]. In a placebo-controlled experimental animal study with the test preparation investigated here [20], an increase in saliva IgA antibodies of the animals after the peroral administration was demonstrated, which is in good agreement with the discussed mechanisms of action of perorally applied bacterial immunostimulants. Studies investigating the release of cytokines by animal and human leukocytes after incubation with the test preparation examined here [21, 22], provided additional information of possible underlying mechanisms of action of the test preparation, in contrast to the antigen-specific action of other bacterial immunomodulators [16, 17]. The panel of cytokines [22] determined in one study with human leukocytes is involved to a major extent in the activation of cell-mediated immunity, so that, despite the concerns raised with in vitro tests, possible effects of the test preparation on the local antibacterial defense under in vivo conditions may be postulated.

It has been also reported for other perorally administered bacterial immunomodulators that even in individuals with underlying immune defects an increase in the gamma-interferon production of CD4-positive T-lymphocytes could be induced after the peroral administration of the preparation [23] illustrating, that perorally applied immunostimulants can develop systemic effects, too.

Acute exacerbations of chronic bronchitis cases are frequently of bacterial origin so that effective antibiotics represent an adequate form of therapy for this common disease of the respiratory tract [24, 25, 26]. However, along with the increasing antibiotic resistance among those pathogens responsible for respiratory tract infections a more critical approach emerged in recent years concerning the uncritical use of antibiotics for the therapy of acute bronchits [26]. Even so the results presented here are of clinical relevance as the preparation reduces the use of antibiotics. Not only would the immune system take benefit from a reduction in antibiotic prescriptions (latent immunosuppression) but the growing development of antibiotic resistance would also be taken into account [26]. In the great majority of cases the tolerability of the test preparation was good and was comparable to that of placebo, which agress with data in the literature on the excellent tolerability of this kind of preparations [27]. In particular the author's hint [27], that atopic patients may also benefit from a peroral immunostimulation with bacterial lysates without additional risk underlines the favourable use/benefit profile of the test preparation.

In summary, the therapeutic management of recurrent, chronic bronchitis with *Enterococcus faecalis* bacteria represents a very promising treatment regimen with a favourable use/benefit ratio. On the one hand it is cost-effective, and on the other hand without serious side effects and therefore suitable for the adequate treatment of these disordes frequently observed by general practioners. Although a possible protection against chronic respiratory tract diseases of allergic genesis, such as asthma by the encounter of frequent bacterial/viral infections is still a matter of debate [28] it appears plausible, that too much infections of the upper and lower respiratory tract may prepare the way for the development of chronic inflammation particularly in adolescence. A reduction of the relapse incidence could therefore counteract the development of chronic respiratory tract diseases already in childhood, being particularly noteworthy to mention under the aspect for the reduction of costs for the therapy of chronic recurrent bronchitis showing an increasing prevalence in the industrialized countries.

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