The Effect of a Homeopathic Preparation on the Clinical Condition of Patients with Corticosteroid-Dependent Bronchial Asthma

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Summary

As part of the research directed at determining less detrimental methods in the treatment of patients with corticosteroid-dependant bronchial asthma, a double-blind, placebo-controlled, randomized study with Engystol® N was performed on 40 patients. Twenty patients received one ampule of Engystol® N subcutaneously every 5 to 7 days. The remaining 20 patients received placebo. The study took place between June and December, 1993. The following were measured for each patient: PEFR, FVC, FEV_1 , granulocyte function, as well as a number of other clinical parameters. The results showed that administration of Engystol® N led to improvement in these clinical parameters when compared to placebo in corticosteroid-dependent patients suffering from bronchial asthma, and that it allowed for a reduction of the dosage of corticosteroids. For the therapy of bronchial asthma, Engystol® N represents an effective medication which is not associated with adverse effects.

Introduction

Despite significant advances in the treatment of bronchial asthma, long term therapy with corticosteroids is necessary for certain patient subgroups. Administration of corticosteroids, however, especially in large doses taken over extended periods of time, leads to numerous and serious adverse reactions.[1] Research into other, less harmful treatment regimes for bronchial asthma is warranted.

In treatment of corticosteroid-dependent bronchial asthma, we originally applied immunosuppressants (cyclosporin A)[2] and immunomodulators (levamisole, calf-thymus extract.)[3,4] Although long term administration of cyclosporin A enabled significant reduction of corticosteroid dosage, other studies show that this therapy is associated with renal damage.[5] Application of immunomodulators lowered only the frequency of recurring viral and bacterial infections. This therapy did not, however, enable reduction in corticosteroid dosage.

Within the context of a search for less harmful methods of asthma treatment, we conducted a clinical trial of Engystol® N including its objective effects on certain immunological parameters.

Methodology and Patient Selection

The study involved 40 corticosteroid-dependent asthma patients within the age range of 24-48 years (mean = 39.) The patients were evenly distributed between males and females (20 in each group.)

The study began in June, 1993 and was completed in December of the same year. Diagnosis of corticosteroid-dependent bronchial asthma was based on the following evidence: case-history data, physical examination, spirometric tests (with determination of PEFR, FVC, and FEV_1), and the patients' history of long term corticosteroid usage. All patients accepted in the study had taken triamcinolone (Polcortolon®, Polfa) 4 to 8 mg/24h for at least 5 years. Furthermore, all patients had suffered from numerous complications from this treatment including muscular atrophy, susceptibility to bruising, generalized weakness and debilitation, and osteoporosis.

Only those patients were included in this study whose FEV_1 exceeded by 50% the normal expected value (FEV_1= forced expiration volume in the first second) and whose PEFR (peak expiratory flow rate) was below 80% of the normal afternoon value. We used the Eutest-2 spirometer to determine FVC (forced vital capacity) and FEV_1, and the Mini-Wright unit to establish the PEFR. Testing for PEFR took place daily, after the patients rose in the morning. The patients used special reporting cards to note the PEFR results and to record the daily corticosteroid usage.

The patients received Engystol® N and the placebos within the context of a double-blind study. Decoding, i.e., revealing to patients and physicians who had received Engystol® N and who had received the placebos, took place only after completion of the program of treatment. Twenty of the patients received Engystol® N in a dosage of 1 ampule administered subcutaneously at intervals of 5 to 7 days. The remaining twenty patients received placebo. In addition to corticosteroids, Engystol® N, or placebos, all patients received methyloxanthine preparations for liquefaction of mucus. Tetracyclines were administered in cases of exacerbation of symptoms.

Post- and pre-study testing was performed to assess the following: morphotic elements in the patients' blood, blood count, urinalysis, serum creatine, urea, potassium, sodium, calcium, magnesium, glucose, GPT (glutamate pyruvate transaminase), GOT (glutamate oxaloacetate transaminase [aspartate aminotransferase]), cholesterol, and lipids.

Likewise, before and after the study, all

patients underwent granulocyte-function tests for the following: *in vitro* migration by Clausen's method,[6] *in vivo* migration by the method of Matusiewicz and Brzezinska,[7] nitroblue tetrazolium reduction by Park's method,[8] and quantitative analysis of production of the superoxide radical O_2 by granulocytes in peripheral blood, by the method of Bellavite *et al.*[9]

Clausen's method

The medium with Parker's solution was supplemented with antibiotics, and a 1% $NaHCO_3$ solution was added to adjust the pH to 7.3. The medium was then mixed with equal amounts of approximately prepared agarose and equine serum from the Warsaw Sera and Vaccine Laboratory. The dissolved medium, at 48°C, was filled into Petri dishes and stored in a refrigerator at 4°C for 30 minutes. Four circular holes, 2.3 mm in diameter, were punched out of the solidified medium and were filled with suitably prepared peripheral-blood leukocytes taken from the study patients.

The Petri dishes were then placed in an incubator, with a controlled supply of air and CO_2. After removal of agarose by means of methanol, and fixation with formalin (40%), the extent of granulocyte migration was calculated after 18 hours by use of the r^2 formula.

Method according to Matusiewicz and Brzezinska

A medium consisting of 2 ml of calf serum, 2 ml of Parker's concentrate, 5 ml of distilled water, and 1 ml of sodium bicarbonate (1%) was placed into a small beaker. The beaker and contents were then heated in a water bath at 50°C. In a second beaker, 10 ml of distilled water was added to 150 mg of agarose, and the mixture was boiled until the agarose completely dissolved.

The contents of both beakers were next mixed and distributed into dishes by means of a pipette. These dishes were then allowed to stand until their contents had hardened. These dishes were made of glass 1 mm thick, and were 3 mm in diameter. A hole 2 mm in diameter was drilled in the bottom of the dish, at its lowest point. The dishes with medium were put into a refrigerator for 10 minutes. The dishes were removed from the refrigerator, and an agarose layer equal in thickness to the thickness of the bottom of the dish was removed through the hole earlier drilled in the bottom of the dish. The recess produced in this manner was then filled with buffered isotonic saline solution.

A window on the skin of the patients was provided by scarifying an area of 0.5 cm^2 on the forearm, with care being taken to avoid bleeding. The scarified area was then covered with a dish and fixed in place with a bandage. The dish was allowed to remain on the patient's forearm for 18 hours. The dish was next removed from the forearm and covered with formalin (40%) for 1.5 to 2 hours. The formalin was then poured off, and the agarose layer removed. The dish was then washed several times with water and allowed to stand to dry. The preparation produced in this manner was stained by Pappenheim's method, and the diameter and surface area of the cell migration zone were calculated.

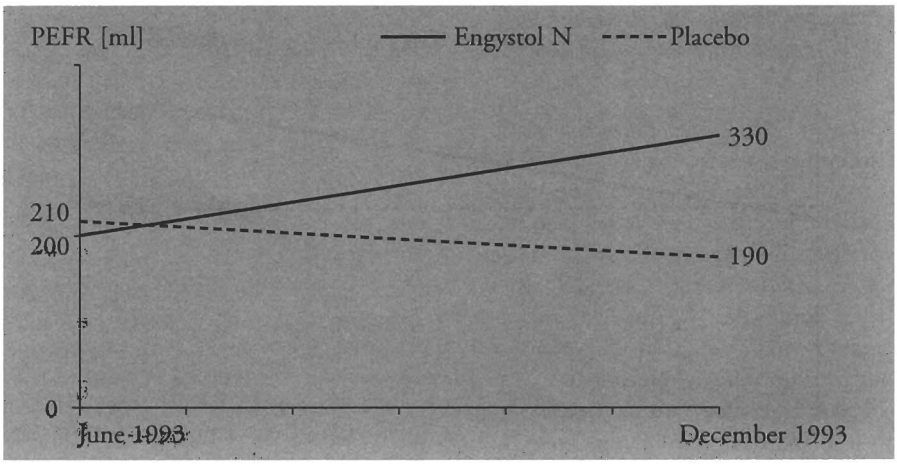

Fig. 1: Mean PEFR values for Engystol® N patients and for placebo group

Park's method

Blood obtained in volumes of 1 ml each from the patient's antecubital vein was mixed in a plastic dish with 0.1 ml of heparin (50µg/ml.) One ml of the blood mixed with heparin in this manner was transferred into another plastic dish. There, a mixture was added consisting of the following: 0.1 ml of phosphate-buffered isotonic saline solution (pH = 7.2) and 0.1 ml of 0.2% solution of nitroblue tetrazolium (NBT; 2 mg/ml.) The preparation was allowed to dry and was then incubated for 15 minutes at 37°C, and for a further 15 minutes at room temperature. Smears were made on microscope slides, air-dried, and stained by the May-Grunwald-Giemsa method. Two hundred granulocytes were counted out per patient. Reduction indices were calculated by dividing the number of formazon-embedding cells by the number of granulocytes present in the specimen, multiplied by 100.

Method according to Bellavite et al

This method is based on the measurement of the degree of reduction of cytochrome C by superoxide radical anions which are produced in granulocytes from the patient's peripheral blood. Three analysis procedures were performed for each sample. The first sample tube served as a control. The second tube served to determine production of superoxide radical anions by non-activated granulocytes. The third tube was used for determination of production of superoxide radical anions by granulocytes by means of opsonized zymosan.

Aliquots of 0.3 ml of cytochrome C obtained from bovine hearts (supplied by the Sigma Company) were added to the tubes. Into the first and second tubes, 0.2 ml of a 0.9% phosphate-

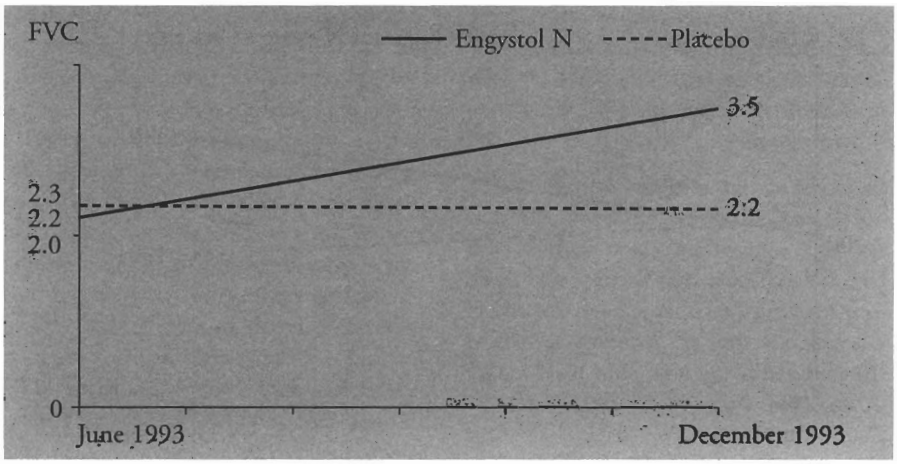

Fig. 2: Mean values of FVC for Engystol® N patients and for placebo group

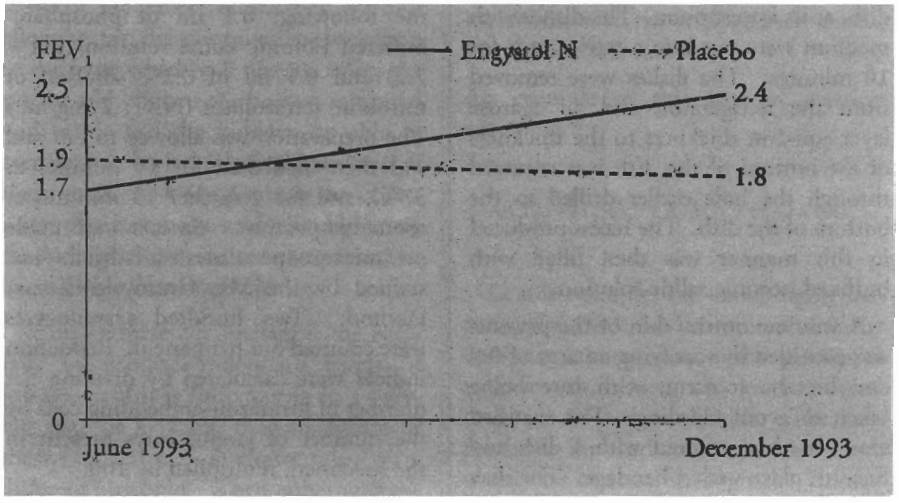

Fig. 3: Mean FEV_1 values for Engystol® N patients and for placebo group

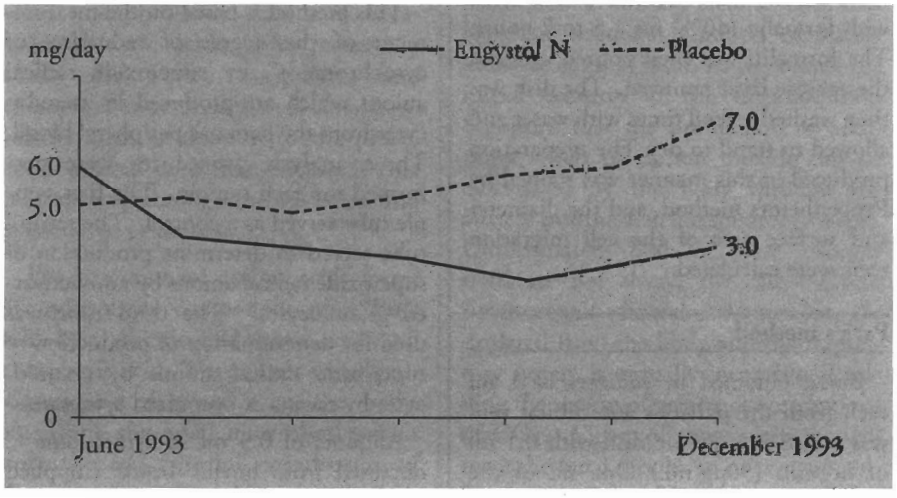

Fig. 4: Mean daily doses of corticosteroids for Engystol® N patients and for placebo group

buffered isotonic saline solution (buffered to pH = 7.2 to 7.4, PBS) was added. Into the third tube 0.1 ml of opsonized zymosan was pipetted.

After an incubation period of 5 minutes at 37°C, 2 ml of hemocuprein (3000 U/ml of a solution of SOD-i, supplied by the Sigma Company) was added to the first tube along with 0.1 ml of the test blood. The tube was then allowed to stand at +4°C. After 10 minutes, 2 ml of hemocuprein (3000 U/ml of a solution of SOD-i, supplied by the Sigma Company) was added to the second, and then to the third tube. All three samples were then centrifuged at 2000 rpm and at +4°C, in a Janetzki K-70 centrifuge.

Absorption measurement of the supernatant was performed at a wave length of 550 nm, in a Specol II spectral photometer. The results were expressed in nmols of O_2 released during one minute by one cell. The data obtained were analyzed statistically by means of Student's t-test.

Results

As shown in Figure 1, the mean PEFR values for asthmatic patients treated by Engystol® N showed a statistically significant increase: from 200 to 330 ml ($p<0.01$.) For the patients receiving placebos, mean PEFR values decreased from 210 to 190 ml ($p<0.01$.)

A similar difference became evident in comparison of the two test groups for the mean FVC values: see Figure 2. Among Engystol® N patients, the mean level increased from 2.2 to 3.5 liters ($p<0.01$.) Among placebo patients, the mean level decreased from 2.3 to 2.2 liters ($p<0.01$.)

Figure 3 reveals that the mean FEV_1 levels for asthmatic patients treated by Engystol® N likewise demonstrated a statistically significant increase: from 1.7 to 2.4 liters ($p<0.01$.) In the placebo group, practically no change was determined, only a slight drop from 1.9 to 1.8 liters ($p<0.01$.)

Figure 4 reveals that a reduction in corticosteroid dosage became possible for those patients treated with Engystol® N: a decrease from 6.0 to 3.0 mg per day. In the placebo group, the daily corticosteroid dosage was required to be

Investigation patients	treatment by Engystol® N		Placebo		Control Group (healthy persons)
	before investigation	post investigation	before investigation	post investigation	
granulocytes of vessels	19.04% ± 6.0	9.13% ± 6.13	18.05% ± 7.23	17.04% ± 8.0	8.10%
diferences between 1st, 2nd, and 3rd, 4th groups	p < 0.01		p > 0.01		

Tab. 1: Ability of granulocytes to reduce NBT in patients with corticosteroid-dependent asthma: results for Engystol® N patients, placebo patients, and healthy persons.

Investigation patients	treatment by Engystol® N		Placebo		Control Group (healthy persons)
	before investigation	post investigation	before investigation	post investigation	
before stimulation	12.03 ± 4.03	9.18 ± 3.4	13.13 ± 6.04	14.03 ± 7.13	7.13 ± 2.03
after stimulation	24.76 ± 7.14	9.14 ± 10.04	25.13 ± 11.04	23.04 ± 13.14	18.04 ± 8.14
diferences between 1st, 2nd, and 3rd, 4th groups	p < 0.01		p > 0.01		

Tab. 2: Quantitative assessment of superoxide radical O_2 generation by peripheral blood granuloctes (nmol/cell/min): results for Engystol® N patients, placebo patients, and healthy persons.

increased from 5.0 to 7.0 mg daily.

As Table 1 shows, corticosteroid-dependant asthma patients demonstrated a significant decrease with regard to the ability of their granulocytes to reduce nitroblue tetrazolium: from 19.04 ± 6.0% down to 9.13 ± 6.13% ($p<0.01$.) In the placebo group, nitroblue tetrazolium reduction decreased only very slightly: from 18.05 ± 7.23% to 17.04 ± 8.0%. The final Engystol® N results compare favorably with the control (healthy) group, with its reduction level of 8.10 %.

Likewise, Table 2 reveals that the ability of peripheral granulocytes to produce superoxide radicals was definitely lower in the Engystol® N group than in the placebo group. The Engystol® N group demonstrated 9.18 ± 3.4 before stimulation in the post-investigation column versus 9.14 ± 10.04 after stimulation in the same column. The placebo group showed 14.03 ± 7.13 before stimulation in the post-investigation column, versus 23.04 ± 13.14 after stimulation in the same column.

Table 3 clearly reveals that an increase in granulocyte migration became evident by the end of the study for the Engystol® N group and for the placebo group. This increase, however, was considerably greater for the Engystol® N group: from 36.04 ± 13.05 mm^2 to 97.09 ± 15.06 mm^2 *in vivo*, and from 21.06 ± 10.0 mm^2 to 40.09 ± 13.05 mm^2 *in vitro*.

Discussion

The present study demonstrates significant improvement in the clinical conditions of the patients treated with Engystol® N. The values obtained from spirometric tests improved significantly.

As a result of clinical improvement, it was possible to reduce the mean daily dosage of triamcinolone from 6.0 to 3.0 mg for those asthma patients who were treated with Engystol® N. Due to the reduction in corticosteroid dosage, it was also possible to achieve alleviation in adverse reactions associated with corticosteroid administration: improvement resulted with respect to bruising, muscular weakness, and depression (improvement in depression symptoms in 5 cases.) The clinical conditions of the placebo patients did not change significantly; the values obtained in spirometric tests remained practically unchanged. For the placebo group, it was even necessary to increase the daily corticosteroid dosage.

The explanation for the clinical improvement after administration of Engystol®N may be associated with the non-specific, anti-inflammatory action of the preparation on the patient's respiratory system. It may also be the result of the capability of the test preparation to inhibit the release of peroxide amino-radicals in granulocytes of peripheral blood. The experiments conducted by Rebuck,[10] as well as our own studies, show that granulocytes account for 50 to 80% of all cells which participate in the inflammatory reactions involved in such

	Engystol N		Placebo		Control group
	before treatment	after treatment	before treatment	after treatment	(healthy persons)
Granulocyte migration *in vivo*	36.40 ± 13.05 mm²	97.09 ± 15.06 mm²	38.01 ± 14.03 mm²	40.03 ± 12.03 mm²	110 ± 20.0 mm²
Granulocyte migration *in vitro*	21.06 ± 10.00 mm²	40.09 ± 13.05 mm²	26.13 ± 9.12 mm²	28.10 ± 10.10 mm²	42.0 ± 13.0 mm²
	p < 0.01		p < 0.01		

Tab. 3: Granulocyte migration in vivo and in vitro in persons with corticosteroid-dependent asthma: results for Engystol N patients, placebo patients, and healthy persons

cases. In the process of migrating to the site of inflammation, such granulocytes release lysosomal enzymes, e.g., elastases, collagenases, phosphatases, and lipases. The enzymes released in this context damage the surrounding tissues and exacerbate the inflammatory process.

On the other hand, the same destructive enzymes stimulate fibroblasts and enhance repair of the damaged tissue, eventually leading to fibrotic alterations in lung tissue. Comparing the results between patients receiving Engystol® N and those receiving placebo, we demonstrated that the ability of the granulocytes to release peroxide aminoradicals was decreased by administration of Engystol® N. This therapy likewise led to a marked decrease in the ability of granulocytes to reduce nitroblue tetrazolium.

Before treatment with Engystol® N, the study patients suffered from 5 to 6 recurrences of upper respiratory-tract infections annually. After 7 months of Engystol® N treatment, the mean frequency of such infections fell to 1 to 2. This reduction may be the result of the specific stimulation of Engystol® N of the migration ability of the granulocytes. Indeed it is precisely among corticosteroid-dependent asthma patients that severe disturbances are observed in the ability of granulocytes to migrate in peripheral blood.[11] These disturbances may be responsible for the recurrences of viral or bacterial infections which are more frequent among asthma patients than among non-asthmatics. Our study revealed a pronounced increase *in vivo* and *in vitro* in the migration ability of the granulocytes among those patients treated with Engystol® N.

To be sure, the favorable clinical and immunological results obtained in this study with Engystol® N for asthma patients are to some degree qualified by the fact that treatment was conducted primarily during the warmer months of the year (June to December.) The clinical condition of asthma sufferers naturally improves spontaneously during the summer. Nevertheless, the study also included two cold months of the year, traditionally unfavorable for asthmatics, November and December, during which time the clinical condition of the Engystol® N patients remained stable, without an increase in corticosteroid dosage. During this same cold period, on the other hand, the placebo patients required an increase in their mean daily doses of corticosteroid, from 5.0 to 7.0 mg. The patients treated with Engystol® N received mean daily doses of 3 mg of triamcinolone during these two cold months.

During the treatment of patients with Engystol® N, no morphological alterations were seen in their blood cells nor did significant changes take place in their urinalysis results. Anomalies involving electrolytes, enzymes, or lipids likewise were not detected.

We conclude that Engystol® N represents an effective and safe drug in the treatment of corticosteroid-dependent bronchial asthma. Its administration enables significant reduction in the required dose of corticosteroids in these patients.

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