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Supplementation with a Soluble Beta-Glucan Exported from Shiitake Medicinal mushroom, *Lentinus edodes* (Berk.) Singer Mycelium: A Crossover, Placebo-Controlled Study in Healthy Elderly

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ABSTRACT: *Lentinus edodes* (Shiitake) is a medicinal mushroom with a long tradition of use in Asia. The major active substance in *L. edodes* is a (1-6,1-3)-beta-glucan (lentinan). No clinical controlled studies have yet investigated the effect of orally administered lentinan on the immune response in healthy, elderly Caucasian subjects. We evaluated the effect and the safety of a beta-glucan from *L. edodes* mycelium, Lentinex®, in healthy, elderly subjects in a double blind, crossover, placebo-controlled trial. Forty-two subjects were randomly allocated to two groups given orally either 2.5 mg/day Lentinex® or placebo for 6 weeks; then after a washout period of 4 weeks, the alternate supplementation was given for 6 weeks. The changes in the number of B-cells were significantly different between the groups. The number of NK cells increased significantly in both groups, but there was no significant difference between the groups. Other factors of the immune response (immunoglobulins, complement proteins, cytokines) were not altered. The safety blood variables (differential cell count, liver function, kidney function, and other blood chemistry) were not influenced by Lentinex®, and the number, nature, and severity of adverse events were similar to placebo. Lentinex® given orally to elderly subjects was safe and induced an increase in the number of circulating B-cells.

KEY WORDS: medicinal mushrooms, Shiitake mushroom, *Lentinus edodes*, Lentinex®, beta-glucan, elderly, immune response, B-cells, NK-cells

ABBREVIATIONS: AE: adverse event; **ANOVA:** analysis of variance; **ALAT:** alanine amino transferase; **ASAT:** aspartate amino transferase; **C:** complement; **CD:** cluster differentiation; **CI:** confidence interval; **GCP:** good clinical practice; **CRP:** C-reactive protein; **γ-GT:** gamma glutamyl transferase; **HDL:** high-density lipoprotein; **HIV:** human immunodeficiency virus; **ICH:** International Conference on Harmonization; **IEC:** independent ethics committee; **IFN:** interferon; **Ig:** immunoglobulin; **IL:** interleukin; **ITT:** intention to treat; **LDL:** low-density lipoprotein; **LEM:** *Lentinus edodes* mycelium; **NK:** natural killer; **NSAID:** nonsteroidal anti-inflammatory drug; **PP:** per protocol; **SAE:** serious adverse event; **TG**: triglycerides; **TNF-α:** tumor necrosis factor-alpha

I. INTRODUCTION

Edible mushrooms may have important salutary effects on health or even in treating disease, and have a long traditional use. This is the case with the medicinal shiitake mushroom *Lentinus edodes* (Berk.) Singer (Lentinaceae, higher Basidiomycetes), which has been used for centuries in Asia.[1] It has also been shown in preclinical studies that extracts from *L. edodes* had antibiotic activity, antiviral activity and antitumor effects.[1]

The main biologically active substance in *L. edodes*, lentinan, a β-1,3 / β-1,6 D-glucan, has been characterized as a high-molecular-weight polysaccharide organized in a triple helix.[2,3] Beta-glucans appear to be able to activate leukocytes directly, stimulating their phagocytic, cytotoxic, and antimicrobial activities, as well as stimulating the production of pro-inflammatory mediators, cytokines, and chemokines, including IL-8, IL-1b, IL-6, and TNF-α.[4]

Extracts from *L. edodes* share the antiviral and antitumor properties, as shown in cellular experi-

mental models and animal studies.[1] The effects seem to occur by stimulating the maturation, differentiation, or proliferation of immune cells involved in host defense mechanisms.[5] Beta-glucans also activate NK-cells and stimulate T helper cells,[6–7] which can be helpful in chronic fatigue syndrome.[8]

Lentinan, injected or given orally, is generally considered safe. Animal studies have evaluated both the safety and immunoenhancing effects of Lentinex® in mice, rats, chickens, and pigs. Lentinex® was found to be safe, and the rats demonstrated a significant increase in B-cells, monocytes, and interferon gamma. No sign of toxicity was observed.[9]

A study performed with Caucasian HIV-positive patients with immune deficiency showed that lentinan injected intravenously was well tolerated.[10] Other studies performed with Asiatic patients with cancer did not report any adverse reactions related to lentinan.»[11,12,13] Except for one study that reported that 4 g powder of fresh whole shiitake mushroom given daily for 10 weeks to healthy subjects increased eosinophil levels in 5 of the 10 subjects, as well as causing an increase in eosinophil granule proteins in serum and stools, which accompanied gastrointestinal symptoms in the same subjects,[14] clinical experience indicates that lentinan acts as an immune system stimulator and that it is safe.

The objective of the current study was to evaluate whether Lentinex®, a fermentation product from *L. edodes* mycelium containing a β-1,6/β-1,3 D-glucan, was able to stimulate the immune response and/or restore some of the reduced immune response in healthy, elderly subjects.[15,16] In addition, the question of safety was also investigated.

II. MATERIALS AND METHODS

A. Lentinex

The product is a liquid from *Lentinus edodes* mycelium containing 16 mg/mL free glucose, 25 µg/mL protein, and 1 mg polysaccharide. The polysaccharide composition is given by glucose/mannose/galactose ratios of 3.4:2.8:1.

B. Subjects (Patients)

Forty-two healthy male and female volunteers over 65 years of age were recruited at one research center. All subjects signed an informed consent form before inclusion. Exclusion criteria included: BMI over or equal to 30 kg/m^2; the use of corticosteroids or NSAIDs; anti-inflammatory drugs, immunemodulating substances; or subjects suffering any clinical condition that rendered them unfit to participate. Subjects with known or suspected drug or alcohol abuse were also excluded.

C. Study Design

This was a randomized, double-blind, crossover study with two arms comparing the active treatment Lentinex® tablets 2.5 mg/day with placebo (cellulose) for 6 weeks. Intravenously administered lentinan was given to patients in doses up to 2 mg/day without toxic effects. Since the oral absorption of lentinan is below the concentration obtained intravenously, and as this was the first study in humans, it was decided to give the subjects a 2.5 mg/day dose of LentinexÒ.

Subjects were chosen 2 weeks prior to treatment start in order to evaluate laboratory results and inclusion/exclusion criteria.

Active or placebo was randomly given for the next 6 weeks. After a 4-week washout period, the subjects received the alternate treatment for another 6 weeks. The subjects were on *ad-libitum* diet and no restrictions in lifestyle or in caloric intake were implemented.

D. Clinical Assessments

Baseline characteristics and demographic data were recorded by the medical staff when entering the study to evaluate the eligibility of the participants. Twelve-hour-fasting blood samples were analyzed to screen healthy subjects for alanine amino transferase (ALAT), aspartate amino transferase (ASAT), C-reactive protein (CRP), gamma glutamyl transferase (γ-GT), high-density lipoprotein (HDL), low-density lipoprotein (LDL), hemoglobin, thrombocytes/platelets, total cholesterol, and triglycerides (TG). These analyses were also performed at each medical visit after randomization. At the randomization visit and all following visits, additional 12-hour-fasting blood samples were analyzed for T helper cell ($CD4^+$), T cytotoxic cell ($CD8^+$), total T cell ($CD3^+$), NK cell ($CD56^+$), and B cell ($CD19^+$) counts, and to determine the levels of immunoglobulins IgM, IgG, IgA, and complement C3. Cytokine analyses (IL-8, IL-10, IL-12)

and tumor necrosis factor (TNF-α) were also performed. Compliance was determined at the end of each treatment period by counting the returned unused tablets. A subject was considered compliant when taking ≥75% of administered dose.

E. Adverse Events

An adverse event (AE) was defined as any unfavorable, unintended event (or symptom) reported by a subject or observed by the investigator during the study. The investigator also classified each AE as serious or nonserious according to International Conference on Harmonization (ICH) guidelines, and gave an opinion on a possible causal relationship to treatment.

F. Statistical Analysis and Determination of Sample Size

A difference of ~30% between Lentinex® and placebo groups for CD4/CD8 was regarded as clinically relevant. A two-sided t-test with significance level 5% and test power 90% gave n = 18 for each treatment group. When adjusted for 10% dropout rate, $2 \times 20 = 40$ subjects were required.

Results are given as mean ± SD and 95% confidence intervals are given for differences between treatment groups (see tables). Changes within treatment groups have been calculated by subtracting the baseline value from the end value, and they have been tested by using the Student's T-test or the Wilcoxon Signed Rank test (if not normally distributed). All analyses and tabulations were carried out using SAS® for Windows Version 8.2.; 5% was chosen as the nominal level of significance.

Statistical analyses for efficacy and safety parameters were performed on all subjects with at least one post-baseline visit (n = 41), defined as the Intention to Treat population (ITT). In addition, statistical analyses for efficacy on the main variables were performed on the per protocol (PP) population, including subjects who completed the treatment period of 16 weeks and did not deviate from the protocol in a manner that would influence the validity of the data, or would result in an extensive loss of information (n = 33).

G. Ethics

The regional Ethics Committee approved the study before it started. The study was conducted in agreement with the Declaration of Helsinki of 1975 as amended in 2000, and in accordance with the International Conference on Harmonization (ICH) guidelines.

III. RESULTS

A. Study Subjects (Patients)

Forty-two patients were randomized and 41 completed the study (97.6%), with one withdrawal in the placebo group (Fig. 1). The baseline characteristics of the whole population are given in Table 1. Complete data for analysis of the efficacy were performed on the PP population, including 33 subjects.

The compliance was similar and high in both treatment groups (98.3% for the supplement and 96.9% for placebo, including one subject with low compliance 49%).

B. Changes in Immune Cellular Response

The number of T helper cells ($CD4^+$) decreased significantly in the placebo group ($P = 0.0357$) but not in the Lentinex® group, whereas there was no difference between the groups (Table 2).{QUERY: AU: Please clarify; this sentence seems self-contradictory.} There were no changes in the number of cytotoxic cells ($CD8^+$). The ratio of T helper cells to T cytotoxic cells was also unchanged, without a difference between the groups. The total number of T cells ($CD3^+$) decreased significantly during the placebo period ($P = 0.0414$), but not during the Lentinex® period (Table 2). No significant difference between the groups was observed. The number of B cells ($CD19^+$) increased nonsignificantly during the supplementation period, whereas it decreased nonsignificantly during the placebo period (Table 2). The difference between groups after six weeks was significant ($P = 0.0368$). The number of NK cells ($CD56^+$) increased significantly in both groups ($P = 0.0465$ and $P = 0.0323$ in the Lentinex® and placebo groups, respectively), but there was no difference between the groups (Table 2).

C. Changes in Immune Humoral Response

The complement C3 decreased significantly in both groups ($P = 0.0418$ and $P = 0.0166$ in the Lentinex® and placebo groups, respectively), but no difference between the groups was observed (Table 3). None of the changes in immunoglobulin

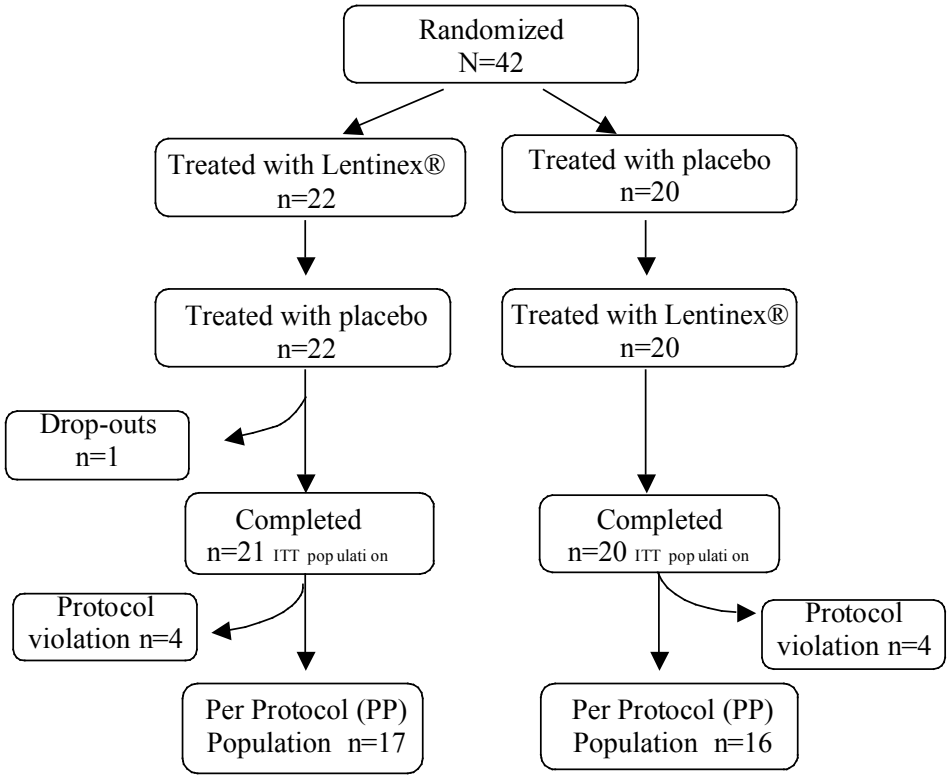

FIGURE 1. Study population .

IgG, IgA, and IgM levels were significant (Table 3). A carryover effect was observed for IgA. There was no correlation between the changes in IgG values and the changes in the number of B cells.

D. Inflammatory Parameters

Changes in cytokine levels were not significant, either within the groups or between the groups (Table 4).

An increase in CRP levels was observed in the active group (P = 0.0206), but not in the placebo group (Table 4). There was, however, no difference between the groups at the end of study.

It would have been expected, if the supplement caused a CRP induction, that significantly more subjects would be induced during the supplement period than during the placebo period. Seven (16.7%) subjects had values above normal after the supplement and 3 (7.3%) after placebo, p = 0.191 (Chi-test) which is not statistically significant. Two subjects had a value above normal only after the washout period (no treatment). These observations do not support a causative relationship to the supplement.

E. Safety

With the exception of the eosinophile levels that increased significantly in the placebo group (P = 0.000) but not in the Lentinex® group, all mean changes were within normal ranges (Table 5). There were no significant changes in diastolic or systolic blood pressure, nor were there any changes in heart rate.

F. Adverse Events

The number and the nature of reported adverse events were similar in the placebo group (n = 27) and in the Lentinex® group (n = 26). The number of subjects reporting adverse events was also similar between the placebo group (n = 22) and the shiitake group (n = 23). Distribution of the severity of the adverse events did not differ between the groups. In the placebo group, 28.6% were classified as "mild," 21.6% as "moderate," and 2.4% as "severe." The corresponding values for the active group were 31%, 26.2,% and 0%, respectively.

Only one case of abdominal distension and one case of dry mouth were regarded as "possibly"

TABLE 1. Baseline Characteristics of Study Population (n = 42)

Variable			Value
Age (years)	Mean ± SD		71.0 ± 5.4
	Range		64.9–84.0
Gender	Female	n (%)	20 (48)
	Male	n (%)	22 (52)
Weight (kg)	Mean ± SD		72.4 ± 12.8
	Range		43–95
Height (cm)	Mean ± SD		171.7 ± 9.0
	Range		157–192
BMI (kg/m^2)	Mean ± SD		24.6 ± 2.6
	Range		17.0–29.0
Smoking (cigarettes/day)	0/day	n (%)	36 (85.7)
	1–10/day	n (%)	4 (9.5)
	11–19/day	n (%)	2 (4.8)
	≥20/day	n (%)	0
Alcohol consumption (units/day)	0/day	n (%)	4 (9.5)
	1–7/day	n (%)	36 (85.7)
	8–14/day	n (%)	2 (4.8)
	>15/day	n (%)	0
Physical exercise (Yes/No)	No	n (%)	10 (23.8)
	Yes	n (%)	32 (76.2)
Physical exercise (hours/week)	Without sweating	mean ± SD	2.57 ± 3.07
	With sweating	mean ± SD	1.12 ± 2.45

Abbreviation: BMI, body mass index

related to the Lentinex® intake, whereas one case of fatigue was "possibly" related to placebo.

IV. DISCUSSION

The widespread use of *L. edodes* and other Chinese medicine is based largely on tradition rather than scientific evidence, and the issues of possible toxicity and putative benefit have not been adequately studied in controlled, clinical trials so far. The current study is, to our knowledge, the first one performed in accordance with good clinical practice (GCP/ICH guidelines), the first one performed in healthy Caucasian subjects, and, additionally, the first one performed where a fermented beta-glucan has been supplemented orally. The choice of elderly subjects as the study population was justified by the fact that potency of the immune response decreases with age.[17]

Additionally, healthy subjects were chosen because we did not want to confound results with possible biases due to diseases related to a compromised immune system. Due to a possible large variation in the responsiveness of the immune response between elderly subjects, we decided to use a crossover design. In this manner, each subject was his/her own control. However, this design had its weaknesses, since period effects were observed for some of the variables. Furthermore, a carryover effect could also be demonstrated. Therefore, one should bear in mind these possible pitfalls when interpreting the results from the current study.

In our study we did not detect any changes in T cell populations except for a significant reduction in the amount of CD+4 cells during the placebo supplementation. The amount of natural killer cells changed similarly during both supplementation periods. However, a significantly higher number of circulating B-cells was observed during the supplementation with the beta-glucan as compared to

TABLE 2. Immune Cellular Response

n = 33	Lentinex pretreatment mean ± SD	Lentinex 6 weeks treatment mean ± SD	Placebo pretreatment mean ± SD	Placebo 6 weeks treatment mean ± SD	Difference (Lent – plac) mean ± SD	95% confidence interval for the difference between Lent and placbo
T helper cells CD4+(%)	53.07 ± 9.51	51.95 ± 9.59	53.62 ± 9.56	52.14 ± 9.12	0.37 ± 6.65	–1.99–2.73
T cytotoxic cells CD-8+ (%)	24.66 ± 9.81	25.08 ± 8.87	24.22 ± 10.41	24.74 ± 10.73	–0.10 ± 3.22	–1.24–1.04
T-cells CD3+ %	72.77 ± 9.52	71.3 ± 10.12	72.79 ± 9.47	71.86 ± 9.43	–0.48 ± 5.97	–2.59–1.64
B-cells CD19+ %	12.54 ± 4.78	12.98 ± 4.94	13.20 ± 5.12	12.63 ± 5.68	1.14 ± 2.76	0.16 –1.86 $p = 0.022$*
NK-cells CD56+ %	11.71 ± 5.92	13.82 ± 7.81	11.33 ± 6.29	13.29 ± 7.59	0.15 ± 7.66	–2.57– 2.87

*Students paired t-test. Lent = Lentinex®, Plac = Placebo, SD = standard deviation

TABLE 3. Immune Humoral Response

n = 33	Lentinex pretreatment mean ± SD	Lentinex 6 weeks treatment mean ± SD	Placebo pretreatment mean ± SD	Placebo 6 weeks treatment mean ± SD	Difference (Lent – plac) mean ± SD	95% confidence interval for difference between Lent and placebo
C3 (g/L)	1.24 ± 0.27	1.13 ± 0.26	1.19 ± 0.25	1.11 ± 0.22	–0.02 ± 0.39	–0.16–0.12
C4 (g/L)	0.23 ± 0.06	0.22 ± 0.05	0.22 ± 0.06	0.22 ± 0.07	–0.01 ± 0.06	–0.03 –0.01
IgG (g/L)	9.94 ± 2.14	10.17 ± 1.97	9.72 ± 2.03	9.99 ± 1.71	0.04 ± 1.63	–0.54 –0.62
IgA (g/L)	2.31 ± 0.86	2.36 ± 0.98	2.34 ± 0.90	2.32 ± 0.93	0.06 ± 0.40	–0.08 –0.20
IgM (g/L)	0.87 ± 0.44	0.87 ± 0.43	0.88 ± 0.44	0.86 ± 0.43	0.02 ± 0.13	–0.03 –0.06

* Students paired t-test. Lent = Lentinex®, Plac = Placebo, SD = standard deviation

TABLE 4. Inflammatory Variables Responses

n = 41	Lentinex pretreatment mean ± SD	Lentinex 6 weeks treatment mean ± SD	Placebo pretreatment mean ± SD	Placebo 6 weeks treatment mean ± SD	Difference (Lent – plac) mean ± SD	95% confidence interval for difference between Lent and placebo
IL-8 (pg/mL)	7.32 ± 5.51	6.39 ± 5.16	5.44 ± 3.12	4.99 ± 2.71	–0.41 ± 6.02	–2.36 –1.54
IL-10 (pg/mL	1.01 ± 3.05	0.83 ± 2.49	0.89 ± 3.18	0.85 ± 2.63	–0.13 ± 1.11	–0.49 –0.23
IL-12 (pg/mL)	68.08 ± 37.9	68.25 ± 37.8	61.28 ± 32.97	64.41 ± 34.90	–2.36 ± 21.97	–9.48 –4.76
TNF-a pg/mL)	1.53 ± 0.57	1.63 ± 0.66	1.64 ± 0.54	1.64 ± 0.62	0.10 ± 0.57	–0.08 –0.28
CRP (mg/mL)	1.93 ± 1.58	4.36 ± 6.84	2.17 ± 2.89	2.78 ± 3.45	1.83 ± 8.44	–0.83 –4.49

*Students paired t-test. Lent = Lentinex®, Plac = Placebo, SD = standard deviation.

the placebo. This is in accordance with published observations.[18]

Inflammatory variables did not seem to be affected during our study. All of the other laboratory

TABLE 5. Changes Within the Supplementation Period (6 Weeks)

n = 42	Lentinex® Mean ± SD	Placebo Mean ± SD	Diff. Lentinex – placebo Mean ± SD	95% confidence interval for difference between Lentinex® and placebo
Hb (g/dL)	0.10 ± 0.61	0.06 ± 0.46	0.04 ± 0.54	–0.19 –0.27
Basophils (10^9/L)	0.01 ± 0.05	0.01 ± 0.05	0.00 ± 0.05	–0.02 –0.02
Eosinophils (10^9/L)	0.01 ± 0.06	0.05 ± 0.08 $p = 0.000$[a]	–0.04 ± 0.07	–0.07 –0.01 $p = 0.011$[b]
Lymphocytes (10^9/L)	–0.05 ± 0.36	–0.09 ± 0.38	0.04 ± 0.37	–0.12 –0.20
Monocytes (10^9/L)	–0.02 ± 0.15	–0.01 ± 0.18	–0.01 ± 0.17	–0.08 –0.06
Neutrophils (10^9/L)	0.30 ± 1.71	0.11 ± 0.97	0.19 ± 1.39	–0.41 –0.79
Platelets (10^9/L)	–2.5 ± 42.1	4.2 ± 28.8	–6.7 ± 36.07	–22.36 –8.96
Leukocytes (10^9/L)	0.28 ± 1.63	0.08 ± 1.14	0.20 ± 1.41	–0.41 –0.81
ALAT (U/L)	0.7 ± 8.9	1.0 ± 7.1	–0.30 ± 8.05	–3.79 –3.19
ASAT (U/L)	–0.3 ± 5.2	0.3 ± 5.7	–0.60 ± 5.46	–2.97 –1.77
γGT (U/L)	2.5 ± 21.5	–1.5 ± 14.4	4.0 ± 18.30	–3.94 –11.94
Creatinine (mmol/L)	0.1 ± 6.5	–1.1 ± 5.2	1.2 ± 5.89	–1.36 –3.76
Bilirubin (mmol/L)	–0.5 ± 4.2	-0.0 ± 4.5	–0.5 ± 4.35	–2.39 –1.39
TG (mmol/L)	0.04 ± 0.371	0.04 ± 0.501	0.00 ± 0.45	–0.19 –0.19
Total cholesterol (mmol/L)	0.10 ± 0.73	0.02 ± 0.75	0.08 ± 0.74	–0.24 –0.40
LDL cholesterol (mmol/L)	0.02 ± 0.56	–0.10 ± 0.63	0.12 ± 0.60	–0.14 –0.38
HDL cholesterol (mmol/L)	0.00 ± 0.20	0.06 ± 0.23	–0.06 ± 0.22	–0.15 –0.03

[a]T-test inside group. [b]Students paired t-test between groups. SD = standard deviation.

safety variables seemed to be unaffected. There were no cases of eosinophily, as was reported after a high daily dose of whole mushroom powder.[13] The finding in that study is probably not relevant for an exported beta-glucan from *L. edodes* mycelium, which contains no pesticides or other unwanted material, which might be the case in whole mushrooms. Since the nature and frequency of adverse events between the treatment groups did not differ, we conclude that Lentinex®, at the given dose, is safe.

In conclusion, the lentinan exported from the mycelium of *L. edodes*, given orally for 6 weeks to elderly subjects, was shown to be safe and revealed a potential to increase the number of circulating B-cells, but did not seem to affect the other immunological and inflammatory markers in this population. It could, however, be possible that longer treatment time and/or higher dose would reveal additional effects.

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