

REVIEW

Effects of orally administered yeast-derived beta-glucans: A review

Anne Berit C. Samuelsen¹, Jürgen Schrezenmeir² and Svein H. Knutsen³

¹ Department of Pharmaceutical Chemistry, Pharmacognosy, School of Pharmacy, University of Oslo, Oslo, Norway

² Clinical Research Center Kiel, Kiel, Germany

³ Nofima, Norwegian Institute of Food, Fisheries and Aquaculture Research, Aas, Norway

Yeast-derived beta-glucans (Y-BG) are considered immunomodulatory compounds suggested to enhance the defense against infections and exert anticarcinogenic effects. Specific preparations have received Generally Recognized as Safe status and acceptance as novel food ingredients by European Food Safety Authority. In human trials, orally administered Y-BG significantly reduced the incidence of upper respiratory tract infections in individuals susceptible to upper respiratory tract infections, whereas significant differences were not seen in healthy individuals. Increased salivary IgA in healthy individuals, increased IL-10 levels in obese subjects, beneficial changes in immunological parameters in allergic patients, and activated monocytes in cancer patients have been reported following Y-BG intake. The studies were conducted with different doses (7.5–1500 mg/day), using different preparations that vary in their primary structure, molecular weight, and solubility. In animal models, oral Y-BG have reduced the incidence of bacterial infections and levels of stress-induced cytokines and enhanced antineoplastic effects of cytotoxic agents. Protective effects toward drug intoxication and ischemia/reperfusion injury have also been reported. In conclusion, additional studies following good clinical practice principles are needed in which well-defined Y-BG preparations are used and immune markers and disease endpoints are assessed. Since optimal dosing may depend on preparation characteristics, dose-response curves might be assessed to find the optimal dose for a specific preparation.

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1 Introduction

Linear or branched 1→3/1→6-linked beta-glucans are compounds that are found in some bacteria, algae, mushrooms, and unicellular fungi such as baker's yeast and *Candida albicans* [1, 2] and as callose in higher plants [3, 4]. Since they are found in microorganisms, these structures are classified as pathogen-associated molecular patterns and can be

recognized by pattern recognition receptors on host immune cells and hence trigger immune responses. The beta-glucans are, therefore, considered immune-modulating compounds that may activate the host immune system and initiate inflammatory processes and thereby provide resistance against infections and cancer development. In the western world, dietary supplements containing beta-glucans are mostly derived from baker's yeast, *Saccharomyces cerevisiae*. Baker's yeast has been used in the production of fermented food and beverages for thousands of years [5] and therefore, certain preparations of yeast-derived beta-glucan (Y-BG) have been approved as novel food ingredients by the European Food Safety Authority [6] and given Generally Recognized as Safe status by US Food and Drug Administration (www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/ucm153925.htm). So far, European Food Safety Authority has not yet approved a health claim on immune function for Y-BG preparations.

Correspondence: Dr. Anne Berit C. Samuelsen, Department of Pharmaceutical Chemistry, Pharmacognosy, School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, N-0316 Oslo, Norway
E-mail: a.b.c.samuelsen@farmasi.uio.no
Fax: +47-22-854-402

Abbreviations: CRP, C-reactive protein; FDA, Food and Drug Administration; ITT, intention to treat; LPS, lipopolysaccharide; PP, per protocol; sIgA, secretory immunoglobulin A; URTI, upper respiratory tract infection; Y-BG, yeast-derived beta-glucans

The history of Y-BG as immunomodulating substances began around 1900 [7], when yeast cells were found to inactivate complement. Later on, this effect was found to be due to an insoluble component in fresh yeast [8] that was named Zymosan. Compositional analyses revealed that the Zymosan preparation contained glucan and also mannan, protein, and fat in addition to small amounts of glucosamine, phosphorus, and magnesium [9]. The beta-glucan component in Zymosan has been shown to bind to pattern recognition receptors such as dectin-1 [10], CR3 [11], Toll-like receptor-2 and -4 mediating phagocytosis and inflammatory processes. The function and signaling of the two main beta-glucan-binding receptors, dectin-1 and CR3 have been described in several reviews [1, 2, 12].

At present, preparations of purified or semi-purified Y-BG are marketed as immunomodulators to provide protection against infections, consumed orally embedded in capsules or in combination with herbs. In a previous review from 2008, only two clinical studies on oral administration of Y-BG were identified [13]. In the recent years, an increasing number of clinical trials have been conducted on effects following oral administration of beta-glucan preparations. Therefore in the present review, an update on the documented effects of orally administered Y-BG is presented. Their relation to beta-glucan structure and solubility is addressed, where data are available, and the perceptions and open questions related to pharmacokinetics, pharmacodynamics, and posology are discussed.

2 Structure and preparation

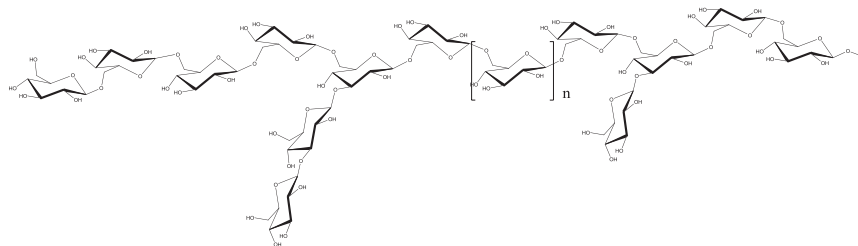
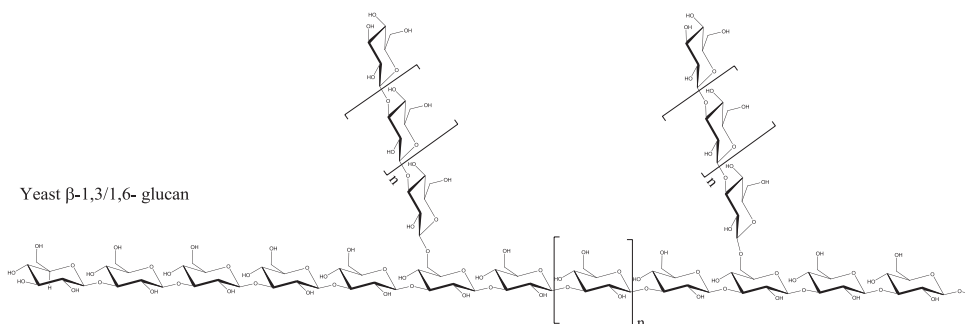
Y-BG are composed of a beta-1→3 D-glucose backbone with side chains linked to O-6 [14]. Although ramifications of Y-BG resemble beta-glucans originating from mushrooms, the latter have shorter side chains. Furthermore, the fungal beta-glucans differ structurally from beta-glucans found in cereals; the latter are nonbranched beta-glucans composed of short 1→4 linked regions linked together by 1→3 bonds. For a review on cereal beta-glucan structure and activities, see [15]. Y-BG from *S. cerevisiae* is in general described as a glucan with beta-1→3 side chains linked to O-6 of the 1→3 linked backbone (Fig. 1). However, it has also been shown that a more complex picture can be drawn based on the occurrence of fractions containing beta-1→6 linked polymers, with a size range from 10 to 100 kDa [16] with one or two beta-1→3 linked units as side chains (Fig. 1) [17–19].

In the native yeast material, it is estimated that branched beta-1→3 glucan and beta-1→6 -glucans account for about 50–55% and 10–15%, respectively of the total cell wall polysaccharides. By the use of a beta-1→3 glucanase and chromatography, a soluble beta-1→6 glucan was isolated from an alkaline insoluble fraction from a wild-type strain of *S. cerevisiae*. NMR and MS confirmed the polymeric beta-1→6 structure, and size exclusion chromatography (Sephadex G75) with dextran standards suggested a molecular mass of 38 kDa [19].

Enzyme systems have been identified in *C. albicans* that can introduce intra chain beta-1→6 linkages on a beta-glucan chain, and even give the basis for cross-linking with other cell wall polysaccharides [20]. Isolated fungal membranes have the capacity to synthesize linear beta-1→3 strands, but additional enzymes may be active in the assembly of glucan structures during cell growth. Although possessing an important role in the yeast cell wall, the function of and the exact composition of the beta-1→6 component are not fully known. The shorter polysaccharides consisting of a beta-1→6 glucan backbone may, according to [21], act as a flexible glue by the capability of covalently cross-linking to beta-1→3-glucan and chitin and mannoprotein found in the cell wall of *S. cerevisiae* [16]. The potential for obtaining Y-BG with further structural variation is due to the successful UV-induced mutations in *S. cerevisiae*. This has given rise to some new strains with 1→6/1→3 linkages deviating from those in the wild-type A364A such as mutant R4 [22].

For a compound produced and isolated from any biological source, it is evident that the structural characteristics such as linkages, ramification, and molecular weights will be strongly dependent on the isolation and purification procedures. Patents have been filed related to the manufacturing of certain preparations with specific characteristics. This includes the isolation of particles retaining the intact three-dimensional cell wall structures and morphology of the yeast [23]. The procedure to obtain a glucan particle or a glucan extract consists of an initial step involving the extraction and purification of an alkaline-insoluble fraction. This simultaneously removes “impurity” constituents such as mannoprotein and other proteins [18]. Relatively harsh conditions are mediated by potassium or sodium hydroxide solutions and may include boiling temperature. This may degrade the beta-glucan chains and intramolecular bounds including those to chitin and proteins in the cell wall. However it might, according to patents [23], be well controlled in such a way that the three-dimensional matrix of beta-glucan particle is kept intact. Then a subsequent purification step may be introduced by applying relatively mild acids in the pH range from 2 to 6 [18]. It is generally known that 1→6 linkages are more acid labile than the corresponding 1→3 linkages and therefore some controlled design of linkage distribution can be accomplished. In addition, certain enzyme systems such as laminarinase and other glucanases, might be introduced to further modify the proportion between 1→6 and 1→3 linkages.

Not all the commercial beta-glucan preparations or products are virtually pure beta-glucans. In addition to other constituent sugars such as mannose, protein and lipopolysaccharide (LPS) can be detected. This issue is not always considered when a certain relation between structure and biological activity is sought for in a system for testing, in vivo or in vitro [24]. Product contamination by bacterial endotoxin (LPS) may cause false-positive results in vitro, since these structures are potent immune stimulatory substances. Enterocytes as well as macrophages and dendritic cells present

Yeast β -1,6/1,3- glucanYeast β -1,3/1,6- glucan**Figure 1.** Structural elements of yeast beta-glucans.

in the intestinal mucosa on the other hand, are low responders to LPS and microorganisms. The gut phagocytes lack the signaling receptors found in phagocytes present in blood and other tissues. They are therefore not producing inflammatory responses in presence of LPS and foreign antigens [25, 26] and presence of LPS in oral preparations can be considered less critical than in preparations meant for injection as well as in *in vitro* studies on monocytes, macrophages, or differentiated dendritic cells.

For a preparation, the reported molecular weight, the degree of branching and side chain length will depend on source as well as the selected production process. To acquire certain analytical data (molecular weight and linkages), proper solubilization is compulsory since this is an indispensable part of qualitative and quantitative analysis of any polysaccharide. If the solubility is inadequate, well defined, and reproducible, descriptive structural data for a certain medical preparation or a powdered formulation may be hard to obtain. However, as noted by Jamas et al. [23], some hydrodynamic parameters such as shape factor and hydrodynamic volume can be determined by viscosimetry. Aggregates of triple helix moieties are further complicating features relevant for beta-glucans preparations, especially important since conformational structures are often considered crucial for some biological effects and responses. Solubilization for physicochemical studies and analytical procedures include DMSO and hot alkali, with the latter having an additional effect of disruption of triple helical structures [27]. It has been demonstrated by size exclusion chromatography and small-angle x-ray scattering that aqueous solutions of scleroglucan and PGG-glucan have rod-like rigid conformations formed by aggregates. In average three and nine aggregated helices were estimated, respectively in the deduced microfibrillar super structures [28]. When in

solution or in a gelatinous state, it is reasonable that both the chemical primary structure and the ordered helical states are fairly stable. This stability is also plausible at elevated temperatures such as boiling during food preparation, and during the subsequent passage in the human digestive tract at varying pHs and shear rates until fermentation by the gut microbiota.

3 Human studies

Search in the literature has identified 12 human studies performed on the effects of orally administrated yeast-derived beta-glucans, Y-BG. The studies are summarized in Table 1.

Six of the studies are addressed to effects on upper respiratory tract infections (URTI) [29–34]. Following heavy exercise, athletes are susceptible to URTI due to a weakened immune system. Physically stressed subjects having run marathon were randomly assigned to daily doses of 250 mg or 500 mg beta-glucan versus placebo ($n = 25$ in each group) for 4 weeks [29]. After 2 weeks, 32 and 24% in the 250 and 500 mg beta-glucan group, respectively, reported symptoms of URTIs versus 68% in the placebo group. After 2 weeks of treatment, 8% in both treated groups and 24% in the placebo group reported such symptoms. The differences were reported as statistically significant. The same preparation and dosages were also tested for effects in self-reported psychologically stressed individuals ($n = 150$). After 2 weeks, five subjects in the 250 mg treated group ($n = 50$) and four in the 500 mg group ($n = 50$) reported symptoms of URTI compared to 16 in the placebo group ($n = 50$). After 4 weeks, four subjects in both treated groups, and 14 in the placebo group reported symptoms [30]. The differences were reported as statistically

Table 1. Human studies on orally administered yeast beta-glucan

Preparation	Subjects	Study design	Study outcome	References
Soluble, 20 kDa, 1:10–1:15 sidechains, LPS < 0.05 EU/mL (SBG Biotec Pharmacon)	Healthy volunteers	O; 100, 200, or 400 mg/day as mouth wash followed by swallowing for 4 days. (<i>n</i> = 6 in each dosage group) 30 days (<i>n</i> = 12)	Increased IgA in saliva after 400 mg/day, serum IgG, TNF- α , IL-6, and IL-1 β unchanged. Could not detect beta-glucan in plasma	[39]
Seventy micrometer particles, 85% insoluble fibers, < 3% fat, < 2% protein (Biolex-Beta®)	Overweight humans (BMI 30–33)	R, DB, PC, CO; 1500 mg/day for 30 days (<i>n</i> = 12)	Increased plasma IL-10 (<i>n</i> = 10) and IL-10 mRNA in adipose tissue (<i>n</i> = 7). Unchanged insulin sensitivity, serum CRP, plasma IL-6 and MCP-1, adipose tissue TNF- α , mRNA, CD68 mRNA, and MRC-1 mRNA.	[42]
Soluble (Imuneks® Mustafa Nevzat Pharmaceuticals)	Seasonal allergic rhinitis patients	R, DB, PC, PG; 20 mg/day (<i>n</i> = 12) or placebo (<i>n</i> = 12) for 12 weeks	Decreased IL-4 and IL-5 and increased IL-12 in nasal lavage fluid and decreased eosinophils present following treatment	[44]
Particulate (Wellmune WGP® Biothera)	Marathon athletes	R, DB, PC 250 (<i>n</i> = 25) or 500 mg/day (<i>n</i> = 25) or placebo (<i>n</i> = 25) for 30 days post marathon	After 2 weeks, 32% (250 mg) and 24% (500 mg) reported symptoms of URTIs versus 68% (placebo). After 4 weeks, 8% in treated groups and 24% in placebo group. Improved psychological symptoms.	[29]
Particulate (Wellmune WGP® Biothera)	Healthy adults during cold/flu season	R, DB, PC, PG; 500 mg/day (<i>n</i> = 21) or placebo (<i>n</i> = 19) for 90 days	No significant differences in incidence of URTIs. Lower average fever score and less missed days at work/school in treated group versus placebo group	[31]
Particulate (Wellmune WGP® Biothera)	Healthy moderate to highly stressed humans	R, DB, PC 250 (<i>n</i> = 50) or 500 mg/day (<i>n</i> = 50) for 30 days	Fewer subjective symptoms of URTIs in both treated groups (8%) versus placebo (28%) after 4 weeks. Improved well-being.	[30]
Particulate (Wellmune WGP® Biothera)	Healthy students during cold/flu season	R, DB, PC; 250 mg/day (<i>n</i> = 48) or placebo (<i>n</i> = 49) for 3 months	Insignificant reduction in number of days with URTI. Self-reported better ability to “breathe easily.” No difference in IL-1 β , IL-8, MIP-1 α and β , G-CSF or MIG, lower MCP-1 in treated group.	[32]
Particulate (Yestimun®, Lieber), 85% Y-BG 10:1:0.6 backbone: side chain: branch	Healthy adults with recurring common colds	R, DB, PC; 900 mg/day (<i>n</i> = 41) or placebo (<i>n</i> = 44) for 26 weeks	Treatment had no effect on total number of common cold during 26 weeks. Significantly less episodes of infection during the 13-week cold season (ITT, <i>p</i> = 0.02; PP, <i>p</i> = 0.045).	[33]
Particulate (Yestimun®, Lieber), 85% Y-BG 10:1:0.6 backbone: side chain: branch	Healthy adults with recurring common cold episodes	R, DB, PC; 900 mg/day (<i>n</i> = 81) or placebo (<i>n</i> = 81) for 16 weeks	Total number of common cold episodes in the treated group (1.06 ± 0.89) was reduced compared with placebo (1.36 ± 0.94) <i>p</i> = 0.041	[34]
Microparticulate (Immutol®, Biotech Pharmacon ASA)	Patients scheduled for coronary artery bypass grafting	O; 700 mg (<i>n</i> = 12), 1400 mg (<i>n</i> = 11), and controls (<i>n</i> = 22) receiving no treatment for 5 days before surgery	Creatine kinase isoenzyme MB was lower in the 1400 mg group (<i>p</i> = 0.028) one day post surgery and troponin T (<i>p</i> = 0.028) 5 days post surgery compared to control	[43]
Immudyne®/MacroForce®	Cancer patients	O; 7.5 mg/day 7 days before surgery and for 15 months (<i>n</i> = 26) versus untreated controls (<i>n</i> = 23)	No relapse in treated group (<i>n</i> = 26) versus 5/23 (22%) relapse in control group	[46]

Table 1. Continued

Preparation	Subjects	Study design	Study outcome	References
Immudyne®/MacroForce®	Postsurgical cancer patients with relapse	O; 15 mg/day (<i>n</i> = 54) versus untreated controls (<i>n</i> = 45)	Treated group: 35 (65%) survived > 3 months, 23 (45%) > 6 months. Control group: 2 (4.4%) survived 3 months and none for 6 months	[46]
Soluble (Imuneks® Mustafa Nevzat Pharmaceuticals)	Female patients with metastatic breast cancer	O; 20 mg/day (<i>n</i> = 23) for 14 days	Stimulated activation and proliferation of peripheral blood monocytes following treatment, i.e., increased monocyte count, increased expression of CD95, and CD45RA on CD14-positive monocytes	[45]

O = open; R = randomized; DB = double blinded; PC = placebo controlled; PG = parallel group; *n* = number of subjects; G-CSF = granulocyte colony-stimulating factor; ITT = intention to treat; LPS = lipopolysaccharide; MCP-1 = monocyte chemoattractant protein-1; MIG = monokine induced by interferon gamma; MIP = macrophage inflammatory protein; PP = per protocol; TNF- α = tumor necrosis factor alpha.

significant. Both studies were, however, not based on prior sample size calculations, primary and secondary endpoints were not defined, adjustment for multiple testing was not done, paired *t*-tests were stated to have been applied for comparisons between independent groups and the diagnosis was not verified by physicians. Furthermore, sample size was low in the first study.

In spite of these weaknesses, the results are promising, suggesting that Y-BG may protect against URTI in both physically and mentally stressed persons. They also suggest that daily doses of 250 mg may be sufficient for exerting effects on URTI.

In a recent study, daily doses of 900 mg particulate Y-BG, taken over a 26-week period, did not show protective effect in healthy individuals with increased susceptibility to common cold based on the primary parameter of total number of episodes ($p_u = 0.41$). The immune status of these subjects was not reported, but the inclusion criterion was at least three common cold episodes within the last 6 months. However, during the common cold period, 13 weeks from November to March, authors reported lower total number of infections in the beta-glucan group (per protocol [PP] population) compared to the placebo group, ($p = 0.045$). The average number of infections was 29% lower in the beta-glucan intention to treat population (ITT) compared to the placebo group ($p = 0.022$) [33]. The study, however, suffered from several drawbacks; it was not based on a sample size calculation, there was no adjustment for multiple testing and the definition of ITT did not seem to follow the predefined definition, since drop-outs during intervention were excluded from ITT and were higher in the glucan group ($n = 5$) compared to the placebo group ($n = 1$).

Similar results were reported from a larger trial with a total of 146 participants given the same particulate preparation 900 mg daily for 16 weeks; Y-BG reduced the average number of common cold infections by 25% compared with placebo in the PP population (1.06 ± 0.89 versus 1.36 ± 0.94 , $p = 0.041$), but failed to reduce it significantly in the ITT population ($p = 0.12$). Y-BG treatment reduced cold-related sleep difficulties, but the duration of URTI episodes was not altered [34]. The study suffered from several weaknesses; lacking adjustment for multiple testing and lack of unequivocal definition of the primary endpoint (total and/or average number of episodes), posthoc exclusion of a site of investigation and exclusion of drop-outs from ITT. Taken together, the results suggest that this preparation of particulate Y-BG may provide some protection from URTI. For providing sufficient evidence, confirmation from well designed and conducted clinical trials with higher sample size is required.

In two randomized, double-blind, placebo-controlled, parallel-group trials conducted during the cold/flu season, healthy individuals received either 500 mg [31] or 250 mg [32] particulate beta-glucan. In the former, a pilot trial in 40 subjects [31], Feldman et al. found no significant differences in the incidence of URTIs, which was the predefined primary goal, between Y-BG and placebo group during a 12-week

intervention period. They, however, found that participants in the treated group had lower average fever score than the placebo group (PP: 0.00 ± 0.00 versus 3.50 ± 3.42 ; $p = 0.042$) and did not miss any days at school or work versus 1.38 ± 1.25 days in the placebo group ($p = 0.026$) [31].

The Fuller study [32] aimed at investigating whether beta-glucan decreases the frequency and severity of URTI symptoms over 90 days during the peak URTI season in 100 healthy university students. Beta-glucan tended to decrease the total number of days with URTI symptoms experienced within the whole group during the 90 days trial. In the placebo group ($n = 49$), symptoms of URTI were registered in 5.5% of the total number of subject days compared to 4.6% in the Y-BG group ($n = 48$), $p = 0.06$. In addition, a self-reported improved ability to “breathe easily” was found in the treated group compared to placebo. None of the individuals experiencing symptoms of URTI tested positive for influenza A (H1N1) infection. Further on, analysis of blood samples showed no difference in IL-1 β , IL-8, macrophage inflammatory protein-1 α and β , granulocyte colony-stimulating factor, or monokine induced by IFN- γ levels in either group. Monocyte chemotactic protein-1 was significantly lowered in the treated group compared with placebo [32].

Taken together, these results indicate that particulate Y-BG may provide some protection from URTI. However, this needs further confirmation from well conducted clinical trials with higher sample size. At present, a metaanalysis on the overall evidence of effects by beta-glucans in general and by specific preparations on URTIs is lacking.

IgA provides protection against infections, and a decreased level of secretory IgA (sIgA) in sport athletes has in some studies been shown to correlate with increased risk of developing URTI [35–37]. Decreased levels of sIgA have also been observed in populations living under chronic stress [38], which may explain an increased susceptibility to infections. Interestingly, sIgA increased in healthy volunteers having received 400 mg soluble Y-BG once daily for four consecutive days, suggesting a protective effect against infections of the oral mucosa. The preparation was administered as a mouthwash for 2 min and then swallowed. The administered Y-BG could not be detected in serum indicating lack of systemic absorption [39]. Experimental data have shown that after oral immunization, IgA plasma cells originating from gut-associated lymphoid tissue are able to migrate to the respiratory mucosa [40, 41] thus providing a possible route for Y-BG activity against URTI.

Daily ingestion of 1500 mg particulate Y-BG for 30 days provided increased plasma levels of the anti-inflammatory cytokine IL-10 and IL-10 mRNA in adipose tissue in overweight to obese subjects with moderately elevated C-reactive protein (CRP) levels. The elevated CRP levels were not due to infections. Y-BG administration did not alter CRP levels, insulin sensitivity, or monocyte chemotactic protein-1 levels [42].

Oral Y-BG showed a possible cardio-protective effect in patients undergoing coronary artery bypass grafting. Patients pretreated with 1400 mg of a microparticulate Y-BG prepara-

tion 5 days before surgery showed lower release of myocardial enzymes, creatine kinase isoenzyme MB and troponin T, compared to controls while inflammatory parameters such as CRP, IL-6, and white blood cell count did not change, except for a significant IL-8 increase on the fifth day post surgery in the group pretreated with only 700 mg Y-BG. The authors conclude that oral pretreatment with Y-BG could not attenuate the inflammatory response that accompanies coronary artery bypass grafting [43].

Y-BG (20 mg/day) orally administered to patients with seasonal allergic rhinitis against olive pollen was associated with decreased levels of Th2 cell derived IL-4 and IL-5 in nasal lavage fluids and increased levels of Th1 cell derived IL-12 while IFN- γ remained unaltered. In addition, the counts of eosinophils decreased in the microenvironment. Authors concluded that Y-BG may have a role as an adjunct to standard treatment in patients with allergic rhinitis [44].

Oral administration of Y-BG has also been applied to cancer patients. A soluble Y-BG preparation administered to breast cancer patients, 20 mg daily for 2 weeks, stimulated proliferation and activation of peripheral blood monocytes. Full blood analysis showed that an increased monocyte count in addition to increased expression of CD95 and CD45RA on CD14-positive monocytes occurred following beta-glucan treatment [45]. In an open study, it was found that oral administration of Y-BG (7.5 mg/day for 15 months) offered a protective effect against cancer relapse after surgery. There were no relapses in the treated group compared to 22% (5/23) in the control group that did not receive additional treatment or placebo. The study included patients with different types of cancer, and the preparations used were not described with regard to Mw and solubility. Y-BG (15 mg/day) was also administered to inoperable cancer patients with relapse. Only 4.4% of the untreated controls survived for 3 months, and none after 6 months whereas in the treated group 65% survived for more than 3 months and 43% were still alive after 6 months. The selection of patients and their allocation to treatment or no treatment were not described [46]. Due to study limitations, additional trials are needed to confirm these results.

Orally administered Y-BG seems to be well tolerated in healthy subjects [31–33, 39]. For short-term treatment at doses of up to 400 mg daily, there were no changes in biochemical- or hematological parameters or on heart rate and blood pressure [39]. Higher doses, 1400 mg daily for 5 days, was well tolerated by patients undergoing coronary artery bypass grafting [43].

After 90 days of daily administration of 250 mg Y-BG, hematologic, biochemical, and liver function tests remained normal, and only few unspecified side effects were reported [32]. At higher doses, 500 mg daily for 90 days, 52% in the treated group and 47% in the placebo group reported side effects. Interestingly, nine subjects (42%) in the treated group and five subjects (26%) in the placebo group reported having experienced infections and infestations (MedDRA code). However, authors did not comment on this and conclude

that the reported adverse effects were not considered to be related to the Y-BG preparation and were of mild-to-moderate severity [31].

After 180 days given 900 mg daily, clinical parameters such as body weight, temperature, heart rate, and blood pressure did not change significantly in healthy subjects, and adverse events reported were considered unlikely connected to beta-glucan intake [33]. Two cases of rashes and one with gastric pressure were probably related to Y-BG intake [34].

4 Animal studies

Animal studies have shown prophylactic effect of Y-BG against bacterial and LPS-induced infections. Prophylactic treatment increased survival time by 30–40% in mice when given Y-BG in high doses (150 or 300 mg/kg) 10 days prior to i.p. infection with *Listeria monocytogenes*. Y-BG normalized bone marrow and spleen granulocyte macrophage progenitor levels in infected animals, and colony-stimulating factor, IL-6, and IL-1 α in bone marrow. IFN- γ production by splenocytes increased relative to untreated infected controls. Lower doses (50 mg/kg) did not show these effects. In addition, it was noted that immunological parameters did not change in noninfected animals [47], indicating that healthy individuals did not respond to the treatment. Y-BG (0.2 and 20 mg/kg) was shown to protect against anthrax (*Bacillus anthracis*) infection in mice [48]. A protective effect was also observed in rats given 20 mg/kg for 2 weeks prior to i.v. injection of *E. coli* LPS. Y-BG provided enhanced recovery of mean arterial blood pressure and protection against kidney and liver injury, measured as decreased creatinine, ASAT, and ALAT levels [49]. Even lower doses (0.1–10 mg/kg) of Y-BG showed significant effects in rats with LPS-induced mastitis; dectin-1 was activated while Toll-like receptor-4 was downregulated in beta-glucan-treated animals. Further on, proinflammatory cytokines tumor necrosis factor alpha and IL-1 β decreased in mammary tissue while serum IL-2 increased [50].

Effects on mucosal immunity in the digestive tract have been observed after oral administration of Y-BG. Mice receiving 25 mg daily (corresponding to 1250 mg/kg) increased the number of intraepithelial lymphocytes in the intestine and the number of CD8+ $\gamma\delta$ T cells (cytotoxic T cells) and IFN- γ [51]. In dogs, oral supplementation of beta-glucan increased serum IgM and decreased serum and mucosal IgA concentrations while IgG concentrations did not change suggesting that the observed effects were due to interaction with the intestinal mucosa [52]. In gnotobiotic pigs on the other hand, there were no changes in serum antibody concentration or in the volume of Peyer's patches after feeding 200 mg Y-BG daily, indicating that the mucosal immune response is dependent on the intestinal microflora [52].

Orally administered Y-BG significantly reduced stress induced IL-12, IL-6, and IFN- γ in mice spleen cells, phagocytosis returned to the normal level and stress-induced cortisone was decreased. Beta-glucan had no effect on

cortisone level of normal unstressed subjects while IL-6 and IL-12 levels increased in both stressed and normal animals [53].

In animal models, orally administered Y-BG has shown additive activity in anticancer treatments. Particulate (400 μ g/day) [54] and soluble Y-BG [55] enhanced the efficacy of monoclonal antibody immunotherapy in mammary tumor bearing mice. In addition, soluble Y-BG (400 μ g/day) administered in combination with the chemotherapeutic alkylating agent cyclophosphamide delayed tumor development in B-cell lymphoma bearing mice compared with animals that received only cyclophosphamide [56]. Similar effect was found when Y-BG was given in combination with peptide vaccination against B-cell lymphoma [57]. Soluble Y-BG alone has not shown the same effect [54, 57] while single treatment with particulate Y-BG (400 μ g/day) retarded tumor growth [55, 58]. The latter effect is related to particulate Y-BG binding to dectin-1 on activated dendritic cells. This promotes Th1 and Tc responses in the tumor microenvironment, leading to tumor regression. The adjuvant effects of soluble Y-BG seem to be due to a dectin-1-independent pathway [55], and antitumor monoclonal antibody mediated cytotoxicity is suggested to be mediated by complement activation and activation of CR3 [55, 59].

Pretreatment with 50 mg/kg Y-BG protected against ischemia/reperfusion injury in kidney in a rat model [60] and reduced infarction size in an ischemia/reperfusion porcine model [61]. In animal models, oral administration of Y-BG has shown to lower the toxic effects of mercury [62], to limit side effects associated with methotrexate treatment [63] and to provide protection against acetaminophen (paracetamol) toxicity [64]. Animal studies suggest low toxicity; the LD₅₀ value of particulate Y-BG was estimated higher than 2 g/kg body weight in rats. No toxic or adverse effects were observed after subchronic administration of doses up to 100 mg/kg for 13 weeks [65].

5 Pharmacokinetics

To what degree and by which mechanisms Y-BG may reach systemic circulation remain controversial. Intestinal uptake may occur via microfold- or membranous- (M-) cells in the Peyer's patches present in the intestine. Both soluble antigens and particulate material (0.3–4 μ m) are taken up by M-cells [66] including yeast particles (3.4 μ m) as demonstrated in minipigs [67]. In mice, both microparticulate (1–2 μ m) and aggregated beta-glucan particles (5–100 μ m) were equally found to activate peritoneal macrophages after oral ingestion [68]. In a human study, intake of a particulate (70 μ m) Y-BG preparation changed immunological parameters in obese individuals [42], but whether the effect was due to intestinal uptake is not known. The particle size of preparations used in other human trials were not defined (Table 1).

Table 2. Pharmacokinetics of orally administered beta-glucans

Beta-glucan	Detection	Subject	Dosage	Bioavailability	Max serum concentration	Detected in organs	References
Soluble laminarin Mw 7.7 kDa	Fluorescein labeled	Mice	1 mg/kg	4.9%	115 ng/mL		[69]
Soluble scleroglucan Mw 1020 kDa	Fluorescein labeled	Mice	1 mg/kg	4.0%	355 ng/mL		[69]
Soluble laminarin Mw 5.3 kDa	¹²⁵ I labeled	Sprague– Dawley rats	250 µg/rat	–	<0.5% ^{a)}	Liver (0.5–1.5%) ^{a)} Kidney (0.2–1%) ^{a)} Stomach and intestines (most)	[70]
Soluble from yeast	Fungitel TM	Mice	20 mg/kg	–	< 10 ng/mL	–	[49]
Particulate from yeast	Fluorescein labeled	Mice	400 µg /mouse	–	–	Spleen, lymph nodes, and bone marrow	[59]
Soluble from yeast 20 kDa	Glucatel TM	Human	100, 200, or 400 mg/day	–	Nonsignificant levels	–	[39]

a) Percentage of ingested amount of beta-glucan.

Uptake from the gut is not restricted to particulate preparations and is independent of dectin-1 [69], which is expressed on phagocytes (macrophages, dendritic cells, and neutrophils). Soluble beta-glucans have been detected at lower concentrations in serum after oral administration to rodents [49,69,70], see Table 2. These beta-glucans were from different sources (yeast, seaweed, and mushroom), with varying molecular weights (5–1000 kDa); administered at different doses (1–20 mg/kg), but all were water soluble. In fact, Rice et al. reported that soluble glucan, but not particulate glucan could be detected in serum after oral administration to mice [69]. Measurable serum concentrations of beta-glucans were not detected in humans following oral ingestion of a soluble preparation [39]. This may of course be due to limitations of the detection method used. Additional human studies on beta-glucan pharmacokinetics are needed and methods to determine the occurrence and quantity of beta-glucans in tissue and blood samples might need improvements. Methods such as specific labeling of the reducing terminal of the chains is a good strategy to minimize structural effects induced by alternative unspecific labeling technology [69]. Pharmacokinetic studies on beta-glucans indicate low systemic uptake in animals (Table 2); 4–5% for soluble fluorescein-labeled laminarin and scleroglucan [69]. Most of the ingested glucan was detected in the stomach and the intestines while small amounts were detected in serum as mentioned above; less than 0.5% of administered ¹²⁵I-labeled laminarin were detected in rat serum [70], 1–10 ng/mL unlabeled Y-BG [49] and up to 115 and 355 ng/mL of laminarin and scleroglucan, respectively [69] were detected in mice serum after oral administration. After intestinal uptake, beta-glucans are transported to lymphoid organs; to the liver, kidneys [70], to the lymph nodes, spleen [58,59], and to the bone marrow [59]. Studies on intraperitoneally administered BG have shown accumulation in liver, spleen, and kidney indicating very long elimination rates [17]. After absorption into Peyer's patches,

beta-glucan particles migrate to layers beneath the basal lamina where they are phagocytized by macrophages and transported out of the Peyer's patch domes [67]. From studies in vitro, it is suggested that the macrophages then migrate to lymphoid organs and degrade the beta-glucans into smaller bioactive soluble fragments within the macrophages before the fragments are released from the macrophages into the environment [59]. This may explain the low-serum concentrations detected in pharmacokinetic studies. The optimal particle size for phagocytosis into macrophages is 1–2 µm [71], and phagocytosis of 3 µm Y-BG and zymosan particles have been demonstrated in vitro [72]. Phagocytosis is initiated by binding to the beta-glucan recognizing receptor dectin-1 on the phagocytes. It has been shown that activation of the receptor requires binding of particulate Y-BGs whereas soluble Y-BG is less effective [72]. Similar observations have been made with beta-glucan from *C. albicans* [73].

It should be noted that immunological effects may be mediated by other mechanisms besides Y-BG uptake to the systemic circulation. Immunological effects may be induced by sensing luminal presence of microbial-derived compounds or metabolites via transepithelial processes of dendritic cells, which then may stimulate neighboring immunocytes being able to enter circulation [74].

6 Posology

The doses administered vary considerably in different human trials ranging from 7.5 mg to 1500 mg daily (Table 1) corresponding to 0.1–20 mg/kg body weight. Studies on particulate Y-BG effect on URTI suggested activities at 250, 500, and 900 mg daily doses, indicating that 250 mg/day may be sufficient. However, comparing dosages for different preparations is not straightforward since different strains of

S. cerevisiae may have been taken into use in addition to different isolation procedures, both affecting the primary structure of the final preparation as mentioned above. In other studies, there are large variations in the dosages applied, and the selected outcome parameters also vary. Increases in IL-10 levels were observed following ingestion of large doses (1.5 g/day) of particulate Y-BG in overweight individuals whereas lower doses were not tested [42]. Soluble preparations were shown to change immunological parameters at significantly lower doses; only 20 mg daily altered immunological parameters in allergic [44] and in breast cancer patients [45].

As mentioned above, the different doses administered in human trials vary, but in animal studies, the range of different dosages varies even more; up to 1250 mg/kg was administered to mice resulting in increases in mucosal immunity [51]. This corresponds to 87.5 g beta-glucan daily given to a 70 kg adult. Furthermore, daily doses corresponding to 10.5 or 21 g to a 70 kg human were necessary for prophylactic effect against bacterial infection while the lower dose corresponding to 3.5 g/day, which still is quite high compared to those used in the human trials, had no effect [47]. The relevance of studies using unrealistically high doses is highly questionable [75]. One might, however, admit considerably higher doses per body weight in rodents due to their higher metabolic activity [76]. Based on a 333-fold higher metabolic rate or energy expenditure in 70 kg weighing humans compared to 30 g weighing mice [76], the highest dose applied in mice translates to 12.5 g in 70 kg weighing humans instead of 87.5 g (when different metabolic activities of both species were not taken into account). On the other hand, doses used in cancer adjuvant trials in animals [54, 56, 57] correspond to those used on humans, 400 mg/day on a body weight basis, and have shown additive effect in animal models, both from particulate and soluble preparations. These doses are quite low and even lower when metabolic activity is considered even when metabolic activity is considered.

7 Conclusion

Results from clinical trials suggest that orally administrated yeast-derived beta-glucan may decrease the number of infections in physically or psychologically stressed persons and in individuals prone to infections of the upper respiratory tract. However, additional well-designed and conducted and sufficiently powered trials with well-defined preparations are needed to confirm this. Whether variations in preparation characteristics may affect product efficacy has yet to be clarified. Other potential health benefits of Y-BG also need further documentation.

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Potential conflict of interest statement: Immitec Norge AS, which is one of the industrial partners in Optifiber and FibeBiotics, is a European supplier of products containing WGP® Wellmune. Leiber GmbH marketing Yestimun is part of the industrial platform of FibeBiotics. The referring to WGP as well as other commercial beta-glucan preparations in the review text is from scientific works performed elsewhere by others.

8 References

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