Preparation, characterization, and biological properties of β-glucans

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ABSTRACT

β-Glucans are soluble fibers with physiological functions, such as, interference with absorption of sugars and reduction of serum lipid levels. β-glucans are found in different species, such as, *Rhynchelytrum repens*, *Lentinus edodes*, *Grifola frondosa, Tremella mesenterica*, *Tremella aurantia, Zea may, Agaricus blazei, Phellinus baummi, Saccharomyces cerevisae* (yeast), and *Agaricus blazei murell* (mushroom). Analysis of the fractions reveals the presence of arabinose, glucose, xylose, and traces of rhamnose and galactose. The presence of β-glucan in these fractions is confirmed by hydrolyzing the polymers with endo-β-glucanase from *Bacillus subtilis*, followed by high-performance liquid chromatography (HPLC) analysis of the characteristic oligosaccharides produced. The 4 M KOH fractions from different tissues are subjected to gel permeation chromatography on Sepharose 4B, with separation of polysaccharides, with different degrees of polymerization, the highest molecular mass (above 2000 kDa) being found in young leaves. The molecular mass of the leaf blade polymers is similar (250 kDa) to that of the maize coleoptiles β-glucan used for comparison. The 4 M KOH fraction injected into rats with streptozotocin-induced diabetes has shown hypoglycemic activity, reducing blood sugar to normal levels for approximately 24 hours. This performance is better than that obtained with pure β-glucan from barley, which decreases blood sugar levels for about four hours. These results suggest that the activity of β-glucans is responsible for the use of this plant extract as a hypoglycemic drug in folk medicine.

Key words: Cell wall, diabetes mellitus, grasses, hypoglycemic activity, rhynchelytrum repens, β-glucans

INTRODUCTION

β-Glucans are carbohydrates (sugars) that are found in the cell walls of bacteria, fungi, yeasts, algae, lichens, and plants such as oats and barley. They are taken as herbal

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medicines, to prevent and treat cancer, lower cholesterol, human immunodeficiency virus (HIV), and diabetes, and to increase the immune system function. Other names for β-Glucans include: Beta Glycans, beta-1, 3-glucan, and beta-1, 3/1, 6-glycan. β-glucan is a soluble fiber derived from the cell walls of algae, bacteria, fungi, yeast, and plants. β-glucan found in yeast and mushrooms contains 1, 3-glucan linkages and occasionally 1, 6 linkages, whereas, the β-glucans from grains (i.e., oats and barley) contains 1, 3 and 1, 4 linkages (1; 2). The yeast-derived beta-1, 3/1, 6 glucan purportedly has greater biological activity than the 1,3/1, 4 counterparts. Different β-glucan linkages are shown Figure 1.[1]

Carbohydrates are extensively used as food for humans and animals. They are very important as sources of raw materials for alcoholic beverages, as food additives, and also in the pharmaceutical industry. Plant carbohydrates present in the food consumed by humans and animals

contain soluble or insoluble fiber-like polymers.[2] There are two types of dietary fibers: Insoluble and soluble, which can be distinguished by their solubility in aqueous solutions. Although the insoluble fibers (cellulose, lignin, and some hemicelluloses) are insoluble in water, the soluble ones (hemicelluloses and pectin) form viscous solutions in water. Soluble fibers can form an unstirred water layer in the gut, which decreases absorption of sugars and lipids. Thus, to some extent, soluble fibers can be used to prevent the postprandial increase of glucose, being therefore useful for the treatment of diabetes at certain levels.[3]

β-Glucans are plant hemicelluloses polysaccharides (soluble fibers) that are recognized as hypocholesterolemic compounds.[4,5] It has been demonstrated in humans that hyperlipidemic individuals may have a decrease of up to 7.5% in serum cholesterol.[6] β-Glucans, as well as several other viscous plant polysaccharides (e.g., guar, locust bean, and pectin), display physiological effects that are typically attributed to a decrease in postprandial glucose levels in the serum. This effect has been related to the property of this polymer to form an unstirred water layer, which by resisting the convective effects of intestinal contractions, decreases sugar absorption by the small intestine.[7] This effect has also been associated with foods such as oats, barley, beans, and gums, which is known to accumulate relatively large amounts of β-glucans.[8-11]

Many tropical grass species, especially those from Africa, such as *Rhynchelytrum repens* (Willd.) C.E. Hubb. (Poaceae), have been considered invaders of the American natural reserves[12] because of their capacity to replace the native species.[13] The plant is used popularly as a phytotherapeutic remedy for the treatment of diabetes in Brazil, and in a recent report the precipitate of the aqueous extract of *R. repens* has been shown to significantly reduce plasma glucose levels when administered to diabetic rats.[14] As high molecular weight polymers are expected to predominate in alcohol precipitate fractions, polymers such as β-glucan are thought to be probable candidates to display biological activity.

## STRUCTURE AND RING SIZE DISTRIBUTION OF THE CYCLIC β-GLUCANS

The (1, 2)-linked D-glucosyl backbone structure of the cyclic, B-(1, 2)-glucans of the Rhizobium and Agrobacterium species was first revealed by methylation analysis, which yielded 3, 4, 6-tri – O-methyl-D-glucose as the only methylated product. The products of periodate oxidation and Smith degradation were also consistent with a 1-(1, 2)-linked backbone. A macrocyclic, unbranched form was proposed because of the absence of reducing and non-reducing terminal residues. The cyclic character of the glucans was unequivocally established by 13C
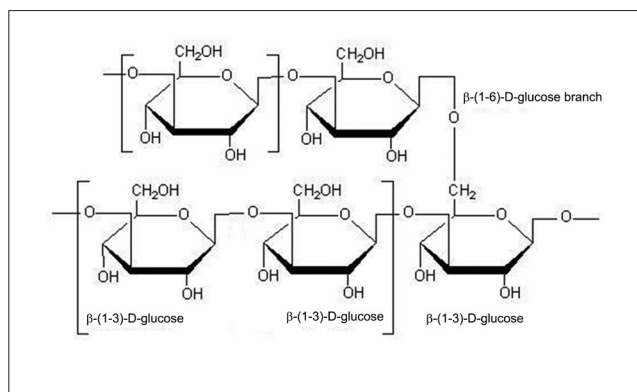
Figure 1: Beta-glucan linkages
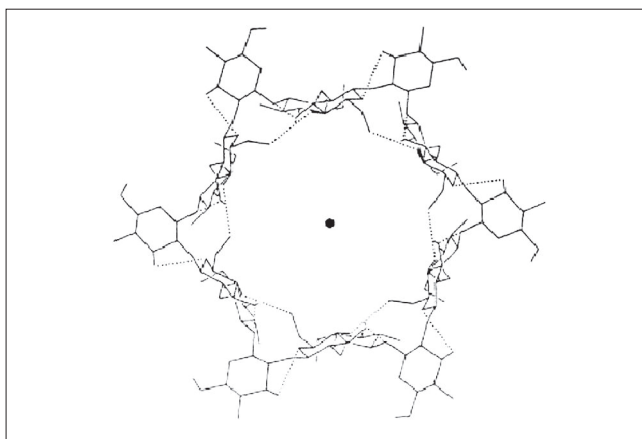
Figure 2: Minimum energy, maximum symmetry conformation of a neutral cyclic ß–(1, 2)-glucan (DP = 18.). Dotted lines indicate hydrogen bonds
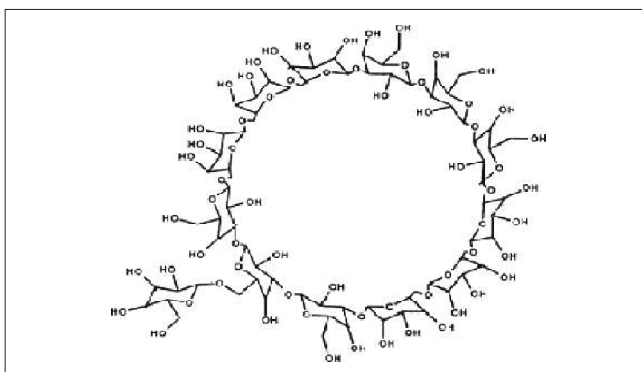
Figure 3: Proposed structure for the unsubstituted cyclic ß –(1, 6)-ß-(1, 3)-glucan of B. japonicum, containing 13 glucose residues

nuclear magnetic resonance spectroscopy and fast atom bombardment mass spectrometry. The β-anomeric configuration at the C-1 carbon atoms was suggested by optical rotation and confirmed by 'H and 13C nuclear magnetic resonance spectroscopy. A proposed structure of the symmetrical cyclic β-(1, 2)-glucan containing 18 glucose residues is shown in Figure 2. The proposed structure of the bradyrhizobial cyclic

Table 1: Properties of membrane-associated glucosyltransferases of the Rhizobiaceae linked to cyclic, 3-glucan biosynthesis

Organism	K_{mUDPG}^{b} (μM)	Metal requirements	Protein-linked intermediate	DP	Linkage
R. meliloti	-[c]	Mn = Mg	Yes	14-25[d]	β-(1,2)
R. phaseoli	33	Mn > Mg	-	17-20	β-(1,2)
A. tumefaciens	-	Mn = Mg	Yes	17-24	β-(1,2
A. radiobacter	50	Mn = Mg	-	17-24	β-(1,2)
R. leguminusarum	-	Mn = Mg	-	17-24	(1,2)
B. japonicum	50	Mn = Mg	ND[e]	13	β-(1,3)-β-(1,6)
B. japonicum	-	Mn > Mg	Yes	≥11	β-(1,2)-β-(1,3)
B. japonicumf	300	Mn = Mg	-		β-(1,2)β-(1,3)-β-(1,6)

[a]Activities have been characterized within crude membrane preparations; [b]UDPG, UDP-glucose; [c]not reported; [d]Reported as 14 – 25 glucose residues on the basis of elution behavior during gel filtration column chromatography; [e]ND, not detected; [f]Because of its growth properties, it was suggested by Cohen and Miller that this strain of *R joponicum* represented a fast growing *Rhizobium* strain

3-glucan (DP = 13) is shown in Figure 3. Table 1 shows the comparison of the ring size distribution of cyclic 3-(1, 2)-glucans from a variety of Agrobacterium and Rhizobium species.[15]

EXTRACTION

Plants of *Rhynchelytrum repens* (Willd.) C.E. Hubb. (Poaceae) are cultivated in a greenhouse at an average temperature of 26°C. After 20 days, the shoots of the plants are collected and subjected to extraction and fractionation of the cell wall carbohydrates. Shoots of *Rhynchelytrum repens* are divided into expanding leaf blades, sheath, stem, and young leaves. The same procedure is simultaneously performed with coleoptiles of maize, to be used as a standard.[16,17] Maize (*Zea mays* L.) caryopsis are soaked in tap water for 15 hours, sown on trays of moist vermiculite, and incubated in the dark at 30°C for two days. The upper two-thirds of the coleoptiles are collected and used for cell wall extraction and fractionation.

Different parts of *R. repens* plants and maize coleoptiles are sequentially extracted with 20 ml of 0.5% ammonium oxalate (pH 7.0), 0.1 M KOH, 1 M KOH, and 4 M KOH, at ambient temperature under an N_2 atmosphere, with continuous stirring. All extractions are performed for one hour, except for the 4 M KOH fractionation, which lasts for 15 hours. After each extraction, the unextracted cell wall components are pelleted by centrifugation, and the supernatant is filtered through nylon meshes. Alkali-soluble fractions are chilled to ice temperature and acidify with glacial acetic acid to pH 5.0. All fractions are dialyzed extensively against deionized water and freeze-dried. Each fraction is analyzed to colorimetric assays for uronic acid and total sugars.[18]

DIGESTION WITH ENDO-β-GLUCANASE

Cell walls from excise coleoptiles and from different plant parts of *Rhynchelytrum repens* are suspended in 100 μl of water and 20 μl of a preparation of *B. subtilis* endo-β-glucanase in 20 mM sodium acetate, and 20 mM NaCl, pH 5.5, is added. The samples are incubated for three hours at 37°C. The products released from the digestion of

purified β-glucan are mainly cellobiosyl-(1→3)-β-d-glucose and cellotriosyl-(1→3)-β-d-glucose, with smaller amounts of cellotetraosyl-(1→3)-β-d-glucose and cellopentaosyl-(1→3)-β-d-glucose. Enzyme reactions are stopped by heating for two minutes in a boiling water bath followed by cooling to ambient temperature and centrifugation at 10,000 *g* for five minutes.[19]

CHROMATOGRAPHY

The oligosaccharides from digestion of the *in vitro* reaction products are separated on a Carbo-Pack PA1 anion exchange column, equilibrated with 0.5 N NaOH, and eluted with a linear gradient of sodium acetate in 0.5 N NaOH, as described by Gibeaut and Carpita and detected with a pulsed amperometric detector.[20] For gel chromatography, the alkali extracts are applied to a 2.5 × 40 cm Sepharose 4B column (Sigma, St. Louis, MO, USA) equilibrated with McIlvaine's buffer (50 mM citric acid-100 mM Na_2HPO_4), pH 5.5. Fractions (3 ml) are collected and 500 μl of each is assayed for sugar using the phenol-sulfuric acid method.[19]

In order to check for the presence of β-glucan in the decoction of *R. repens* shoots, the water-soluble extract is subjected to precipitation with ethanol (three volumes) at 5°C for 18 hours. The material is centrifuged for 15 minutes at 18,000 *g*; the pellet is solubilized in 80% ethanol, and stirred and centrifuged as mentioned earlier. This procedure is repeated once more. The precipitated fractions are concentrated under reduced pressure for complete drying, resuspended in distilled water, and submitted to enzymatic hydrolysis, followed by high-performance anion exchange chromatography with pulsed amperometric detection analysis.

On the basis of the results obtained with the sequential extractions of different plant parts, the hypoglycemic effects are studied only with the combined 4 M KOH fractions, as these are the richest β-glucan containing fractions. All 4 M KOH freeze-dried fractions are pooled, solubilized in hot water, and then injected intraperitoneally into Wistar rats with streptozotocin-induced diabetes.

IDENTIFICATION OF BIOCHEMICAL AND FUNCTIONAL PROPERTIES OF β-GLUCANS

The content of β-glucans in barley can be affected by genetic and environmental factors, but generally falls between 3 and 6%. The content of arabinoxylans can be equally high in barley (3 – 6%). β-Glucans are unbranched homopolymers of D-Glc*p*, linked via β-1→3 and β-1→4 linkages. The linkage arrangement is not completely irregular; consecutive blocks of β-1→4 linkages, generally two or three, but sometimes up to 20, are separated by single β-1→3 linkages randomly. β-glucans extracted at higher temperature (65°C or 95°C) generally have a higher ratio of β (1→4)/(1→3) linkages than those extracted at 40ºC. The ratio of cellotriose to cellotetraose DP3/DP4 and the presence of longer cello oligosaccharides (DP > 9) is higher in β-glucans extracted at higher temperatures.

We have demonstrated that partially degraded β-glucans are capable of forming a gel-like network structure. Such structures are not detected in solutions of intact β-glucans. This behaviour is attributed to aggregation of the chains along the cellulose-like fragments in the chains of β-glucans. During malting, a majority of β-glucans gets degraded. We have examined the content and composition of non-starch polysaccharides in the commercial malt obtained from cv. Harrington. We have determined that the malt contained only 0.5% of β-glucans out of which 0.23% is soluble, whereas, approximately 0.38% remained insoluble in water, but could be extracted with alkali.[21]

DETERMINATION OF MOLECULAR WEIGHT OF β-GLUCANS

SEC-Calcofluor Detection
A high-performance size-exclusion chromatography system (HPSEC) has been set up, with detection based on the specific binding of Calcofluor to β-Glucans for determination of the amount and molecular weight of β-glucans in different cereal extracts. To calibrate the HPSEC system, purified β-glucans are fractionated into a narrow molecular weight range and the average molecular weight is determined before analysis on the HPSEC system. The detector response is similar for β-glucans from oats and barley and appears to be independent of the molecular weight. Four different methods for extraction of β-Glucans from different cereal products have been tested: Two alkaline, one with hot water and added amylase, and one with water and added xylanase. Inactivation of the endogenous glucanase is crucial for the stability of the extracts, even when extracting at high temperature or pH. Yields have varied widely between the different extraction methods, but the average molecular weight and molecular weight distribution are similar. Extractions with sodium hydroxide generally give a higher yield and molecular weight of β-Glucans in the extracts.[22]

IN VIVO STUDY USING A STREPTOZOTOCIN MODEL

The induction of diabetes and the animal treatment procedures are based on the study by Pepato *et al.* Male Wistar rats weighing 180 – 200 g are used for this experiment. The animals are housed in individual cages in a room with a 12/12 hour light/dark cycle and an ambient temperature of 22–25°C. They are fed a commercial stock diet containing (w/w) 9% fibers, 23% protein, 65% carbohydrates, and also containing adequate amounts of vitamins and mineral nutrients. The animals are used for the experiment after an acclimatization period of at least one week before the experimental sessions. Induction of diabetes is performed by injection of streptozotocin (40 mg/kg body weight; Sigma) dissolved in a citrate buffer with pH 4.5, into the dorsal vein of the penis of rats previously fasted for 14–16 hours. After the injection the animals are placed in metabolic cages with free access to water and to the same type of food they had received before administration of the drug.

The experimental groups are non-diabetic control rats, streptozotocin-induced diabetic control rats, streptozotocin-induced diabetic rats treated with a pooled fraction of 4 M KOH, and streptozotocin-induced diabetic rats treated with pure barley β-glucan (DGLUC) purchased from Sigma. Plasma glucose is determined by an enzymatic method (glucose-oxidase) before and two, four, six, eight, and 24 hours after administration of the extracts. In both treatments, 4 M KOH and DGLUC, the animals received injections of 100 mg extract per kg body weight and non-diabetic control rats and streptozotocin-induced diabetic control rats received saline solution. All extracts were administered intraperitoneally.

Data are analyzed statistically by analysis of variance (ANOVA) and the significance is assessed by the Tukey test, and *P* values of less than 0.05 are considered significant. Data are reported as means ± SEM for each group (*N*=10).[21,22]

HOW DOES β-GLUCAN WORK?

β-glucan taken orally differs from other food substances. This type of glucan is acid resistant so it passes through the stomach virtually unchanged. Macrophages in the mucous lining of the intestinal wall pick up the beta glucan particles through the beta glucan receptors. Immediate activation of these cells follows, and they are later able to travel back to the local lymph nodes (Payer's Patch) as part of their natural antigen-presenting function, to release cytokines and induce systematic immune activation. β-glucans may lower blood cholesterol by preventing the absorption of cholesterol from food in the stomach and intestines, when it is taken by mouth. When given by injection, beta glucans

may stimulate the immune system by increasing chemicals, which prevent infections.

ABSORPTION OF β-GLUCAN

For best results, β-1, 3-D glucan should be taken on an empty stomach. Enterocytes reportedly facilitate the transportation of β-1, 3 glucans and similar compounds across the intestinal cell wall into the lymph where they begin to interact with macrophages to activate immune function. Radio-labeled studies have verified that both small and large fragments of β-glucans are found in the serum, which indicates that they are absorbed from the intestinal tract. M cells within the Payer's Patches physically transport the insoluble whole glucan particles into the gut-associated lymphoid tissue (GALT).[23]

CLINICAL APPLICATIONS

β-glucans as Immunomodulating Agents

β-glucans are potent immunomodulators with effects on both innate and adaptive immunity. The ability of the innate immune system to quickly recognize and respond to an invading pathogen is essential for controlling infection. Dectin-1, which is a type II transmembrane protein receptor that binds β-1,3 and β-1,6 glucans, can initiate and regulate the innate immune response.[24-26] It recognizes β-glucans found in the bacterial or fungal cell wall, with the advantage that β-glucans are absent in human cells. It then triggers effective immune responses including phagocytosis and proinflammatory factor production, leading to the elimination of infectious agents.[27,28] Dectin-1 is expressed on cells responsible for the innate immune response and has been found in macrophages, neutrophils, and dendritic cells.[29] The Dectin-1 cytoplasmic tail contains an immunoreceptor tyrosine-based activation motif (ITAM) that signals through the tyrosine kinase in collaboration with Toll-like receptors 2 and 6 (TLR-2/6).[27,30,31] The entire signalling pathway downstream to dectin-1 activation has not yet been fully mapped out, but several signalling molecules have been reported to be involved. They are NF-κB (through Syk-mediate pathway), signalling adaptor protein CARD9, and the nuclear factor of activated T cells (NFAT).[32-34] This will eventually lead to the release of cytokines including interleukin (IL)-12, IL-6, tumour necrosis factor (TNF)-α, and IL-10. Some of these cytokines may play an important role in the cancer therapy. On the other hand, the dendritic cell-specific, ICAM-3-grabbing, non-integrin homolog, SIGN-related 1 (SIGNR1), is another major mannose receptor on macrophages that cooperates with Dectin-1 in the non-opsonic recognition of β-glucans for phagocytosis.[35] Furthermore, it has been found that blocking of TLR-4 can inhibit the production of IL-12 p40 and IL-10, induced by purified Ganoderma glucans (PS-G), suggesting a vital role of TLR-4 signalling in glucan-induced dendritic cell maturation. Such an effect is also operated via the augmentation of the IκB kinase, NF-κB activity, and MAPK phosphorylation.[36]

Immune Activation Induced by β-glucans

β-glucans can act on a variety of membrane receptors found on the immune cells. They may act singly or in combination with other ligands. Various signalling pathways are activated and their respective simplified downstream signalling molecules are shown. The reactor cells include monocytes, macrophages, dendritic cells, natural killer cells and neutrophils. The immunomodulatory functions induced by β-glucans involve both innate and adaptive immune responses. β-glucans also enhance opsonic and non-opsonic phagocytosis and trigger a cascade of cytokines release, such as tumour necrosis factor (TNF-α) and various types of interleukins (ILs).

Other possible receptors and signalling pathways induced by β-glucans are less definite at the moment. For example, lentinan, a form of mushroom derived β-glucans, has been found to bind to a scavenger receptor found on the surface of myeloid cells, and triggers phosphatidylinositol-3 kinase (PI3K), Akt kinase, and p38 mitogen-activated protein kinase (MAPK) signalling pathways. However, no specific β-glucans scavenger receptor has been identified so far. [37] *Candida albicans* derived β-glucans, but not other forms of pathogenic fungal β-glucans, can bind to the LacCer receptor and activate the PI-3K pathway in controlling the neutrophil migration. but such an activation pathway may involve other molecules found in the Candida derived β-glucans.[38]

Furthermore, two other receptors known as scavenger and lactosylceramide also bind β-glucans and can elicit a range of responses. β-glucans can enhance endotoxin clearance via the scavenger receptors, by decreasing TNF production, leading to improved survival in rats subjected to *Escherichia coli* sepsis.[39-42] β-glucans binding to a lactosylceramide receptor can enhance myeloid progenitor proliferation and neutrophil oxidative burst response, leading to an increase in leukocyte anti-microbial activity. It is also associated with the activation of NF-κB in human neutrophils.[43]

β-glucans act on a diversity of immune-related receptors particularly in Dectin-1 and CR3, and can trigger a wide spectrum of immune responses. The targeted immune cells of β-glucans include macrophages, neutrophils, monocytes, NK cells, and dendritic cells. The immunomodulatory functions induced by β-glucans involve both innate and adaptive immune responses. β-glucans also enhance opsonic and non-opsonic phagocytosis. Whether β-glucans polarize the T cells subset toward a particular direction remains to be explored.

Diabetes

Both oat and fungal β-glucans reduce blood glucose concentrations after oral administration, as seen in animal experiments and clinical trials. In diabetic rats, orally ingested fruiting bodies and the acidic polysaccharides of both *Tremella mesenterica* and *T. aurantia* reduce blood glucose concentrations. β-glucans prepared by hot water extraction

of *Agaricus blazei* basidiocarps show antihyperglycemic, antihypertriglyceridaemic, antihypercholesterolemic, and antiarteriosclerotic activity in diabetic rats. Oat β-glucans have been used in several clinical trials to reduce glucose. Studies show that oat β-glucan lowers postprandial glycemia. It has also been shown that oat bran flour is more effective than oat bran crisp, explained by the thrice higher β-glucan content in oat bran flour.

Hypertension

β-glucan has been seen to reduce hypertension. In genetically modeled rats with spontaneous hypertension (SHR), a diet containing 5% Shiitake (*Lentinus edodes*) or maitake (*Grifola frondosa*) causes a decrease in the mean systemic blood pressure. Moreover, consumption of whole maitake basidiocarps and the water-soluble extract also led to a decrease in blood pressure in Zuker fatty rats, in a diabetes rat model.

Hypertriglyceridemia

Diabetes-associated dyslipidemia is a major risk factor for CVD. The dyslipidemia is caused either by insulin resistance or adipocytokines. In diabetes, the adipose cells are insulin-resistant, thus, the insulin-mediated uptake of free fatty acids in skeletal muscles is impaired. Increased circulating free fatty acids flux to the liver, resulting in increased triglyceride synthesis and the assembly of very low-density lipoprotein (VLDL). Thus, the characteristic of dyslipidemia in patients with diabetes is hypertriglyceridemia. Hyperglycemia and low insulin may also contribute to VLDL production (Hobbs 2006). In diabetes, adiponectin is reduced, which increases the muscle-free fatty acid uptake and reduces the plasma-free fatty acid level. This mechanism is independent of insulin-resistance. In addition, high-density lipoprotein (HDL) may also decrease. β-glucan has been shown to decrease LDL cholesterol and increase HDL, to possibly alleviate dyslipidemia and reduce CVD.

Oats was first found to have a cholesterol-lowering effect and the active component was identified as β-glucans. Oats reduced both serum total cholesterol and LDL cholesterol compared to the control. In 20 hypercholesterolemic male patients, oat bran was seen to be better than wheat bran in lowing cholesterol. Barley was also seen to have a similar effect (Davy *et al.*).[44]

Cancer

β-glucans, like lentinan (derived from the Shiitake mushroom) and Polysaccharide-K, have been used as an immunoadjuvant therapy for cancer since 1980, primarily in Japan. There is a large collection of research, which demonstrates that β-glucans possess anti-tumor and anti-cancer activities. In a mouse model study, beta 1,3 glucan in conjunction with interferon gamma inhibited tumors and liver metastasis.[22] In some studies, β-1,3 glucans enhanced the effects of chemotherapy. In a cancer experiment, using

a mouse model, administration of cyclophosphamide in conjunction with beta-1,3 glucans derived from yeast resulted in reduced mortality.[23] In human patients with advanced gastric or colorectal cancer, the administration of beta-1,3 glucans derived from shiitake mushrooms in conjunction with chemotherapy resulted in prolonged survival time.

Preclinical studies have shown that a soluble yeast β-glucan product, Imprime PGG, when used in combination with certain monoclonal antibodies or cancer vaccines, offers significant improvements in long-term survival versus monoclonal antibodies alone. This benefit, however, does not result from Betafectin enhancing the specific killing action of the antibody. The anti-tumor activity is caused by a unique killing mechanism that involves neutrophils that are primed with Betafectin and that are not normally involved in the fight against cancer. Recent research by Hong *et al.*, demonstrates that this mechanism of action is effective against a broad range of cancers when used in combination with specific monoclonal antibodies that activate or cause the complement to be bound to the tumor.[45]

Multinational research has successfully demonstrated that the oral form of yeast β-1,3-D glucan has similar protective effects as the injected version, including defense against infectious diseases and cancer.[46-49] Of late, orally delivered glucan has been found to significantly increase proliferation and activation of monocytes in the peripheral blood of patients with advanced breast cancer. The technology has wide applicability in cancer therapy. Each form of the cancerous tumor cell has specific antigens on the cell surface, some of which are common to other types of cancer. (e.g., Mucin 1 is present on about 70% of all types of cancer cells). Different immunotherapies target different antigens for binding monoclonal antibodies to tumor cells. This has resulted in the development of hundreds of monoclonal antibodies, many targeting a different specific antigen on cancer cells. In research studies, Betafectin has improved the effectiveness of all complement activating monoclonal antibodies tested, including breast, liver, and lung cancer (company data). The magnitude of success varies based on the specific monoclonal antibody used and the type of cancer.

Prevention of Infection

There have been numerous studies and clinical trials conducted with soluble yeast β-glucans and the whole glucans particulate. These studies have ranged from the impact of β-glucans on post-surgical nosocomial infections to the role of yeast β-glucans in treating anthrax infections. Post-surgical infections are a serious challenge following major surgery, with estimates of 25–27% infection rates post-surgery. Alpha–Beta Technologies conducted a series of human clinical trials in the 1990s, to evaluate the impact of β-glucan therapy on controlling infections in

high-risk surgical patients. In the initial trial 34 patients were randomly (double-blind, placebo-controlled) assigned to treatment or placebo groups. The patients who received PGG-glucan had significantly fewer infectious complications than the placebo group (1.4 infections per infected patient for the PGG-glucan group vs. 3.4 infections per infected patient for the placebo group). Additional data from the clinical trial revealed that there was decreased use of intravenous antibiotics and shorter stays in the intensive care unit (ICU) for patients receiving PGG-glucan versus patients receiving placebo.

There have been studies with humans and animal models that further support the efficacy of β-glucan in combating various infectious diseases. One human study demonstrated that the consumption of oral whole glucan particles increased the ability of immune cells to consume a bacterial challenge (phagocytosis). The total number of phagocytic cells and the efficiency of phagocytosis in healthy human study participants increased when a commercial particulate yeast β-glucan was consumed which shows the potential for yeast β-glucan to increase the reaction rate of the immune system to infectious challenges. The study concluded that oral consumption of whole glucan particles represented the fact that it was a good enhancer of natural immunity.[49,50]

Antimicrobial Effect
β-Glucan from oats has been demonstrated to have antimicrobial effects against *E. coli* and *B. subtilis*. On comparing cationic and native β-glucans, the latter has been seen to inhibit the growth of these bacteria by approximately 35%, while the cationic one has been seen to cause 80% inhibition in both microorganisms, indicating that β-glucan amination promotes antimicrobial effects. In this same study, cationic β-glucan has been seen to be more effective against *E. coli* (Gram-negative) than *B. subtilis* (Gram-positive), which can be explained by the interaction of the polycations with the negatively charged bacterial surface, altering membrane permeability and thereby inhibiting growth.[51]

Radiation Exposure
β-glucan is a well-known biological response modifier (BRM), isolated from the yeast cell wall polysaccharides and is made up entirely of glucose β (1,3), linked together in linear chains with a variable frequency of β (1,6)-linked side chains. A specific hematopoietic activity was first demonstrated with β-glucan in the mid-1980s, in an analogous manner, as a granulocyte monocyte colony stimulating factor (GM-CSF). The anti-infective activity of β-glucan combined with its hematopoiesis stimulating activity resulted in the enhanced survival of mice receiving a lethal dose of 900–1200 cGy of radiation. *In vitro* studies showed that β-glucan could enhance granulocyte and megakaryocyte colony formation by hematopoietic stem progenitor cells when used in combination with GM-CSF and interleukin-3 (IL-3), respectively.

Septic Shock
β-glucan reduces septic shock by the mechanisms of the immune enhancing ability. Early research by Onderdonk *et al.* investigated the ability of yeast β-glucan to reduce septic infections using *in vivo* models. Onderdonk *et al.* found that mice challenged with *E. coli* or *S. aureus* bacteria are protected against septic infections when they are injected with PGG-glucan four to six hours prior to infection. Additional research further supports that yeast β-glucan reduces septic shock by killing bacteria present in blood.[50] A study by Kernodle *et al.* has demonstrated that preventative dosing of yeast β-glucan, prior to infection with *S. aureus*, prevented sepsis in a guinea pig model. Research on the use of yeast β-glucan immunomodulators as a means of treating and preventing bacterial sepsis is well-documented. Recent reports on glucan and sepsis revealed another possible mechanism — glucan protects against oxidative organ injury.[52]

Chemoprotective Effects
In recent times, several *in vitro* studies have demonstrated that β-glucans of different origin have effective protective activity against different mutagenic agents. The barley β-glucan was found to have a protective effect against damage induced by methyl methanesulfonate (MMS), in the CHO-K1 cell line (deficient in drug metabolism). The effects of the inducers MMS and 2-aminoanthracene (2AA) in the HTC cell line (proficient in drug metabolism), using different treatment protocols (pre-treatment, simultaneous, simultaneous with pre-incubation and post-treatment), indicated that the simultaneous protocol with preincubation provided the greatest reduction in DNA damage, suggesting that the β-glucan may react with mutagenic agents impeding their interaction with the DNA.[53] The protective effect against 2AA and MMS, in lower concentrations, was also seen in CHO-K1, in the presence or absence of a DNA polymerase inhibitor (Ara-C).[54]

Surgery
There have been numerous studies and clinical trials conducted with the soluble yeast β-glucan particle and the whole glucan particle. Immunomodulators that enhance macrophage function have been shown to be beneficial in humans, as well as in animal models. One such study that looked at this correlation examined wound tensile strength and collagen biosynthesis, and positive effects were observed.

Wound Healing
Macrophage activity is known to play a key role in wound healing from surgery or trauma. In both animal and human studies, therapy with β-glucan has provided improvements such as fewer infections, reduced mortality, and stronger tensile strength of scar tissue.

Allergic Rhinitis

This disease is caused by an IgE-mediated allergic inflammation of the nasal mucosa. Orally administered yeast glucan decreases the levels of IL-4 and IL-5 cytokines responsible for the clinical manifestation of this disease, while it increases the levels of IL-12. Based on these studies, glucan may have a role as an adjunct to standard treatment in patients with allergic diseases.

Arthritis

Using paramagnetic resonance spectroscopy, yeast-derived glucan is found to cause a decline in oxidative tissue damage during the progress of arthritic diseases, suggesting a role in the treatment of arthritis.

ADDITIONAL APPLICATIONS

It is known that cereals, mushrooms, and yeast facilitate bowel motility and can be used in the amelioration of intestinal problems, particularly constipation. Non-digestible β-glucans, forming a remarkable portion of these materials, are also able to modulate mucosal immunity of the intestinal tract. In the central nervous system, β-glucans activate microglial cells. These cells act as scavengers of the brain cell debris and play a positive role in Alzheimer's disease, AIDS, ischemia injury, and multiple sclerosis.[55,56]

Influence of certain cereals (barley, oats) and edible mushrooms on the decrease of the levels of serum cholesterol and liver low-density lipoproteins, lead to lowering of atherosclerosis and cardiovascular disease hazards, which is also mediated by β-glucans.[57] It is known that cereals, mushrooms, and yeast facilitate bowel motility and can be used in amelioration of intestinal problems, particularly obstipation.[58,59] Non-digestible β-glucans, forming a remarkable portion of these materials, are also able to modulate mucosal immunity of the intestinal tract.[60]

Studies have shown that β-glucans found in baker's yeast and certain fungi have anti-cancer properties. In Japan, β-glucans like Lentinan and Polysaccharide-K, isolated from certain medicinal mushrooms have been used for over 20 years in intravenous forms and are approved for use as adjuncts to chemotherapy. There are phase III trials in the U.S. using β-glucans with other cancer drugs. At this time, no β-glucans have been approved by the FDA for use in the treatment of disease. Other β-glucans, such as β-D-glucan, can play an important role in the diagnosis of toxic mycosis caused by fungi that contain compounds, such as, the *Candida* and *Aspergillus* species. β-glucan is also promoted as a dietary supplement for weight loss, these claims are not well supported by research although β-glucans can have some effect on the effective glycemic index and insulin response.

β-D-glucan's Role in Diagnostics

β-D-glucan (properly known as (1→3) β-D-glucan, but also incorrectly called 1, 3-β-D-glucan or even just glucan) forms part of the cell wall of certain medically important fungi (medicinal mushrooms), especially the *Aspergillus* and *Agaricus* species. An assay to detect the presence of (1→3) β-D-glucan in the blood has been produced by Fungitell and is marketed as a means of diagnosing invasive fungal infection in patients. One of the limitations of the assay is the presence of fungal contaminants in amoxicillin-clavulanate, which may result in false-positive results in those patients receiving these antibiotics.

SAFETY CONCERNS WITH β-GLUCANS?

β-glucans may be safe for most adults when taken by mouth or when the injectable solution is used for a short period of time. Injections that have microparticles are not safe. There is not enough information to know whether β-glucans are safe when applied to the skin.

The potential side effects of β-glucans, when taken by mouth, are not known. When used by injection, β-glucans can cause chills, fever, pain at the injection site, headache, back and joint pain, nausea, vomiting, diarrhea, dizziness, high or low blood pressure, flushing, rashes, decreased number of white blood cells, and increased urine. People with AIDS who take beta glucans have developed thickening of the skin of the hands and feet.[55,56]

DRUG INTERACTIONS

β-Glucans have drug interactions with diclofenac [Cataflam(R), Voltaren(R)]; etodolac [Lodine(R)]; fenoprofen [Nalfon(R)]; flurbiprofen [Ansaid(R)]; ibuprofen [Motrin(R)]; indomethacin [Indochron E-R(R), Indocin(R)]; ketoprofen [Orudis(R), Oruvail(R)]; ketorolac [Toradol(R)]; meclofenamate [Meclomen(R)]; Nabumetone [Relafen(R)]; naproxen [Anaprox(R), Naprelan(R), Naprosyn(R)]; oxaprozin [Daypro(R)]; piroxicam [Feldene(R)]; sulindac [Clinoril(R)]; tolmetin [Tolectin(R)]; and Aspirin [Disprin(R), Bufferin (R)].

SIDE EFFECTS

β-glucans have the side effects such as, breathing problems or tightness in the throat or chest, chest pain, skin hives, rash, or itchy or swollen skin.[61]

CONCLUSIONS

β-glucans are sugars that are found in the cell walls of the bacteria, fungi, yeasts, algae, lichens, and plants, such as oats and barley. They are sometimes used as medicine. β-glucans are used for high cholesterol, diabetes, cancer, and HIV/AIDS. β-glucans are also used to boost the immune

system in people whose body defense has been weakened by conditions such the chronic fatigue syndrome, or physical and emotional stress; or by treatments such as radiation or chemotherapy. β-glucans are also used for colds (common cold), flu (influenza), H1N1 (swine) flu, allergies, hepatitis, Lyme disease, asthma, ear infections, aging, ulcerative colitis and Crohn's disease, fibromyalgia, rheumatoid arthritis, and multiple sclerosis. Healthcare providers sometimes give β-glucans by IV (intravenously) or by injection into the muscle, to treat cancer and to boost the immune system in people with HIV/AIDS and related conditions. β-glucans are also given by IV to prevent infection in people, after surgery. Healthcare providers sometimes give beta glucans via a shot under the skin (subcutaneously) for treating and reducing the size of skin tumors resulting from cancer that has spread. In manufacturing, β-glucans are used as food additives in products such as salad dressings, frozen desserts, sour cream, and cheese spreads. There are several β-glucan supplement products that claim β-glucans taken by mouth can only be absorbed if the product is prepared by a special patented process that 'micronizes' β-glucan particles to a size of one micron or less. However, there is no reliable evidence to support such a claim.

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