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Oral administration of a new soluble branched β-1,3-D-glucan is well tolerated and can lead to increased salivary concentrations of immunoglobulin A in healthy volunteers

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Summary

The soluble branched yeast β-1,3-D-glucan (SBG) belongs to a group of carbohydrate polymers known to exert potent immunomodulatory effects when administered to animals and humans. A new oral solution of SBG has been developed for local application to the oropharyngeal and oesophageal mucosa in order to strengthen the defence mechanisms against microbial and toxic influences. In the present study oral administration of SBG has been investigated primarily for assessment of safety and tolerability in an early phase human pharmacological study (phase I). Eighteen healthy volunteers were included among non-smoking individuals. The study was an open 1 : 1 : 1 dose-escalation safety study consisting of a screening visit, an administration period of 4 days and a follow-up period. Groups of six individuals received SBG 100 mg/day, 200 mg/day or 400 mg/day, respectively, for 4 consecutive days. The dose increase was allowed after a careful review of the safety data of the lower dose group. No drug-related adverse event, including abnormalities in vital signs, was observed. By inspection of the oral cavity only minor mucosal lesions not related to the study medication were seen in seven subjects. Repeated measurements of β-glucan in serum revealed no systemic absorption of the agent following the oral doses of SBG. In saliva, the immunoglobulin A concentration increased significantly for the highest SBG dose employed. SBG was thus safe and well tolerated by healthy volunteers, when given orally once daily for 4 consecutive days at doses up to 400 mg.

Keywords: β-1,3-D-glucan, IgA, oral, phase I, safety

Introduction

A group of carbohydrate polymers, commonly referred to as β-glucans, has been reported to exert immunomodulatory effects when administered to animals and humans, possibly through an effect on the innate immune system [1,2]. These homopolysaccharides are extractable from bacteria, fungi and plants, and they are made up by β-D-glucopyranosyl units linked together with a β-1,3-glucan backbone. Their molecular weight, degree of branching and nature of branches are believed to determine their putative immunomodulatory efficacy [3,4]. The microbial derived β-glucans are considered to belong to the group of compounds commonly known as pathogen-associated molecular patterns (PAMPs), which are conserved structures on several groups of microorganisms. The PAMPs act on the immune system through pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) [4] and the recently identified proinflammatory non-opsonic receptor for β-glucans, Dectin-1 [5,6], on phagocytic cells.

Dectin-1 plays an important role in the inflammatory response to pathogens by enhancing TLR-mediated activation of nuclear factor κB (NF-κB), which leads in turn to activation of the proinflammatory cytokines interleukin (IL)-12 and tumour necrosis factor (TNF)-α in macrophages and dendritic cells [7,8]. Furthermore, β-D-glucan derived from yeast seems to augment maturation of antigen-presenting dendritic cells [9]. Most interesting is the ability of orally administered β-glucan to enhance the cytotoxicity of antitumour monoclonal antibodies directed against tumour markers such as epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER)-2 and the cell differentiation antigen CD20 in xenograft tumour models [10]. Given the favourable

pharmacodynamics of oral β-glucan treatment, the therapeutic potential should be exploited in pharmaceutical formulations suitable for oral administration in humans.

A soluble form of β-glucan derived from *Saccharomyces cervisiae* has been shown to enhance neutrophil antimicrobial functions *in vitro* [11], reduce staphylococcal abscess formation in a guinea pig model [12] and reduce serious postoperative infections in patients undergoing high-risk gastrointestinal surgery in humans [13]. In the majority of experiments in animals the β-glucans have been administered through intraperitoneal, intravenous or subcutaneous injections. There is, however, increasing evidence that β-glucans are also active when applied to mucosal surfaces or skin, with an adjuvant effect in an experimental nasal spray vaccine [14] or as an aid to early wound repair [15–17]. Prevention and treatment of radiation-induced oral mucositis and ulceration thus represent an attractive therapeutic option for these agents. In keeping with this notion β-glucans exert radioprotective [18–20], myeloproliferative [21–23] and anti-inflammatory properties [24–26] in addition to promoting an increased anti-infective state of the innate immune system [12,27,28].

Recently, the new highly purified homopolysaccharide solution of yeast β-1,3-D-glucan (SBG) by Biotec Pharmacon (Tromsø, Norway) entered clinical investigation. In a series of preclinical experiments SBG showed no mutagenic or chromosomal toxicity, and there were no acute or delayed toxicity in mice, rats and pigs after oral or parenteral administration (data on file). Based on the already existing documentation on β-glucans, the Commission of the European Communities recently designated SBG as an orphan medicinal product for prevention of oral mucositis in head and neck cancer patients (EU/3/05/294, http://pharmacos.eudra.org). The present phase I study is the first trial with SBG in humans. The study was designed primarily to estimate safety and tolerability in healthy volunteers. Secondary objectives were to estimate the systemic absorption of soluble yeast β-glucan after oral administration, and to measure immunologcal parameters in blood and saliva. The present study demonstrates for the first time that SBG oral solution is well tolerated when given orally once daily for 4 consecutive days at doses up to 400 mg.

Materials and methods

The study was conducted in accordance with the Declaration of Helsinki of 1964 (revised version of Edinburgh 2000) and the Notes for Guidance on Good Clinical Practice (CPMP/ICH/135/95), and applicable regulatory requirements. The study protocol was approved by the Regional Ethics Committee and the Norwegian Medicines Agency (NMA), and all participants provided a written informed consent before study entry.

This was an open 1 : 1 : 1 dose-escalation safety study consisting of a screening visit, an administration period of 4 consecutive days and a follow-up period with visits on days 5 and 8. Eighteen healthy, non-smoking volunteers (age range 20–30 years) were included after having signed an informed consent. The SBG study drug (Biotec Pharmacon, Tromsø, Norway), which has been derived from Baker's yeast (*S. cerevisiae*) cell walls, is a chemically well-defined solution of an endotoxin-free (< 0·05 EU/ml), 2% (w/v) underivatized aqueous soluble branched β-1,3-D-glucan, with an average molecular weight of approximately 20 kDa. SBG has a branched structure with a backbone of β-1,3-linked glucose and side chains of mainly β-1,3-linked nature anchored to the backbone by β-1,6-linkages for approximately every 10–15th glucosyl units. The hazy solution was without any smell and taste, and it was dispensed by the hospital pharmacy in glass bottles containing 5, 10 and 20 ml SBG (concentration 20 mg/ml in water), respectively.

Three groups, each with six individuals, received either 100 mg/day (group 1), 200 mg/day (group 2) or 400 mg/day (group 3). Selection of dose range was based on previous experience with β-glucans in animal models [12] and humans [13,29]. Drug administration to groups 2 and 3 was started after the completion and approval of safety analyses from the previous dose levels. The SBG preparation was administered as a mouthwash for 2 min and then swallowed. This regimen was identical for the three groups, with daily morning doses of SBG for 4 consecutive days. The participants had to refrain from alcohol during the study period, and were not allowed to drink tea or coffee in the morning on study days scheduled for oral examination. Before blood sampling at screening and day 5, they were fasting overnight (10 h). The subjects did not eat bread or other yeast containing products for at least 12 h prior to blood sampling for β-glucan analysis.

Safety variables

Routine haematological and clinical chemistry data and urinalysis were obtained. Vital signs were recorded and the oral cavity was inspected. Abnormal findings were classified according to the NCI–CTC grading system.

Drug measurements

The plasma concentration of β-1,3 glucan was measured on days 1 (before and 1 h after drug administration), 2, 5 and 8 using a commercial chromogenic assay (Glucatell™, Association of Cape Cod Inc., MA, USA). The limit of detection was 5 pg/ml. The concentration of β-1,3 glucan in pooled control plasma from healthy volunteers varied from 5 to 17 pg/ml.

Immunological parameters

Immunoglobulins (total IgA and IgG) and cytokines (IL-1β, IL-6 and TNF-α) were measured in blood and saliva

obtained before and after drug administration (days 4 and 5). The saliva samples were collected with absorbent wicks (MucoSafe™) and extracted in 1 ml buffer solution and stored at −70°C until analysed. Total IgG and IgA in blood and saliva were measured by standard enzyme-linked immunosorbent assay (ELISA) methods using Nunc Immuno Plates (MaxiSorp F96; Nunc A/S, Roskilde, Denmark) coated with, respectively, goat anti-human IgG and IgA (Sigma-Aldrich Inc., St Louis, MO, USA) after which non-specific protein-binding sites were blocked with phosphate-buffered saline (PBS) containing 5% non-fat dry milk (Oxoid, Hampshire, UK). Serum, extracts of saliva and standards [Human Standard Serum Nor-01, or Purified Human IgA1 Lambda (dimer); both from Nordic Immunological Laboratories, Tilburg, the Netherlands] were serially diluted twofold in the blocking solution during application on the ELISA plates, and incubated at 4°C overnight before washing and application of peroxidase-conjugated goat antibodies to either human IgG or IgA (Sigma-Aldrich). Bound IgG and IgA were detected with o-phenylenediamine dihydrochloride (Sigma-Aldrich), and the concentrations were based on standard curves after corrections for dilutions made during extraction from wicks and preparation for ELISA. Quantification of IL-1β, IL-6 and TNF-α were carried out using solid phase chemiluminiscence ELISA kits according to the manufacturer's instructions (QuantiGlo Immunoassays, R&D Systems, Minneapolis, MN, USA).

Data analysis and statistics

Data from all subjects who had received at least one dose of study medication were included in the analyses of safety. Descriptive statistics are presented as means, standard deviations (s.d.) and ranges. Baseline laboratory values were defined as the mean of predose values on days 0 and 1. The data were entered into a SAS computer database for further analysis and statistical evaluation. The Wilcoxon's signed rank sum test was used to analyse changes in plasma drug concentrations and immunology parameters. *P*-values < 0·05 were considered significant.

Results

Eighteen healthy, non-smoking volunteers (mean age 24·6 years, range 20–30, six females and 12 males, all Caucasians) were included after screening procedures and having signed an informed consent. None of the subjects received concomitant medication at baseline. Seventeen of the 18 subjects completed the trial. Prior to receiving SBG the range of the β-1,3 glucan concentrations for the participating subjects varied from 0 to 20 pg/ml in plasma, the concentration of β-1,3 glucan never exceeded 20 pg/ml in samples obtained during the study. No significant differences between the concentrations on days 5 or 8 and the prestudy value were found. For example, in dose group 1 there was an increase in the mean β-glucan concentration of 3·2 pg/ml, in dose group 2 of 0·3 pg/ml, whereas in dose group 3 no increase was observed in the day 8-values compared with the prestudy values. These results indicate that no systemic absorption of β-1,3 glucan occurred after the oral administration of SBG.

No adverse events were considered to be related to the study medication, and no abnormalities in vital signs were observed. Inspection of the oral cavity revealed minor mucosal lesions in seven subjects considered by the odontologist to represent normal variations, and no further action was taken. Five subjects had an upper respiratory infection, including one with additional herpes labialis. One person receiving the lowest dose had to be excluded due to soft tissue injury acquired accidentally during physical training. He had a transient rise in creatine kinase (CK) up to 8800 U/l on day 3.

The laboratory data suggested no relationship to the study medication. Increased values of C-reactive protein, fibrinogen and abnormal differential counts of leucocytes were found in subjects who reported upper respiratory tract infections. Except for the person experiencing explainable, increased CK-values, no laboratory parameters of NCI-CTC grades 3 (severe) or 4 (life-threatening) were observed. Other haematological and biochemical parameters remained within the normal range during the study.

Mean values of IgG and IgA in serum and saliva for dose group 1 and 3 are presented in Table 1. The saliva IgA concentration increased from 39·6 μg/ml (18·5–72·7) at baseline to 105·4 μg/ml (49·5–219·4) after completion of four consecutive daily doses of 400 mg ($P < 0·05$). Similar increases were not seen at lower doses (100 mg/day or 200 mg/day). The administration of the lowest dose (100 mg/day) or the highest dose (400 mg/day) for 4 days did not influence the concentration of IgG in serum or saliva. In serum there was no significant influence on the concentrations of TNF-α and IL-6 (data not shown). IL-1β was measured only in saliva (mean

Table 1. Concentrations of IgG and IgA in serum and saliva at baseline (day 1) and after completion of oral branched yeast β-1,3-D-glucan (SBG) administration (day 5). SBG was administered for 4 consecutive days, and the immunoglobulin levels of the low-dose (100 mg/day) and high-dose (400 mg/day) groups were compared.

	Oral SBG dose			
	100 mg/day		400 mg/day	
	Serum (g/l)	Saliva (μg/ml)	Serum (g/l)	Saliva (μg/ml)
IgG				
Day 1	7·2 ± 2·9	12·3 ± 8·5	6·4 ± 1·8	5·8 ± 4·0
Day 5	5·8 ± 3·4	6·2 ± 5·7	5·8 ± 1·2	6·5 ± 3·2
IgA				
Day 1	1·6 ± 0·7	50·2 ± 19·1	2·1 ± 1·3	65·8 ± 29·4
Day 5	1·5 ± 0·9	39·6 ± 21·1	2·2 ± 1·3	105·4 ± 73·9*

The listed values represent means ± s.d. for each dose group. *$P < 0·05$, saliva IgA day 5 compared to day 1.

values ranging from 15·6 to 72·0 pg/ml), and no change in concentrations was found after 4 days of treatment.

Discussion

Our study represents the first trial with a new soluble yeast SBG product in humans, and the study was designed to estimate the initial safety and tolerability in healthy subjects. The SBG solution was administered for 4 consecutive days to mimic a short prophylaxis or treatment course for oropharyngeal mucositis. No systemic absorption could be detected following the oral administration of SBG when analysing serum samples obtained at days 2, 5 and 8 with the highly sensitive and specific Glucatell β-1,3 glucan assay. The serum β-1,3 glucan concentrations remained low (≤ 20 pg/ml) throughout the study. In a previous study using the same β-1,3 glucan detection assay 30 healthy volunteers had mean serum concentrations in the range 0–86 pg/ml (mean ± s.d. = 17 ± 34), with only two sera containing > 60 pg/ml [30], indicating that the values in our study were within the range seen normally in healthy individuals. In clinical practice, a β-1,3 glucan level of ≥ 60 pg/ml has been defined as positive for invasive fungal infections [31].

This study was performed primarily to assess the clinical, haematological, biochemical and mucosal tolerance to oral SBG. The only serious event we registered during the study period was due to an accidental sports injury. When administered at dose levels of 100, 200 and 400 mg daily for 4 consecutive days, the SBG solution were well tolerated by the healthy subjects. There were no significant changes in the biochemical and haematological parameters analysed. Furthermore, SBG had no effect on heart rate and blood pressure. The low levels of β-1,3 glucan detected in serum samples drawn during the course of the study for all three dose groups indicate a lack of systemic drug accumulation. However, the study was not designed to determine whether any level of systemic absorption of β-1,3 glucan takes place following oral administration. The single time-point measurements could not preclude potential systemic adsorption and subsequent sequestration of β-1,3 glucan in the reticuloendothelial system. Furthermore, it was beyond the scope of this trial to disclose the pharmacokinetics of SBG in saliva. However, this is an interesting topic that should be addressed in forthcoming trials. No effect on blood concentrations of the cytokines and immunoglobulins was observed.

The statistically significant increase in saliva IgA concentration in the high dose-level group was seen only on day 5, which indicates a slow responding effect possibly enhanced by repeated SBG doses. Owing to a limited number of measurements the duration of the IgA response could not be determined by this experiment. However, any increase in total saliva IgA effected by SBG is appealing, because salivary IgA seems to act as a barrier for oral colonization by microorganisms such as *Candida albican*s by deferring the adhesion to oral surfaces [32]. Once established, oral candidiasis seems to lower the salivary IgA content and contribute to the maintenance of the stomatitis [33]. Furthermore, failure in secretion of salivary IgA is associated with recurrent parotitis in children [34]. Thus, oral SBG may provide increased protection of the oral mucosa to diverse microorgansims, which could be useful for immunocompromized patients and patients receiving radiotherapy that involves the oral cavity. The present study has demonstrated the feasibility and tolerability of SBG oral solution. Further studies are required to determine the therapeutic potential of this new oral drug.

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