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 therapeu-
tic potential should be exploited in pharmaceutical for-
mulations suitable for oral administration in humans.

A soluble form of β-glucan derived from Saccharomyces
cervisiae has been shown to enhance neutrophil antimicro-
bial 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 adminis-
tered 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]. Pre-
vention 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 newly highly purified homopolysaccharide
solution of yeast β-1,3-D-glucan (SBG) by Biotec Pharma-
con (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 exist-
ing 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 vol-
unteers. Secondary objectives were to estimate the systemic
absorption of soluble yeast β-glucan after oral administra-
tion, and to measure immunological 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 Com-
mitee 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 con-
sisting 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) underiva-
tized 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 glu-
cose 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 partic-
pants 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 contain-

Safety variables

Routine haematological and clinical chemistry data and uri-

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™, Associ-
ation 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 Cauca-
sians) 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 odontol-
gist 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, fibrino-
gen and abnormal differential counts of leucocytes were found in subjects who reported upper respiratory tract infec-
tions. 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 IgG in saliva and serum 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.

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.

<table>
<thead>
<tr>
<th>Oral SBG dose</th>
<th>Serum (g/l)</th>
<th>Saliva (μg/ml)</th>
<th>Serum (g/l)</th>
<th>Saliva (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/day</td>
<td>7·2 ± 2·9</td>
<td>12·3 ± 8·5</td>
<td>6·4 ± 1·8</td>
<td>5·8 ± 4·0</td>
</tr>
<tr>
<td>400 mg/day</td>
<td>5·8 ± 3·4</td>
<td>6·2 ± 5·7</td>
<td>5·8 ± 1·2</td>
<td>6·5 ± 3·2</td>
</tr>
<tr>
<td>IgA</td>
<td>Day 1</td>
<td>1·6 ± 0·7</td>
<td>50·2 ± 19·1</td>
<td>2·1 ± 1·3</td>
</tr>
<tr>
<td></td>
<td>Day 5</td>
<td>1·5 ± 0·9</td>
<td>39·6 ± 21·1</td>
<td>2·2 ± 1·3</td>
</tr>
</tbody>
</table>

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 1,3 glucan detection assay 30 healthy volunteers had mean 68)

However, the study was not designed to determine whether dose groups indicate a lack of systemic drug accumulation. Samples drawn during the course of the study for all three 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 albicans* 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 microorganisms, 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.

**Acknowledgements**

The study was sponsored by Biotec Pharmacon ASA, Tromsø, Norway, and monitored by Mericon AS, Skien, Norway. The authors are indebted to Eva Vognild at Mericon AS for proficient and thorough trial monitoring and Rolf Engstad at Biotec Pharmacon for useful help in preparing the manuscript. The authors would also like to thank Inger Lise Haugen at the Norwegian Institute of Public Health and Anne K. Axelsen at the Institute of Microbiology for skilful performance of ELISA analyses. Finally, the authors are grateful to Birgitte Lid Adamsen, Ingrid Fellesdal and May Ellen Lauritsen for competent execution of technical study procedures at the Clinical Research Unit.

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