Glucomannan in prevention of oxidative stress and inflammation occurring in adjuvant arthritis

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Abstract

OBJECTIVES: The aim of this study was to evaluate the effects of a biological response modifier, glucomannan (GM), isolated from *Candida utilis*, on the progress of adjuvant arthritis in Lewis rats. **METHODS**: Adjuvant arthrithis was induced in Lewis rats by a single intradermal injection of *Mycobacterium butyricum*. GM was administered in two different doses of 5 and 7.5 mg/kg b.w. The treatment involved daily oral or intraperitoneal administration of the substance from day 0, i.e. the day of immunization to the end of the experiment - day 28. Cyclosporin A was used as a therapeutic standard in daily oral dose of 2.5 mg/kg b.w. The following parameters were monitored: hind paw volume, total antioxidant status, protein carbonyl groups, activity of N-acetyl-beta-D-glucosaminidase in plasma, lysozyme and peroxidase activity of peritoneal macrophages and activity of gamma-glutamyltransferase in homogenates of spleen, hind paw muscle and hind paw joint. **RESULTS**: Beneficial action of GM was revealed mainly in hind paw volume decrease. Further decrease of the activity of the enzyme gamma glutamyltransferase (GGT) in the spleen, hind paw joint and muscle tissue homogenates, decrease of the plasmatic activity of N-acetyl-beta-D-glucosaminidase (NAGA), and finally suppression of lysozyme and peroxidase activity assessed in peritoneal macrophages were observed in arthritic animals treated with GM. All these findings speak in favor of the anti-inflammatory activity of glucomannan. Moreover, a significant improvement of the arthritis induced suppression of total antioxidant status and decrease of the level of the arthritis-associated protein carbonyls in plasma were detected. **CONCLUSIONS**: The important characteristics of GM isolated from *Candida utilis*, such as good water solubility and relatively small molecular weight, along with the observed *in vivo* anti-inflammatory and antioxidant effects, appear to be promising features for its prospective use as a natural agent in prevention and supplementary therapy of rheumatoid arthritis.

ORIGINAL ARTICLE

Abbrevations

AA	- adjuvant arthritis
b.w.	- body weight
CS	- Cyclosporine A
GGT	- γ-glutamyl transferase
γ-GPN	- γ-glutamyl-p-nitroanilide
GM	- glucomannan
HPV	- hind paw volume
IL	- interleukin
i.p.	- intraperitoneal
MDA	- malondialdehyde
NAGA	- N-acetyl-beta-D-glucosaminidase
RA	- rheumatoid arthritis
ROS	- reactive oxygen species
p.o.	- oral
SASc	- Slovak Academy of Sciences
TAS	- total antioxidant status
TBARS	- thiobarbituric acid reacting substances
TNF	- tumor necrosis factor

INTRODUCTION

In recent decades, polysaccharides isolated from botanical sources (mushrooms, algae, lichens and higher plants) have attracted a great deal of attention in the biomedical arena because of their broad spectrum of therapeutic properties and relatively low toxicity. While our understanding of the mechanism of action of these substances is still developing, it appears that one of the primary mechanisms involves nonspecific induction of the immune system (Tzianabos, 2000). Plant and mushroom polysaccharides reveal immunomodulatory effect that depends on polysaccharide structure and molecular weight (low molecular weight – inhibition**,** high molecular weight – activation) (Schepetkin & Quinn, 2006). Yeast cell wall comprises 80–90% carbohydrates and the majority (50–60% w/w) of carbohydrates is represented by the branched polymers of glucose [(1→3)-β-D- and (1→6)-β-D-glucans], while the minor part is represented by non-branched *N*-acetyl-(1→4)-β-D-glucosamin (chitin) and the polymers of D-mannose (mannans) that are usually covalently bound to proteins (Chauchan *et al*., 2002). Yeast β-D-glucans represent a heterogeneous group of polymers composed of 3-linked glucopyranosyl residues (main chain) with the presence or absence of 6-linked β-D-glucopyranosyl residues (side chains). They represent approx (50–60% w/w) of the cell wall (Dijkgraaf *et al*., 2002). This group of polysaccharides is traditionally viewed as efficient immunomodulators.

Glucomannans, another yeast cell wall component, are functionally and structurally very similar to other yeast mannans, differing only in the presence of terminal glucopyranosyl units in the side-chains. However, their biological activities appear to be more similar to those of cell-wall glucans than to those of cell-surface mannans, possibly due to the terminal glucosyl units (Kogan, 1993). Potent antimutagenic, anticlastogenic, and bioprotective activities of *Candida utilis* glucomannan against chemical compounds with different modes of action were documented (Vlčková *et al*., 2004). The protective effect of glucomanann *in vivo* was studied after p.o or i.p. administration in the model of cyclophosphamide-induced mutagenicity prior to cyclophosphamide injection (Chorvatovičová *et al*., 1999). A recent study described the efficient action of glucomannan as antimutagen, anticlastogen, DNA-breaks inhibitor or inducer, and as cytotoxic/cytostatic effect enhancer. Several possible mechanisms of the observed bioprotectivity including free radical scavenging were suggested (Miadoková *et al*., 2006).

Polysaccharides as glucans and glucomannans have been shown to exhibit various biological activities, which are mediated by interaction with cell surface receptors. GM could be recognized by several types of receptors including complement receptor 3, the mannose receptor, toll-like receptors, and other lectin receptors, which are widely expressed on leukocytes and mediate cellular response to different types of pathogen-associated molecular patterns. Mannan/glucan binding sites have also been described on other cell types, including fibroblasts (Kougias *et al*., 2001). The relationships between the structure of glucans and glucomannans and their immunomodulatory activities still remains unclear.

Oxidative stress and inflammation contribute to the pathogenesis of RA in an interactive mode. One of the major origins of oxidative stress in inflammatory diseases are neutrophils. Free radicals produced by neutrophils play an important role in inflammatory and immune processes involved in many diseases such as allergies, infections (Jančinová *et al*., 2006) and rheumatoid arthritis (Cross *et al*., 2006; Nosáľ *et al*., 2007). Therefore it is important to study how both processes – oxidative stress and inflammation – could be affected by new experimental therapies. Animal models of RA are experimental tools to study new therapies. One of such models is adjuvant arthritis (AA) induced by a single intradermal injection of heat-inactivated *Mycobacterium butyricum* in incomplete Freund´s adjuvant. The aim of this study was to evaluate the effects of a biological response modifier, glucomannan (GM), isolated from *Candida utilis*, on the development of adjuvant arthritis in Lewis rats.

MATERIAL AND METHODS

Preparation of glucomannan

The yeast strain *Candida utilis* was used as a biological source of GM. Strain CCY 29-38-18 was obtained from the *Collection of Yeast and Yeast-like Microorganisms* (Institute of Chemistry, Slovak Academy of Sciences, Bratislava). GM was isolated from cell wall glycoproteins using extraction with 2% KOH and purification with Fehling reagent as described previously (Kogan *et al*., 1988).

Characterization of glucomannan

Elemental analysis revealed 0.075% nitrogen. The molar mass of the polysaccharide was 33 kDa, as determined

by gel filtration. According to specific optical rotation measurement, the polysaccharide contained predominantly α-glycosidic linkages. IR spectrum contained absorption bands at 810 and 970 cm^{-1}, which are characteristic of α-D-mannans (Kato *et al*., 1973). The ^{13}C spectra of the polysaccharide and assignment of the observed carbon peaks that corresponded to the GM structure were in agreement with those described by Kogan *et al*. (1993).

Experimental design and methods used

After approval by the local ethics committee, AA was induced in male Lewis rats (Breeding Farm Dobrá Voda, Slovakia), weighing 150–170 g each, by a single intradermal injection of heat-inactivated *Mycobacterium butyricum* in incomplete Freund´s adjuvant (Difco Laboratories, Detroit, MI, USA). In each experimental group 6–8 animals were used.

Inflammatory, arthritic, and oxidative stress parameters were assessed. The experiments included healthy intact animals as reference controls, arthritic animals not treated with GM, and arthritic animals with GM administration. The treatment involved daily oral (5 and 7.5 mg/kg b.w.) or intraperitoneal (dose 5 mg/kg b.w.) administration of the substance starting from day 0, i.e. the day of bacterial inoculation, until the end of the experiment, day 28. Cyclosporin A (CS) was selected as a reference drug. The daily oral dose of 2.5 mg/kg was used.

We monitored a clinical parameter: *change of the hind paw volume (HPV)*. The HPV increase was calculated as the percentage of increase of HPV on day 28 in comparison to the beginning of the experiment. This basic parameter of arthritis progression was measured for both substances (GM and CS), all modes of administration and doses of GM. All treatments and control were compared to untreated arthritis (100%). The measurement of biochemical parameters was performed as described below: *TAS in plasma* was measured by the 2,2`-azinobis(3-ethylbenzothiazoline 6-sulfonate) assay (Rice-Evans & Miller, 1994). *Protein carbonyl groups*, the marker of oxidative modifications of proteins, were established in plasma using a classical carbonyl reagent 2,4-dinitrophenylhydrazine (Levine *et al*., 1994). *TBARS* in plasma were measured spectrophotometrically at 535 nm (Brown & Kelly, 1996). The activity of *cellular GGT* in spleen and hind paw joint and muscle tissue homogenates was measured by the method of Orlowski (Orlowski & Meister, 1970) as modified by Ondrejickova (Ondrejickova *et al*., 1993). The activity of *NAGA* and protein level in plasma were assayed according to standard methods used in our previous studies (Barrett, 1977; Lowry *et al*., 1951). For *lysozyme and peroxidase activity s*uspension of rat intraperitoneal macrophages (2×10^6 cells per ml) was ultrasonically disintegrated and centrifuged. The supernatant was mixed with the suspension of *Micrococcus luteus* ATCC 4698 (OD_{410}=0.8) in phosphate buffer. The lysozyme activity was measured spectrophotometrically (λ=410 nm) in 96 wells/plate over a 20-minute period. The peroxidase reaction was stopped after 20 min with H_2SO_4 and changes in A_{490} were measured (MR 5000 spectrophotometer, Dynatech) (Bukovský *et al*., 1998).

Statistics

The data were expressed as arithmetic mean with SEM, while lysozyme and peroxidase activity was expressed by SD. The arthritis group (AA) was compared to healthy control animals (CO), and treated arthritis groups were compared to untreated arthritis animals (AA). For significance calculations the Student's t-test was used with the following significance designations – ns (not significant); * $p<0.05$ (significant); ** $p<0.01$ (very significant); *** $p<0.001$ (extremely significant).

RESULTS AND DISCUSSION

Oxidative stress has been implicated in various pathological conditions involving several diseases and aging (Dalle-Donne *et al*., 2006; Dhalla *et al*., 2000; Jenner 2003; Sayre *et al*., 2001; Valko *et al*., 2007). The pathogenesis of RA is associated predominantly with the formation of free radicals at the site of inflammation. Oxidative injury and inflammatory status in various rheumatic diseases was corroborated by increased levels of iso-prostanes and prostaglandins in serum and synovial fluid compared to controls. Oxidative conditions in synovial tissue are also accompanied by a higher incidence of p53 mutations (Firestein *et al*., 1997). RA is an autoimmune disease that causes chronic inflammation of the joints and the surrounding tissue accompanied with the infiltration of activated T cells and macrophages (Bauerova & Bezek, 1999). It is known that macrophages are one of the primary cellular targets through which immunomodulators, such as fungal polysaccharides, induce immunomodulatory, anti-tumorigenic, wound-healing and other therapeutic effects (Schepetkin & Quinn, 2006).

In our experiments we evaluated the *in vivo* antioxidant ability of GM. The total antioxidant status of plasma was assessed together with a parameter of lipid peroxidation – TBARS levels in plasma as well as with a parameter of oxidation of plasmatic proteins – level of protein carbonyls. The enhancing effect of GM on TAS was very strong – it reverted the TAS decreased in untreated arthritis to the level of healthy animals. As lipid peroxidation measured by TBARS was not affected by GM administration, the antioxidative properties of GM are based probably on its inhibitory activity against oxidation of plasmatic proteins occurring in AA (Figure 1). This effect was more pronounced at intraperitoneal administration than at oral administration. This observation was in agreement with the action of GM on the basic arthritic parameter – the change of the HPV (Figure 2). Again, the i.p. administration was more effective than the oral administration. Moreover,

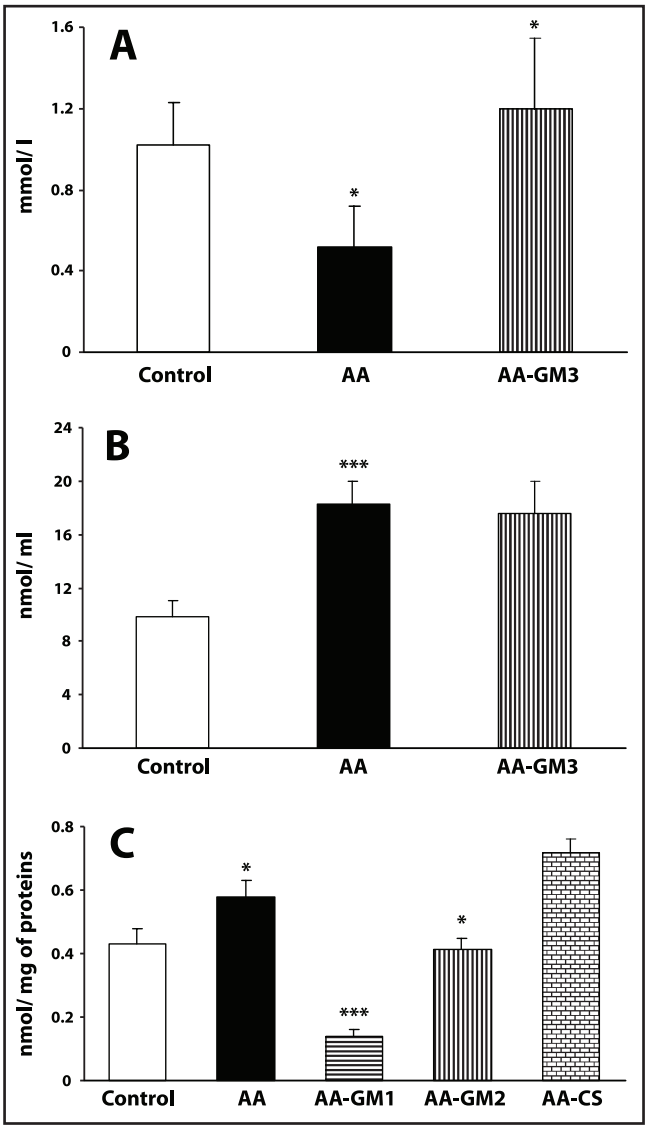

Figure 1. Effect of glucomannan on TAS (**A**), TBARS (**B**), protein carbonyls in plasma (**C**). Comparison of control, arthritic (AA) and arthritic animals treated with glucomannan (AA-GM1) i.p. dose of 5 mg/kg b.w.; (AA-GM2) p.o. dose of 5 mg/kg b.w.; (AA-GM3) p.o. dose of 7.5 mg/kg b.w. and with Cyclosporine A (AA-CS) p.o. dose of 2.5 mg/kg b.w.

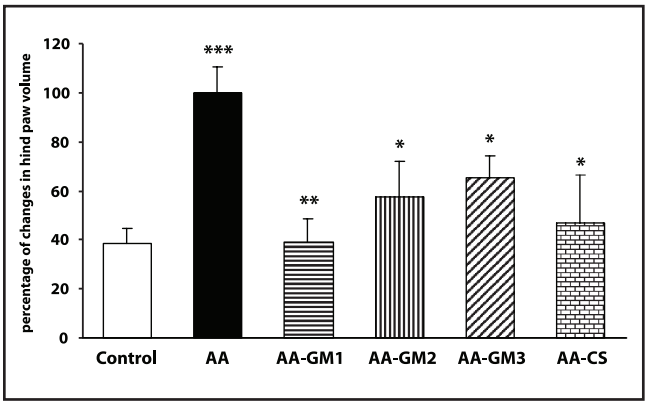

Figure 2. Effect of glucomannan on changes of hind paw volume. Comparison of control, arthritic (AA) and arthritic animals treated with glucomannan (AA-GM1) i.p. dose of 5 mg/kg b.w.; (AA-GM2) p.o. dose of 5 mg/kg b.w.; (AA-GM3) p.o. dose of 7.5 mg/kg b.w. and with Cyclosporine A (AA-CS) p.o. dose of 2.5 mg/kg b.w. The AA group represents 100%.

the lower dose of 5 mg/kg b.w. appeared to be more efficient than the higher one of 7.5 mg/kg. This finding appears to substantiate the hypothesis that the lower dose of GM could better inhibit the progression of AA than the larger one. Large doses of GM exert immunostimulatory activities, which could be so intensive that they would overcome the antioxidative properties of the molecule of GM. As in these experiments no significant differences were established between the dose of 5 and of 7.5 mg/kg b.w., a larger dose range should be evaluated in further investigations. In any case, effects of all modes of GM administration were comparable to those of CS on the change of HPV. CS did not exhibit antioxidative effect, yet it was more efficient in lowering NAGA activity (Figure 3).

GM in the oral dose of 5 mg/kg suppressed the arthritis-induced increased lysozyme and peroxidase activity of peritoneal macrophages (Figure 3). The anti-inflammatory effect of GM was further supported by inhibition of GGT activity measured in the homogenate of hind paw muscles. A similar significant effect was observed also in hind paw joint homogenate and spleen homogenate for GM administered in the dose of 7.5 mg/kg (Figure 4).

Although the biological activities of glucomannans are supposed to be more comparable with those of the cell-wall glucans than those of cell-surface mannans, in the experiments performed we observed immunosuppressant properties of the GM isolated from *Candida utilis*. This finding was supported by decrease of the activity of the evaluated inflammatory enzymes and by suppression of oxidative stress occurring in AA. The observed anti-arthritic effect should be assessed in future studies related to the relationship between molecular structure and immunomodulating properties of this polysacccharide.

The important characteristics of GM isolated from *Candida utilis*, such as good water solubility and relatively small molecular weight, along with the observed *in vivo* anti-inflammatory and antioxidant effects, appear to be promising features for its prospective use as a natural agent in prevention and supplementary therapy of RA.

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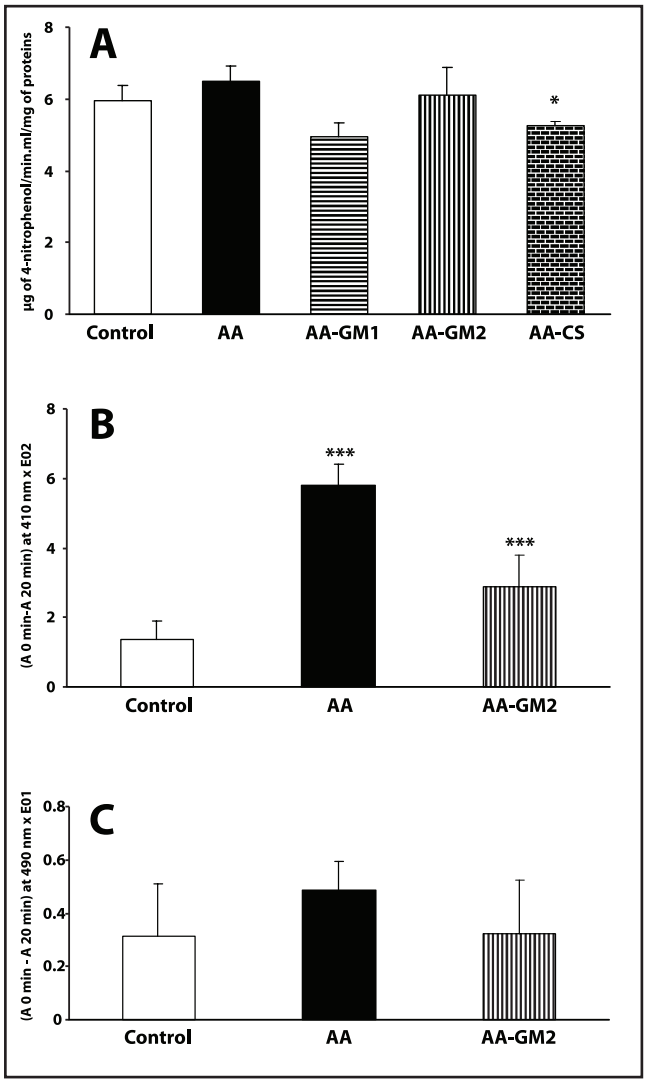

Figure 3. Effect of glucomannan on activity of NAGA in plasma (A), and lysozyme (B) and peroxidase (C) activity of peritoneal macrophages. Comparison of control, arthritic (AA) and arthritic animals treated with glucomannan (AA-GM1) i.p. dose of 5 mg/kg b.w.; (AA-GM2) p.o. dose of 5 mg/kg b.w. and with Cyclosporine A (AA-CS) p.o. dose of 2.5 mg/kg b.w.

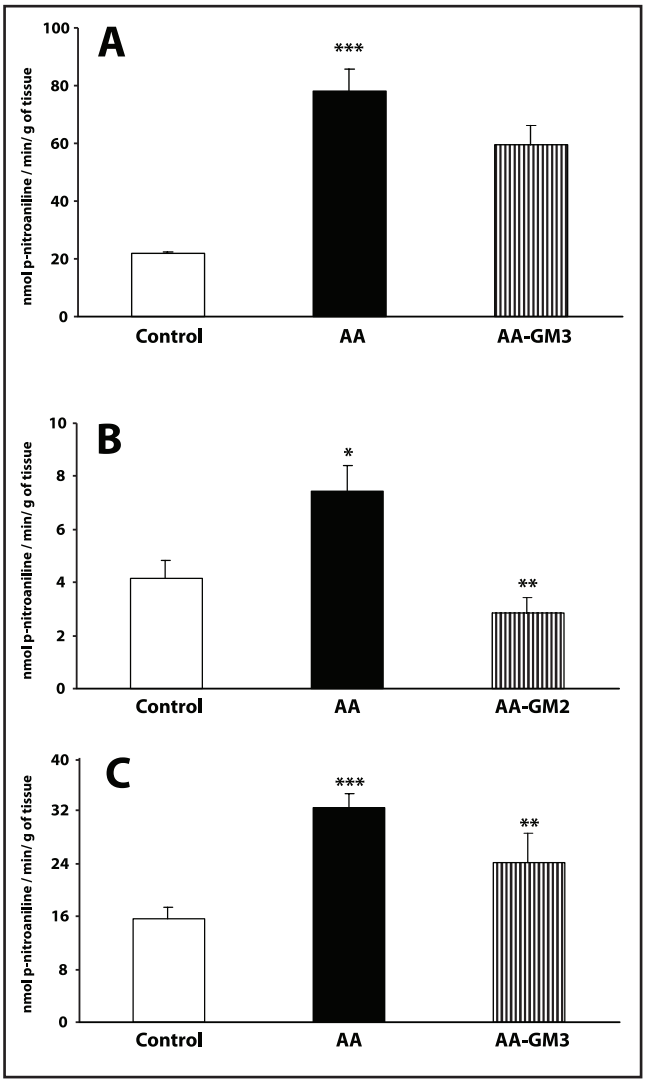

Figure 4. Effect of glucomannan on activity of GGT in spleen (A), hind paw muscle (B) and hind paw joint (C). Comparison of control, arthritic (AA) and arthritic animals treated with glucomannan (AA-GM2) p.o. dose of 5 mg/kg b.w.; (AA-GM3) p.o. dose of 7.5 mg/kg b.w.

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