Carbohydrate Polymers 84 (2011) 153–161

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Effect of oat and barley β-glucans on inhibition of cytokine-induced adhesion molecule expression in human aortic endothelial cells: Molecular structure–function relations

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ARTICLE INFO

*Article history:*
Received 8 September 2010
Received in revised form 5 November 2010
Accepted 9 November 2010
Available online 16 November 2010

*Keywords:*
Oat and barley β-glucans
Anti-inflammatory activity
Aortic endothelial cells
Cell adhesion molecules
Molecular structure

ABSTRACT

Endothelial cell adhesion molecules have been recognized as an early step in inflammation and atherogenesis. The inhibition of TNF-α-induced expression of vascular cell adhesion molecule (VCAM-1) and intracellular cell adhesion molecule (ICAM-1) in human aortic endothelial cells (HAEC), following pretreatment with mixed-linkage (1 → 3), (1 → 4)-β-D-glucans (β-glucans) isolated from oat and barley, and the possible molecular structure–function relations, have been explored. The apparent molecular weight (Mw) of β-glucans varied in the ranges of 0.71–2.42 × $10^5$ (barley) and 0.36–2.55 × $10^5$ (oat); a higher ratio of 3-O-β-cellobiosyl-D-glucose to 3-O-β-cellotriosyl-D-glucose oligomers in the polymeric chains was also found for barley β-glucans. Analysis of variance showed that polysaccharide concentration, Mw and fine structure of β-glucans affected the VCAM-1 expression ($P < 0.05$); the reduction of VCAM-1 expression in stimulated HAEC was also dose-dependent (concentration range of 3.75–200 μg/ml β-glucans) and significant at polysaccharide levels > 6.25 μg/ml ($P < 0.05$). The inhibition of VCAM-1 expression was greater for barley β-glucans compared to oat, displaying a maximum anti-inflammatory activity at Mw ~1.40 × $10^5$. Instead, the expression of ICAM-1 was suppressed ($P < 0.05$) only at high polysaccharide concentrations (>100 μg/ml), with maximum activity at Mw ~2.50 × $10^5$. Structural differences between the oat and barley β-glucans did to seem to influence the ICAM-1 expression.

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## 1. Introduction

Atherosclerosis, the underlying condition of coronary artery disease (CAD), is one of the major health problems, among elderly individuals, in most Western societies (Meng, 2006). Endothelial dysfunction has been shown to be associated with anatomically overt CAD, while the inflammatory process, in terms of interaction of endothelium with the immune system, plays an important role in the pathogenesis of atherosclerosis (Feletou & Vanhoutte, 2006). The cytokine-induced expression of adhesion molecules is among the numerous signalling pathways which have been implicated in the immune response of endothelium (Han, Quon, & Koh, 2007). The adhesion molecules, namely vascular cell adhesion molecule (VCAM-1) and intracellular cell adhesion molecule (ICAM-1), activated by pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), seem to participate in the initiation of this interaction.

Cell walls of cereal grains, mostly barley and oat and in lesser extent rye, sorghum and wheat, are rich in mixed-linkage (1 → 3), (1 → 4)-β-D-glucans, which are linear homopolysaccharides of D-glucose residues interlinked via β-(1 → 3) and β-(1 → 4) linkages; their structure consists of consecutively (1 → 4)-linked β-D-glucose in blocks (i.e. oligomeric cellulose segments) that are separated by single (1 → 3)-linkages (Lazaridou & Biliaderis, 2007).

Oat and barley derived β-glucans have been implicated with cardiovascular protection in humans, exerting their effects mainly by lowering the levels of serum total cholesterol and low-density-lipoprotein (LDL)-cholesterol (Ames & Rhymer, 2008; Brown,

*Abbreviations:* CAD, coronary artery disease; VCAM-1, vascular cell adhesion molecule; ICAM-1, intracellular cell adhesion molecule; TNF-α, necrosis factor alpha; LDL-cholesterol, low-density-lipoprotein-cholesterol; GI, gastrointestinal; IL-1β, interleukin 1β; HAEC, human aortic endothelial cells; NAGREF, National Agricultural Research Foundation; OBC, oat bran concentrate; EBM, endothelial cell basal medium; hEGF, human epidermal growth factor; FBS, fetal bovine serum; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid); HBS, Hepes Buffer Saline; DMEM, Dulbecco's minimal essential medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Mw, molecular weight; HPSEC, high performance size exclusion chromatography; RI, refractive index; HPAEC, high-performance anion-exchange chromatography; PAD, pulsed amperometric detector; EGM, endothelial growth medium; PBS, phosphate buffer saline; DP, degree of polymerization; BARS, brachial artery reactivity studies.

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doi:10.1016/j.carbpol.2010.11.019

Rosner, Willett, & Sacks, 1999). As result, the US Food and Drug Administration (FDA) approved health claims for the use of oat and barley β-glucan based foods for lowering the risk of heart disease suggesting a dosage of 3 g β-glucan per day, with a recommendation of 0.75 g of β-glucan per serving (Anonymous, 1997, 2005). Recently, a similar health claim has also been approved by European Food Safety Authority (EFSA), stating that oat and barley β-glucans can contribute to maintenance of normal blood cholesterol concentrations at a dosage of at least 3 g per day in one or more servings (EFSA, 2009). Furthermore, supplementation with either oatmeal and whole-grain oat or wheat cereal products has been shown to prevent the postprandial impairment of vascular reactivity in response to a high-fat meal diet (Katz, Nawaz, Boukhalil, Giannamore, et al., 2001; Katz, Nawaz, Boukhalil, Chan, et al., 2001). Another known mechanism of soluble dietary fibers, such as cereal β-glucans, by which they can prevent endothelial dysfunction and decrease coronary heart disease risk is through their ability to attenuate postprandial elevations in insulin and glucose levels (Dikeman & Fahey, 2006; Vogel, 1999). Indeed, consumption of cereal β-glucans has been linked to lower postprandial glucose and insulin responses (Cavallero, Empilli, Brighenti, & Stanca, 2002; Wood, 2002). It is important also to mention that the hypoglycemic and hypocholesterolemic physiological actions of cereal β-glucans were found to largely depend on concentration and molecular weight of the solubilized polysaccharide in the gastro-intestinal (GI) tract (Lazaridou & Biliaderis, 2007; Wood, 2002).

There is a growing body of both *in vitro* and *in vivo* studies indicating that (1 → 3), (1 → 4)-β-D-glucans can also modulate various aspects of mammalian immune response. It has been demonstrated that mixed-linkage (1 → 3), (1 → 4)-β-D-glucans can stimulate the immune system showing anti-inflammatory activity, as well as exert antimicrobial and antitumor function under *in vitro* conditions or after oral, intraperitoneal, intragastrical or parenteral administration (Cheung & Modak, 2002; Davis et al., 2004; Delaney, Carlson, Frazer, et al., 2003; Di Renzo, Yefenof, & Klein, 1991; Estrada et al., 1997; Estrada, Van Kessel, & Laarveld, 1999; Hong et al., 2004; Porter, Morel, & Coles, 2006; Yun et al., 1998). Interestingly, the *in vitro* secretion of interleukin 1β (IL-1β) by rat monocytes stimulated by barley β-glucan was dependent on the molecular weight and dose of the polysaccharide (Porter et al., 2006). Additionally, an *in vitro* study showed that fecal water from ileostomic patients consuming oat β-glucans enhances the immune response of cytokine-stimulated enteric epithelium (Ramakers et al., 2007). In this context, previous studies have shown that the source (yeast, fungi, bacteria) and the structural and physical features of microbial (branched) β-(1 → 3), (1 → 6)-glucans, i.e. primary structure (degree of branching, polymer charge, linkage pattern and ratio), molecular weight, solubility and chain ordering, can considerably affect the physiological functionality on the immune system responses (Bohn & BeMiller, 1995; Giavasis & Biliaderis, 2007; Kulicke, Lettau, & Thielking, 1997; Zhang, Li, Xu, & Zeng, 2005).

To our knowledge, however, the effects of cereal β-glucans on the early stage of inflammatory process in endothelium leading to atherogenesis, have not yet been examined. The objective of the present study was to explore the anti-inflammatory potential of cereal β-glucans on immune stimulated endothelium *in vitro* and examine possible relationships between molecular structure of the polysaccharides and physiological function. Therefore, in this work the ability of barley and oat β-glucans, differing in molecular structure (molecular weight and distribution of cellulosic oligomers in polymeric chain), to inhibit the expression of adhesion molecules (VCAM-1 and ICAM-1) in TNF-α-activated human aorta endothelial cells (HAEC) and their effect on cell viability have been examined.

## 2. Materials and methods

### 2.1. β-Glucan sources

Eight mixed-linkage (1 → 3), (1 → 4) β-D-glucan samples varying in their molecular and structural features were used. Samples oat 255, oat 144 and oat 106 were β-glucans extracted from whole oat flours of three registered Greek oat varieties, Pallini, Kassandra and Flega, respectively; these oat cultivars, belonging to two different oat species (Pallini and Flega to *Avena sativa* L, and Kassandra to *Avena byzantina*), were obtained from the National Agricultural Research Foundation (NAGREF), Cereal Institute, Thessaloniki, Greece. Sample oat 36 was isolated from an oat bran concentrate (OBC) that was β-glucanase treated and was provided by CEBA (Lund, Sweden); the β-glucan and protein contents of the initial concentrate were 25.4% (dry basis, d.b.) and 17.7% (d.b.), respectively. The preparation bar 242 was isolated from whole barley flour of a registered Greek cultivar of normal covered barley seeds, Thessaloniki, provided by NAGREF, whereas bar 142, bar 113 and bar 71 samples were products derived from bar 242 by mild acid-hydrolysis.

### 2.2. Chemicals and cell culture

The HAEC culture, as well as the endothelial cell basal medium (EBM) and Single Quots Bulletin kit, containing human epidermal growth factor (hEGF), hydrocortisone, gentamycin, amphotericin B, bovine brain extract and fetal bovine serum (FBS), were purchased from Clonetics® (Cambrex Corporation, Athens Greece); all other cell culture materials, such as 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) buffered saline solution (HBS), trypsin/EDTA solution and Dulbecco's minimal essential medium (DMEM), were obtained from Invitrogen Life Technologies (Thessaloniki, Greece). TNF-α (T0157), (±)-α-tocopherol (vitamin E, T3251), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT, M5655) and the peroxidase substrate *o*-phenylendiamine hydrochloride (FAST™OPD, P9187) were obtained from Sigma–Aldrich (Athens, Greece). VCAM-1 (BBA4) and ICAM-1 antibodies (BBA3) were obtained from R&D System (Thessaloniki, Greece) and sheep anti-mouse IgG secondary antibody (NA931) was purchased from Amersham (Athens, Greece). All other chemicals were of analytical grade and purchased from Sigma–Aldrich, Fluka and BioRad (Athens, Greece).

### 2.3. Isolation of cereal β-glucans

The isolation-purification protocols and the acid hydrolysis procedures followed for the production of the oat and barley β-glucan isolates are described in detail in Fig. 1. Oat and barley grains from the Greek cultivars were ground in a Camas mill to pass a 0.8 mm screen. The reflux of oat and barley flours and of OBC with 82% (v/v) aqueous ethanol at 85 °C removed most of the lipids and aimed at the inactivation of endogenous β-glucanases. The aqueous extraction of β-glucans at a temperature (52 °C) below the gelatinization temperature of starch resulted in very little starch solubilization. The purification procedure involved a dual-enzyme digestion with a heat stable α-amylase (Termamyl 120L, Novozymes A/S, Bagsvaerd, Denmark) and porcine pancreatin (Sigma–Aldrich Chemie Gmbh, Steinheim, Germany), followed by removal of starch and protein hydrolyzates by exhaustive dialysis. After the precipitation of the polysaccharide with two volumes of ethanol, the material was suspended in 2-propanol, filtered and dried to obtain soluble oat (oat 255, oat 144, oat 106 and oat 36) and barley (bar 242) β-glucan isolates. Samples of barley β-glucans

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