Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 21 (2010) 47–54
Journal of Nutritional Biochemistry

Oligomerized grape seed polyphenols attenuate inflammatory changes due to antioxidative properties in coculture of adipocytes and macrophages

Takuya Sakurai[a], Kentaro Kitadate[b], Hiroshi Nishioka[b], Hajime Fujii[b], Takako Kizaki[a], Yasumasa Kondoh[c], Tetsuya Izawa[d], Hitoshi Ishida[e], Zsolt Radák[f], Hideki Ohno[a,*]

[a]*Department of Molecular Predictive Medicine and Sport Science, Kyorin University, School of Medicine, Tokyo 181-8611, Japan*
[b]*Amino Up Chemical Co., Ltd., Sapporo 004-0839, Japan*
[c]*Department of Surgery, Tokai University School of Medicine Tokyo Hospital, Tokyo 151-0053, Japan*
[d]*Department of Health and Sport Science, Doshisha University, Kyoto 610-0394, Japan*
[e]*Third Department of Internal Medicine, Kyorin University, School of Medicine, Tokyo 181-8611, Japan*
[f]*Laboratory of Exercise Physiology, School of Sport Science, Semmelweis University, Alkotas u. 44, Budapest H-1123, Hungary*

Received 17 May 2008; received in revised form 29 September 2008; accepted 2 October 2008

Abstract

Macrophage infiltration of white adipose tissue (WAT) is implicated in the metabolic complications of obesity. In addition, inflammatory changes through dysregulated expression of inflammation-related adipokines such as tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) in WAT are considered to be one of the causes of insulin resistance. Recently, enhanced oxidative stress in adipocytes has been reported to be implicated in dysregulated expression of inflammation-related adipokines. Polyphenols are well known as potent natural antioxidants in the diet. In the present study, we investigated the antioxidative effects of an oligomerized grape seed polyphenol (OGSP) on inflammatory changes in coculture of adipocytes and macrophages. Coculture of HW mouse white adipocytes and RAW264 mouse macrophages markedly increased the production of TNF-α, MCP-1 and plasminogen activator inhibitor-1 compared with control culture. Treatment of HW cells with OGSP significantly attenuated the dysregulated production of adipokines. Moreover, OGSP significantly suppressed coculture-induced production of reactive oxygen species (ROS). Although enhanced release of free fatty acids (FFAs) by coculture was not altered by OGSP, FFA-induced ROS production in HW cells was significantly attenuated by OGSP. Furthermore, OGSP significantly reduced increases in the transcriptional activity of nuclear factor-κB and activation of extracellular signal-regulated kinase by coculture. Thus, these results suggest that the antioxidative properties of OGSP attenuate inflammatory changes induced by the coculture of adipocytes and macrophages.

Keywords: Oligomerized grape seed polyphenol; Adipocytes; Anti-inflammatory effects; Antioxidative effects; Adipokines

1. Introduction

Obesity is associated with numerous diseases, including diabetes and cardiovascular disease [1]. Recently, it has been suggested that obesity is associated with chronic inflammatory response [2,3]. Indeed, an increased number of infiltrating macrophages and dysregulated secretion of some inflammation-related adipokines has been observed in the white adipose tissue (WAT) of obese subjects [4–6]. For example, expression levels of the pro-inflammatory adipokines tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1) are up-regulated in the WAT of obese mice [5,7,8]. Overexpression of MCP-1 in adipocytes promotes macrophage infiltration in WAT, and exposure of adipocytes to TNF-α and MCP-1 results in blockage of insulin-induced glucose uptake in adipocytes [8–10]. Moreover, in addition to impairing insulin signaling, it has been reported that TNF-α regulates expression of other adipokines, such as MCP-1 and plasminogen activator inhibitor-1 (PAI-1), in adipocytes [10,11]. TNF-α up-regulates PAI-1 expression in adipocytes via the pathway that involves activation of signaling molecules, such as extracellular signal-regulated kinase (ERK), protein kinase C

* Corresponding author. Tel.: +81 422 44 4427; fax: +81 422 44 4427.
E-mail address: ohnoh2o@kyorin-u.ac.jp (H. Ohno).

0955-2863/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2008.10.003

and nuclear factor-κB (NF-κB) [11]. The level of PAI-1 in plasma is increased in obese patients, and many reports have shown that increased PAI-1 levels contribute to the development of obesity [12]. These reports indicate that the inflammatory response in WAT is one of the potential mechanisms of obesity-induced insulin resistance.

A previous report has demonstrated that adipocytes generate reactive oxygen species (ROS) and that the levels of oxidative stress are increased in the WAT of KKAy mice, which act as a diabetic model [13]. Increased oxidative stress in adipocytes causes dysregulated expression of adipokines, including MCP-1 and PAI-1 [13–15]. Moreover, increased oxidative stress in adipocytes has been found to impair insulin signaling [16]. In fact, prolonged exposure of 3T3-L1 adipocytes to micromolar concentrations of H_2O_2 inhibits insulin-induced translocation of glucose transporter 4 to the plasma membrane, thereby inhibiting glucose uptake [17].

Grapes are one of the most widely consumed fruits in the world. Grape seeds are rich in polyphenols, including catechin, epicatechin and procyanidin [18]. One of the characteristics of grape seed polyphenols (GSPs) is that they are mixtures of monomers, dimers and oligomers of catechin and/or epicatechin, while tea polyphenols are only monomers of catechin and epicatechins with gallate substitution [18–20]. In general, it is thought that high-molecular-weight forms of polyphenols are more difficult to absorb than those with low molecular weights. Actually, Fujii et al. [21] have found that oligomerized forms of purified GSPs are more conducive to higher absorption rates in vivo than non-oligomerized forms. In addition, recent studies have shown that procyanidin in grape seeds possesses antioxidative and anti-inflammatory activities; for example, grape seed procyanidin acts as an anti-inflammatory agent in endotoxin-stimulated RAW264 macrophages by inhibiting NF-κB [19].

In the present study, we hypothesized a possibility that oxidative stress is implicated in inflammatory response of adipocytes and that polyphenols may possess anti-inflammatory effects through their antioxidative effects. Thus, we investigated the antioxidative effects of oligomerized grape seed polyphenols (OGSPs) developed by our group [21,22] on inflammatory changes in coculture of adipocytes and macrophages.

2. Materials and methods

2.1. Oligomerized grape seed polyphenol

OGSP was obtained by oligomerizing the purified GSP polymers using a modification of a patented technology previously described [21]. In brief, the process involves the eluate extraction of powdered dried fruits with 80% (v/v) methanol. The filtrate is subjected to a DAIMON HP-20 column, and after washing with H_2O, the eluate is evaporated to dryness, yielding a dark brown powder consisting of a mixture of proanthocyanidins. The resulting mixture is combined with L-cysteine hydrochloride monohydrate and L-ascorbic acid in H_2O and heated at 60°C for 48 h. The reaction mixture is filtered through a DAIMON HP-20 column, washed with H_2O and eluted with 40% (v/v) ethanol. Evaporation of the eluate yields a reddish brown powder, the oligomeric proanthocyanidin–cysteine complexes. The structure of the major components of OGSP was confirmed, by analysis of mass and nuclear magnetic resonance spectra, to be 4-*S*-cysteine derivatives of procyanidins B-1 and B-2, that is, epicatechin-(4β-8)-epicatechin-(4β-8)-catechin and epicatechin-(4β-8)-epicatechin-(4β-8)-epicatechin, respectively.

2.2. Cell culture

HW mouse white preadipocytes were kindly provided by Professor M. Saito (Tenshi University, Sapporo, Japan) [23]. These cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). Differentiation to adipocytes was induced by treatment with 1 μM dexamethasone and 0.5 mM 3-isobutyl-1-methylxanthine for 48 h. The treated cells were maintained in DMEM containing 10 μg/ml of insulin and 50 nM 3-3′-5-triod-L-thyronine for 72 h to accumulate the triglyceride content. Fully differentiated cells were treated with OGSP (10 and 20 μg/ml) for 24 h. The murine macrophage cell line RAW264 (RCB0535) was purchased from RIKEN Cell Bank (Tsukuba, Japan). Cells were cultured in DMEM supplemented with 10% FCS.

2.3. Cytotoxicity assay

The cytotoxic activity of OGSP for HW cells was determined by measuring the level of lactate dehydrogenase (LDH) in culture medium released from HW cells. The level of LDH was measured by using an LDH Cytotoxicity Detection Kit (Takara Bio Inc., Shiga, Japan) according to the manufacturer's protocol.

2.4. Measurement of ROS production in adipocytes

Measurement of ROS production in the HW cells was performed as described previously [13]. ROS production was measured by nitroblue tetrazolium (NBT) reduction. HW cells were incubated for 60 min in PBS (137 mM NaCl, 8.1 mM Na_2PO_4, 2.68 mM KCl and 1.47 mM KH_2PO_4) containing 0.2% NBT. Formazan was dissolved in 50% acetic acid, and the absorbance was monitored at 560 nm using a spectrophotometer.

2.5. Measurement of inflammation-related adipokines in culture medium

The levels of TNF-α, MCP-1 and PAI-1 in culture medium were measured using a Mouse TNF-α ELISA Kit (BioSource, Nivelles, Belgium), Mouse MCP-1 ELISA (Bender Medsystems, Burlingame, CA, USA) and Mouse PAI-1 Total Antigen Kit (Innovative Research, Peary Court Novi, MI, USA), respectively.