Food Chemistry 145 (2014) 198–204

Contents lists available at ScienceDirect

Food Chemistry

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

AMP-activated protein kinase mediates insulin-like and lipo-mobilising effects of β-glucan-rich polysaccharides isolated from *Pleurotus sajor-caju* (Fr.), Singer mushroom, in 3T3-L1 cells

G. Kanagasabapathy [a,c], K.H. Chua [a,c], S.N.A. Malek [b,c], S. Vikineswary [b,c], U.R. Kuppusamy [a,c,*]

[a] Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[b] Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
[c] Mushroom Research Centre, University of Malaya, 50603 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 22 January 2013
Received in revised form 25 June 2013
Accepted 13 August 2013
Available online 21 August 2013

Keywords:
Pleurotus sajor-caju
Lipogenesis
Lipolysis
Polysaccharides
Oxidative stress

ABSTRACT

Mushrooms have been used to treat various diseases for thousands of years. In the present study, the effects of *Pleurotus sajor-caju* mushroom on lipogenesis, lipolysis and oxidative stress in 3T3-L1 cells were investigated. The β-glucan-rich polysaccharides (GE) from *P. sajor-caju* stimulated lipogenesis and lipolysis but attenuated protein carbonyl and lipid hydroperoxide levels in 3T3-L1 cells. This extract caused an increase in the expression of 5′-AMP-activated protein kinase subunit γ-2 (PKRAG2) and 5′-AMP-activated protein kinase subunit γ-3 (PKRAG3) when compared to control (untreated) cells. Moreover, GE induced the expressions of hormone-sensitive lipase, adipose triglyceride lipase enzymes, leptin, adiponectin and glucose transporter-4 in 3T3-L1 cells which may have contributed to the lipolytic and insulin-like activities observed in this study. These findings suggest that GE is a novel AMPK activator that may be valuable in the formulation of nutraceuticals and functional food for the prevention and treatment of diabetes mellitus.

1. Introduction

The increasing worldwide incidence of diabetes mellitus (DM) in adults constitutes a global public health burden. The prevalence of DM was estimated to be 2.8% of the general population in the year 2000. It is predicted to be doubled by the year 2030 to about 380 million (Kanagasabapathy et al., 2012) and India, China and the United States will have the largest numbers of people with diabetes (Frode & Medeiros, 2008). Hence, there is a clear need for effective pharmaceutical intervention for treating DM (Umar, Ahmed, Muhammad, Dogarai, & Mat Soad, 2010). The most effective hypoglycemic drugs are insulin, sulphonylurea derivatives, biguanides, thiazolidinediones and alpha glucosidase inhibitors. However, these agents have undesirable side effects such as obesity, high blood pressure and heart disease (Slovacek, Pavlik, & Slovackova, 2008).

Adipose tissue (composed of adipocytes) plays a critical role in lipid metabolism, glucose homeostasis and energy balance. The primary role of adipose tissue is to store energy in the form of triglycerides when energy intake exceeds energy expenditure and to release it in the form of free fatty acids in starvation. Adipocyte differentiation, known as lipogenesis, is the anabolic process of fat cell development (Lee et al., 2010) while lipolysis is the catabolic process that causes the breakdown of triglycerides stored in fat cells to fatty acids and glycerol (Jeon et al., 2004). Insulin favours lipid storage through the activation of lipogenesis, lipoprotein lipase synthesis and export to the vascular endothelium and triglyceride esterification through the production of glycero-phosphate from glucose. By contrast, adrenergic hormones (glucagon and epinephrine) activate lipolysis through binding to a β-adrenergic receptor and production of cellular cAMP. In adipose tissue, lipolysis (fasting and exercise) activates AMP-activated protein kinase (AMPK). AMPK acts as a fuel sensor and regulates glucose and lipid homeostasis in adipocytes (Kong, Kim, & Kim, 2009). Once activated, AMPK phosphorylates a number of proteins and modulates the transcription of genes implicated in the regulation of energy metabolism to switch on catabolic pathways that produce ATP and switch off anabolic pathways that consume ATP (Daval, Foufelle, & Ferre, 2006).

Mushroom polysaccharides have been studied extensively because they are known to possess many medicinal properties, such as antitumor, immune-modulating, antioxidant, anti-hypercholestrolemic (Oyetayo, 2008), antiviral, antibacterial, anti-inflammatory and anti-diabetic activities (Fukushima, Ohashi, Fujiwara, Sonoyama, & Nakano, 2001). In Malaysia, the widely

* Corresponding author at: Department of Biomedical Science, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. Tel.: +60 3 79674900; fax: +60 3 79676600.
E-mail address: umah@um.edu.my (U.R. Kuppusamy).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.08.051

cultivated 'edible fungal food' is the *Pleurotus* mushroom, commonly referred to as oyster mushroom (cendawan tiram). Oyster mushrooms have been discovered to have definite nutritive and medicinal values with good quality proteins, carbohydrates, iron, vitamins and very little lipid or starch. Reports on medicinal properties of *P. sajor-caju* polysaccharides are still scant but chemical characterisations of the polysaccharides are well-known. Pramanik, Mondal, Chakraborthy, Rout, and Islam (2007) have reported that polysaccharides from hot water extract of *P. sajor-caju* consist of soluble glucans (α and β) and heteropolysacharides. The polysaccharides are identified as (1→3),(1→6)-β-glucans.

Therefore, the present study was undertaken to investigate the effects of β-glucan-rich polysaccharides of *P. sajor-caju* (GE) on lipogenic and lipolytic activities in 3T3-L1 cells, as well as the oxidative stress level during these processes. In order to elucidate the underlying mechanism, the protein and gene expression studies were carried out using the adipocytes treated with GE and insulin as positive control. The findings of this study may be valuable in the formulation of nutraceuticals and functional food for the prevention or adjuvant therapy for diabetes mellitus.

2. Materials and methods

2.1. Mushroom samples

All necessary permits and permission for the collection of materials for the described field of study were obtained and the party involved is duly acknowledged. Fresh fruiting bodies of *P. sajor-caju* (10 kg) were grown and collected from a mushroom farm in Semenyih, Selangor Darul Ehsan, Malaysia. Authentication of *P. sajor-caju* was carried out by the Mushroom Research Centre (MRC), University of Malaya and a voucher material (KUM 50082) for this study was deposited at the MRC culture collection.

2.2. Isolation, purification and determination of β-glucan in polysaccharide (GE) from hot-aqueous extract of P. sajor-caju

The isolation and purification of polysaccharide were based on the method described by Roy, Maiti, Mondal, Das, and Islam (2008) except that, in this study, the polysaccharide was not further purified by gel permeation chromatography. The β-glucan level in GE was estimated using a β-glucan kit (specific for mushroom and yeast) purchased from Megazyme International (Ireland). The enzyme kit, contains exo-1,3-β-glucanase, β-glucosidase, amyloglucosidase, plus invertase, glucose determination reagent (GOPOD-glucose oxidase, peroxidase, 4-aminoantipyrine) and glucose standard solution. To estimate the total glucan content, GE was hydrolysed with 37% hydrochloric acid (v/v) for 45 min at 30 °C and continued for 2 h at 100 °C. After neutralisation with 2 M potassium hydroxide, hydrolysis (onto glucose) was done using a mixture of exo-β-(1-3)-D-glucanase plus β-glucosidase in sodium acetate buffer (pH 5.0) for 1 h at 40 °C. To measure the total glucan content, glucose oxidase–peroxidase mixture was added to GE and incubated for 20 min at 40 °C. The absorbance was measured using a spectrophotometer (Bio-Tek Instruments Inc, USA) at 510 nm. The α-glucan content was estimated according to the same method as described above, after enzymatic hydrolysis with amyloglucosidase and invertase. The β-glucan content was calculated by subtracting the α-glucan from the total glucan content.

2.3. Reagents and cells

3T3-L1 cells were purchased from the American Type Culture Collection (ATCC, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin and insulin were purchased from Sigma Chemical Co. (USA). Dexamethasone and 1-methyl-3-isobutyl xanthine (IBMX) were purchased from ICN (USA). Amphotericin B (Fungizone®) was purchased from Flow Lab (Australia). Thiazolyl blue tetrazolium bromide (MTT) was purchased from Bioworld (USA). Isopropanol was purchased from Thermo Fisher Scientific Inc. (USA).

2.4. Assessment of viable cells

The cells were seeded at a density of 3×10^4 cells/ml. Preadipocyte suspension (90 μl) was transferred into sterile 96-well tissue culture plates and incubated for 24 h at 37 °C and 5% CO_2. Then, aliquots (10 μl) of different concentrations of GE were added to the culture wells to yield final concentrations of one to 1000 μg/ml and the plates incubated for 24 and 48 h, respectively. Milli-Q water was used as the negative control. The viability of preadipocytes was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). The absorbance was measured at 560 nm and 700 nm (reference), using a spectrophotometer. The percentage of cell viability was calculated as stipulated below:

$$\text{Cell viability } (\%) = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{control}}} \times 100$$

2.5. Differentiation and lipogenesis assay

For the differentiation process, cells were seeded at a density of 3×10^4 cells/ml in each well of a 24-well culture plate. Confluent preadipocytes were differentiated with part-1 differentiation media (DM1) (10% FBS, 2 mM fungizone, 2 mM penicillin–streptomycin, 1 μM dexamethasone, 0.5 mM IBMX, 10 μg/ml insulin and DMEM media) for two days at 37 °C and 5.0% CO_2. On day three, DM1 was removed and the cells were replenished with part-2 differentiation media (DM2) (10% FBS, 2 mM fungizone, 2 mM penicillin–streptomycin, 10 μg/ml insulin and DMEM media). On day five, the cells were replenished with growth media (GM) (FBS, 2 mM fungizone, 2 mM penicillin–streptomycin and DMEM media). Each concentration of GE (0.01–1000 μg/ml) was tested in triplicate. After incubating for 48 h, the adhering cells were washed with PBS and fixed with formalin, rinsed with 60% isopropanol, stained with Oil Red O working solution (0.3% in 60% isopropanol) and incubated in the dark for 2 h. Subsequently, the cells were washed with phosphate buffered saline (PBS). The cells were then dissolved in 100% isopropanol and the absorbance was measured at 510 nm, using a spectrophotometer. Insulin was used as the positive control while Milli-Q water was used as the negative control. The percentages of lipogenic activity were calculated as stipulated below:

$$\text{Lipogenic viability } (\%) = \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}}}{\text{Abs}_{\text{control}}} \times 100$$

2.6. Lipolysis assay

Fully differentiated adipocytes were treated with various concentrations (1–1000 μg/ml) of GE. After 4 h of treatment, lipolysis assay was performed according to the protocol provided in the lipolysis kit purchased from ZenBio (USA). This method is based on a coupled reaction, which measures non-esterified fatty acids and glycerol released by adipocytes. The glycerol and free fatty acids were quantified by an enzyme-based reaction, which yields a proportional quantity of hydrogen peroxide (H_2O_2), which is measurable once modified into chromogenic compounds. Isoproterenol