Inhibitory Effects of Marine Algae Extract on Adipocyte Differentiation and Pancreatic Lipase Activity

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Abstract - Obesity, which is characterized by excessive fat accumulation in adipose tissues, occurs by fat absorption by lipase and sequential fat accumulation in adipocyte through adipocyte differentiation. Thus, inhibition of pancreatic lipase activity and adipocyte differentiation would be crucial for the prevention and progression of obesity. In the present study, we attempted to evaluate anti-adipogenic activity of several algae extracts employing preadipocytes cell line, 3T3-L1 as an in vitro assay system. The effects on pancreatic lipase activity in vitro were also evaluated. Total methanolic extracts of Cladophora wrightiana and Costaria costata showed significant inhibitory activity on adipocyte differentiation as assessed by measuring fat accumulation using Oil Red O staining. Related to pancreatic lipase, C. wrightiana and Padina arborescens showed significant inhibition. Further fractionation of C. wrightiana, which showed the most potent activity, suggested that CHCl₃ and n-BuOH fraction are responsible for adipocyte differentiation inhibition, whereas n-BuOH and H₂O fraction for pancreatic lipase inhibition. Our study also demonstrated that n-BuOH fraction was effective both in early and middle stage of differentiation whereas CHCl₃ fraction was effective only in early stage of differentiation. Taken together, algae might be new candidates in the development of obesity treatment.

Key words - Marine algae, C. wrightiana, Adipocyte differentiation, Pancreatic lipase, 3T3-L1 cells, Obesity

Introduction

Obesity is a major health problem in both developed and developing countries (Brug and Crawford, 2009). It is no longer considered only a cosmetic problem but associated with several pathological disorders, including diabetes, hypertension, atherosclerosis and cancer (Kopelman, 2000). Obesity is characterized by abnormal increase in the number and/or size of adipocytes in adipose tissue (Rosen and Spiegelman, 2006; Bastard et al., 2006). Therapeutics for obesity can be developed by various ways such as lipase inhibition, suppression on food intake, stimulation of energy expenditure, inhibition on adipocyte differentiation and regulation on lipid metabolism (Yun, 2010). Synergic action of these mechanisms is preferred for the most effective way to treatment of obesity.

Energy intake starts from fat absorption through digestion of fat into monoglycerides and fatty acids. Lipase is a key enzyme for lipid absorption. Among lipase, pancreatic lipase is responsible for the hydrolysis of 50 - 70% of total dietary fats (Birari and Bhutani, 2007). Reduction of fat absorption by the inhibition of pancreatic lipase is known to be beneficial for the regulation of obesity (Yun, 2010). Orlistat, a specific pancreatic lipase inhibitor, has been clinically used for the prevention of obesity (Hill et al., 1999; Ballinger and Peikin, 2002). Absorbed fat is further accumulated into adipose tissue. Adipose tissue is as a metabolic and endocrine organ which plays an essential role in regulating energy balance. In obesity, adipocytes undergo abnormal growth characterized by increased numbers of fat cells storing their lipids through excessive adipocyte differentiation. Therefore, inhibition of fat absorption and/or fat accumulation by the disturbance of lipase and adipocyte differentiation is suggested to be important therapeutics in obesity.

Marine algae are rich sources of bioactive compounds with diverse biological activities (Kim and Karagozlu, 2011). Because of harsh underwater environment, algae produces characteristic constituents by unique metabolic pathways compared to aerial plants. Marine algae exhibit
diverse biological activities, such as anticancer, anticoagulant, antiviral, antioxidant, and anti-inflammatory activity (Athukorala et al., 2007; Artan et al., 2008; Park et al., 2005; Kim et al., 2009; Kim and Thomas, 2011; Mayer and Gustafson, 2008). Recently, beneficial effects of marine algae against obesity have been reported (Maeda et al., 2007; Kong et al., 2010; Park et al., 2011). In the present study, we attempted to investigate the antiadipogenic activity of algae extract by measuring adipocyte differentiation and pancreatic lipase activity.

**Experimental**

**Plant material**—The marine algae samples were collected by hand from a depth of 10 m using scuba equipment off the shore in the South Korea. *Hypnea flexicilis* and *S. micracanthum* were collected from Wando, Cheonnam Province, Korea, at April, 2010. *C. costata, A. clathratum* and *D. viridis* were collected from Goseong, Gangwon Province, Korea, at March, 2010. *C. wrightiana, M. papulosa, Pachymeniopsis lanceolata, P. athescens* and *H. clathratus* were collected from Seogwipo, Jeju Province, Korea, at May, 2010. They were identified by the herbarium of Wildlife Genetic Resources Center, National Institute of Biological Resource, where voucher specimens, *C. costata* (BAE20100330E3), *A. clathratum* (BAE20100330E2), *D. viridis* (BAE20100330E1), *C. wrightiana* (BAE20100508E7), *M. papulosa* (BAE20100508E6), *Pachymeniopsis lanceolata* (BAE20100508E5), *P. athescens* (BAE20100508E4), and *H. clathratus* (BAE20100508E3) were deposited.

**Extraction and isolation**—Fresh algae were dried for three days at the room temperature. Then, they were extracted 3 times with MeOH at room temperature for 2 days, which yielded the methanolic extract. The methanolic extract was then suspended in H2O and partitioned successively with n-hexane, CHCl3, EtOAc and n-BuOH to yield n-hexane, CHCl3, EtOAc, n-BuOH and H2O fractions.

**Sample preparation**—Fresh algae were washed two or three times with tap water to remove salts and epiphytes from the surface and air dried for two or three days at the room temperature. Its extract and fractions were dissolved in DMSO and diluted with water. Sample solutions were filtered in membrane filter (0.2 µm) before treatment.

**Culture of 3T3-L1 cells**—3T3-L1 mouse embryo fibroblasts were obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) until confluence. Two days after confluence (day 0), cells were stimulated to differentiate with differentiation medium containing DMEM with 10% FBS, 0.5 mM 3-isobutyl-1-methyl-xanthine, 1 µM insulin and 1 µM dexamethasone for 2 days (day 2). Cells were then maintained in DMEM supplemented with 10% FBS and 1 µM insulin for another 2 days (day 4), followed by culturing with DMEM with 10% FBS for an additional 4 days (day 8). All media contained 100 IU/mL penicillin and 100 µg/mL streptomycin. Cells were maintained at 37 ºC in a humidified atmosphere of 95% air-5% CO2. The cultures were treated with test samples for whole culture period (day 0 - 8) for general experiment. In some experiment, test samples were treated after differentiation was induced (fully differentiated cells) for three day. For the evaluation of time dependent activity, test samples were treated for indicated time-periods.

**Oil Red O staining**—Lipid droplets in cells were stained with Oil Red O (Choi et al., 2011). Eight days after differentiation induction, cells were washed three times with PBS and fixed with 10% formalin at room temperature for 1 hr. After fixation, cells were washed once with PBS and stained with freshly diluted Oil Red O solution (3 parts of 0.6% Oil Red O in isopropyl alcohol and 2 parts of water) for 15 min. Cells were then washed twice with water and visualized. For quantitative analysis, Oil Red O staining was dissolved with isopropyl alcohol and optical density was measured at 520 nm by ELISA plate reader.

**Assessment of pancreatic lipase activity**—Pancreatic lipase inhibitory activity was evaluated using previously reported methods with a minor modification (Nakai et al., 2005; Lee et al., 2010). Briefly, enzyme solution was prepared by the reconstitution of porcine pancreatic lipase (Sigma, St. Louis, MO) in 0.1 M Tris-HCl buffer (pH 8). Then, test sample was mixed with enzyme buffer, and incubated for 15 min at 37 ºC. After incubation, 10 mM p-nitrophenylbutyrate (p-NPB) was added and the enzyme reaction was allowed to proceed for 15 min at 37 ºC. Pancreatic lipase activity was determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using a microplate reader. Relative pancreatic lipase activity (%) was calculated as (activity of compound w/ substrate – negative control of compound w/o substrate) / (activity of w/o compound and w/ substrate – negative control of w/o compound and substrate) × 100.

**Statistical analysis**—The evaluation of statistical significance was determined by the Student’s t-test with a value of p < 0.05 or less considered to be statistically significant.