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Anti-obesity effects of seaweeds of Jeju Island on the differentiation of 3T3-L1 preadipocytes and obese mice fed a high-fat diet

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ABSTRACT

The seaweeds were collected from the coast of Jeju Island, South Korea. We investigated ethanol extracts from seaweed as potential antiobesity agents by testing their effect on adipogenic differentiation in 3T3-L1 cells. Among the red algae extracts tested, the *Plocamium telfairiae* extract (PTE) showed the highest inhibitory effect on lipogenesis in adipocytes and, thus, was selected as a potential antiobesity agent. PTE treatment significantly decreased the expression of the adipogenic-specific proteins peroxisome proliferator-activated receptor-γ, CCAAT/enhancer-binding protein-α, sterol regulatory element-binding protein 1, and fatty acid-binding protein 4 compared with that in the untreated 3T3-L1 cells. PTE also inhibited high-fat diet (HFD)-induced obesity in male C57BL/6 mice. Oral administration of PTE significantly reduced the body weight, fatty liver, amount of white adipose tissue, and levels of triglyceride and glucose in the tested animals. Taken together, these data demonstrate that PTE can be developed as a therapeutic agent for obesity.

1. Introduction

Obesity is a medical condition of excess body fat accumulation that may impair health (Wu et al., 2010). According to the World Health Organization, overweight and obesity are the fifth leading risk for global death, and at least 2.8 million adults die each year as a result of being overweight or obese (Chandrasekaran et al., 2012). The fundamental cause of obesity is an energy imbalance between calories ingested and calories expended as a result of changes in diet and physical activity (Kim et al., 2011). Obesity and overweight lead to a number of chronic diseases including cardiovascular disease, type 2 diabetes, some types of cancer, and osteoarthritis (Park et al., 2012).

Sibutramine and orlistat are used as antiobesity drugs, but they have some adverse effects such as headache, thirst, insomnia, constipation, and steatorrhea (Ho et al., 2006; Sung et al., 2011). Therefore, many researchers have tried to find natural remedies to combat life-threatening obesity. Some studies reported that natural bioactive substance from the seeds of *Hunteria umbellata*, *Panax ginseng*, and *Embelia ribes* have antiobesity effects in high-fat diet (HFD)-fed mice (Adeneye et al., 2010; Lee et al., 2013a,b; Bhandari et al., 2013).

Marine algae have been used as a traditional food in Asian countries, especially China and Korea (Kang et al., 2013a,b; Rioux et al., 2009). Marine algae have been reported to contain diverse bioactive substances including polyphenols, polysaccharides, and amino acids (Kang et al., 2013a,b), and ethanol extracts from red algae show various biological activities such as asthmatic, antidiabetic, and antioxidant effects (Jung et al., 2009; Kim et al., 2008; Wang et al., 2009). The present study evaluated the antiobesity effect of ethanol extracts from red algae on 3T3-L1 cells and HFD-fed mice.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), bovine serum (BS), Phosphate-buffered saline (pH 7.4; PBS) and penicillin–streptomycin (PS) were from Gibco BRL (Grand Island, NY, USA). Antibodies to peroxisome proliferator activated receptor gamma (PPARγ), fatty acid binding protein 4 (FABP4) and CCAAT/enhancer binding protein (C/EBPα) were purchased from Cell Signaling Technology (Bedford, Massachusetts, USA). Antibody

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to sterol regulatory element binding protein 1C (SREBP-1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). 3-isobutyl-1-methylxanthine (IBMX), dexamethasone, insulin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were from Sigma Chemical Co. (St. Louis, MO, USA). Thiobarbituric acid-reactive substances (TBARS), total cholesterol, determined in the tissue using a commercial available kit from Sigma Chemical Co. (St. Louis, MO, USA). All chemicals and reagents used were of analytical and obtained from commercial sources.

2.2. Preparation of 70% ethanol extract from marine algae

Marine algae were collected from the coast of Jeju Island, South Korea. Salt, sand and epiphytes were using tap water. Then the samples were rinsed carefully with fresh water and freeze-dried. Dried seaweeds were ground and sifted through a 50-mesh standard testing sieve. The marine algae powder (2 g) was extracted three times with 70% ethanol (100 ml) and filterated. After filtration, 70% ethanolic extracts were evaporated to dryness under vacuum and dissolved in DMSO, and then used for experiments, adjusting the final concentration of DMSO in culture medium to <0.1% (Tables 1–3).

2.3. Animals

Male C57BL/6 mice, weighing 19–22 g, were purchased from Jung Ang Lab Animal Inc (Seoul, Korea). Animals were acclimated to temperature (22 °C) and humidity (55%) controlled rooms with a 12-h light/dark cycle for 1 week prior to use. Mice were randomly divided into 3 groups: group 1, normal diet; group 2, HFD; and group 3, HFD plus algae extract (100 mg/kg, mouse). Each group consisted of 10 mice. Starting after the fifth week, HFD-fed mice were treated with 100 mg/kg algae extract or saline for 9 weeks, and body weights and survival rates were measured for 14 weeks. After 14-weeks, the mice were anesthetized and blood samples were collected to determine biochemical parameters. The blood samples of mouse were collected to determine the triglyceride content. The livers and white adipose tissues immediately fixed in 10% formalin, and then stained with haematoxylin and eosin (H&E). The remaining livers and white adipose tissues were froze in liquid nitrogen and stored at − 70 °C for biochemical assays. Blood glucose levels were determined by detecting serum glucose content. Glucose contents were measured in tail blood using a Glucometer (Accu-Chek Instant: Boehringer Mannheim, Seoul, Korea). All experiments were performed in accordance with the experimental animal guidelines of Jeju National University animal center.

Table 1
List of brown algae collected from Jeju Island, Korea.

No.	Scientific name
1	*Ishige foliacea*
2	*Undaria pinnatifida*
3	*Myelophycus caespitosus*
4	*Padina arborescens*
5	*Sargassum thunbergii*
6	*Dictyota coriacea*
7	*Ishige okamurae*
8	*Hijikia fusiforme*
9	*Sargassum borneri*
10	*Sargassum piluliferum*
11	*Sargassum muticum*
12	*Myagropsis myagroides*
13	*Sargassum macrocarpum*
14	*Sargassum giganteifolium*

Table 2
List of green algae collected from Jeju Island, Korea.

No.	Scientific name
1	*Codium contractum Kjellman*
2	*Ulva conglobata*
3	*Enteromorpha linza*
4	*Ulva pertusa Kjellman*
5	*Enteromorpha intestinalis*

2.4. Cell culture and differentiation

3T3-L1 preadipocytes were obtained from American Type Culture Collection (Rockville, MD, USA) were cultured in DMEM containing 1% PS and 10% bovine calf serum (Gibco BRL) at 37 °C under a 5% CO_2 atmosphere. To induce differentiation, 2-day post confluent preadipocytes (designated Day 0) were cultured in MDI differentiation medium (DMEM containing 1% PS, 10% FBS, 0.5 mM IBMX, 0.25 μM dexamethasone and 5 μg/ml insulin) for 2 days. The cells were then cultured for another 2 days in DMEM containing 1% PS, 10% FBS and 5 μg/ml insulin. Thereafter, the cells were maintained in post differentiation medium (DMEM containing 1% PS and 10% FBS), with replacement of the medium every 2 days. To examine the effects of test samples on the differentiation of preadipocytes to adipocytes, the cells were cultured with MDI in the presence of test samples. Differentiation, as measured by the expression of adipogenic markers and the appearance of lipid droplets, was complete on Day 8.

2.5. Cell viability assay

The cytotoxicity of seaweed against 3T3-L1 cells was assessed via a colorimetric MTT assay. 3T3-L1 preadipocytes plated on 24-well plate were treated with seaweeds (100 μg/ml) at 37 °C for 48 h. MTT stock solution (100 μl; 2 mg/ml in PBS) was then added to each well to a total reaction volume of 600 μl. After 4 h of incubation, the plates were centrifuged (800 × g, 5 min), and the supernatant were aspirated. The formazan crystals in each well were dissolved in 300 μl of DMSO, and the absorbance was measured with an ELISA plated reader at 540 nm.

2.6. Determination of lipid accumulation by Oil Red O staining

To induce adipogenesis, 3T3-L1 preadipocytes were seeded on 6-well plates and maintained for 2 days after reaching confluence. Then media was exchanged with differentiation medium (DMEM containing 10% FBS, 0.5 mM IBMX, 0.25 μM Dex and 10 μg/ml insulin) and cells were treated with test samples. After two days, the differentiation medium was replaced with adipocyte growth medium (DMEM supplemented with 10% FBS and 5 μg/ml insulin), which was refreshed every 2 days. After adipocyte differentiation, the cells were stained with Oil Red O, an indicator of cell lipid content with slight modifications. Briefly, cells were washed with

Table 3
List of red algae collected from Jeju Island, Korea.

No.	Scientific name
1	*Corallina pilulifera Postels et Ruprecht*
2	*Acrosorium flabellatum Yamada*
3	*Geliduum amansii*
4	*Lomentaria denticulate*
5	*Martensia denticulate*
6	*Plocamium telfairiae*
7	*Laurencia okamura Yamada*
8	*Carpopeltis angusta*