Effects of the Crude Polysaccharides from *Poria cocos* on the Proliferation and Differentiation of 3T3-L1 Cells

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Abstract: To study the effects of crude polysaccharides from *Poria cocos* (PCCP) on proliferation and differentiation of 3T3-L1 preadipocyte. 3T3-L1 cells were treated with culture medium contained different concentrations of PCCP (0, 50, 100 and 200 µg mL⁻¹) for 48 h and subsequently cell proliferation was assayed by MTT Method. The extent of the differentiation was determined by fluorescence spectrophotometry and Oil Red O staining on days 8 after treatment with culture medium supplemented with different concentration PCCP (0, 50, 100 and 200 µg mL⁻¹). As the results, the different concentration PCCP (50, 100 and 200 µg mL⁻¹) inhibited to some extent proliferation of 3T3-L1 preadipocytes when compared with the control. But the difference is not significant (p<0.05). And PCCP stimulated cell differentiation into adipocyte at lower concentration (50 and 100 µg mL⁻¹) (p<0.05) while it inhibited cell differentiation into adipocyte at high concentration (200 µg mL⁻¹) (p<0.05). It can be concluded from the results that the PCCP was involved in regulation of adipogenesis and lipogenesis.

Key words: *Poria cocos*, polysaccharides, 3T3-L1, proliferation, differentiation, China

INTRODUCTION

Adipose tissue mainly comprises adipocytes which is involved in the regulation of energy balance and homeostasis (Rosen and Spiegelman, 2006). Adipose tissue dysfunction plays a crucial role in the pathogenesis of obesity-related insulin resistance and type 2 diabetes (Goossens, 2008; Savage *et al.*, 2007). Thus, adipocytes are emerging as a potential therapeutic target for type 2 diabetes (Danforth, 2000). *Poria cocos* is a medicinal fungus of the family polyporaceae that grows on the roots of old dead pine trees (Lu *et al.*, 2010). *Poria cocos* is one of the most important traditional Chinese medicines widely used in China. *Poria cocos* alone or in combination with other herbs is often used to treat diabetes as well as other disorders (Jia *et al.*, 2003; Lau *et al.*, 2008; Li *et al.*, 2011). Besides antidiabetes activity, numerous reports indicate that extract of *Poria cocos* have antitumor, antioxidant and antiinfective activities in biological systems (Wu *et al.*, 2004; Tai *et al.*, 1995; Zhang *et al.*, 2005).

Chemical compounds found in *Poria cocos* include triterpenes and β-pachymarone, a polysaccharide composed of β-pachymaronose, pachymaric acid and pachomic acid (Lu *et al.*, 2010; Tai *et al.*, 1995). Polysaccharides as one of the most important components of *Poria cocos* exhibit various bioactivities. It has been reported that polysaccharides extracted from *Poria cocos* have antitumor and immunomodulatory activities (Lee *et al.*, 2004; Huang *et al.*, 2007). To date, the effect of the polysaccharides from *Poria cocos* on proliferation and differentiation of 3T3-L1 has not been researched. In the present study, researchers evaluated the effect of the PCCP on the proliferation and differentiation in 3T3-L1 preadipocytes. Understanding of the effects of the PCCP on proliferation and differentiation of 3T3-L1 preadipocyte would allow for control of certain diseases such as diabetes.

MATERIALS AND METHODS

Cell culture: The 3T3-L1 cell lines were obtained from the Center of Cell Culture Collection of Academia Sinica (Shanghai, China) and the cells were cultured by Dulbecco’s Modified Eagle’s Medium (DMEM, Hyclone) supplemented with 10% newborn calf serum (NBS, Gibco) and antibiotics in a 5% CO₂ humidified atmosphere at 37°C. About 2 days after postconfluence (day 0), cells were induced to differentiate by treatment with an induction medium containing 170 nM insulin, 1 µM Dexamethasone (Dex, Sigma) and 0.25 mM

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MTT cell viability assay: MTT (3-(4,5-dimethylthiazol-2-

Measurement of lipid accumulation: Lipid content was measured using a commercially available kit according to the manufacturer's instructions (AdipoRed assay Reagent, Lonza). AdipoRed, a solution of the hydrophilic stain Nile Red is a reagent that enables the quantification of intracellular lipid droplets. In brief, cells were induced to differentiation with culture media containing different concentration of the PCCP (0, 50, 100, 150 and 200 μg mL⁻¹) for 48 h periods (day 0-2, 2-4, 4-6 or 6-8) during the adipogenic phase. The medium was changed every 2 days. On day 8, the intracellular lipid content was measured by AdipoRed assay. Cells were washed with PBS (pH 7.4) and 200 μL of PBS was added to the wells. About 5 μL of AdipoRed reagent was added to each well. After 10 min, the plates were placed in the fluorometer and fluorescence was measured with an excitation wavelength of 485 nm and emission wavelength of 572 nm.

Oil Red O staining: To visualize intracellular lipids, cells treated with culture media contained different concentration PCCP for 8 days were stained with Oil Red O dye (Sigma, USA). In brief, the cells were washed twice with PBS fixed with 10% ice-cold formalin in PBS for 1 h, washed 3 times with PBS and then dried. The fixed cells were stained by Oil Red O for 1 h. After three washes with distilled water, the cells were photographed under a microscope (Olympus IX71, Japan) equipped with CCD video camera.

Data analysis: The statistical significance of variations in cell viability and lipid content was calculated by Duncan's test using PROC GLM (SAS Institute Inc, Cary, NC).

RESULTS AND DISCUSSION

The effect of PCCP on cell viability: To evaluate the effect of the PCCP on the viability of 3T3-L1 preadipocytes, 3T3-L1 preadipocytes were treated with various concentrations of the PCCP (0, 50, 100, 200 μg mL⁻¹) for 48 h. MTT was carried out at 48 h. According to the test results (Fig. 1) when the absorbance values obtained in basal culture mediums (without PCCP) were accepted as 100%, the mean cell viability values obtained after PCCP treatments at 50, 100 and 200 μg mL⁻¹ were 98.76, 97.75 and 94.70%. The PCCP showed a decrease in cell viability when compared to the control group. But the difference is not significant (p>0.05).

The effect of PCCP on lipid accumulation: To evaluate the effect of the PCCP on lipid accumulation, 3T3-L1 cells were treated with different concentration PCCP (0, 50, 100 and 200 μg mL⁻¹) during differentiation. Lipid content was
measured on day 8 by AdipoRed assay. In Fig. 2, the PCCP promoted lipid accumulation at low concentration (50 and 100 μg mL⁻¹) in 3T3-L1 cells while it inhibited lipid accumulation at high concentration in 3T3-L1 cells. For example, 50 and 100 μg mL⁻¹ of the PCCP increased lipid content by 63.83% and 52.70%, respectively when compared to the control group (p<0.05). In contrast, 200 μg mL⁻¹ of the PCCP inhibited lipid content by 10.10% when compared to the control group but the difference was not significant (p>0.05).

**Oil Red O staining:** Oil Red O staining were used to monitor lipid droplet accumulation in these cells treated with different concentration PCCP. Figure 3A shows the staining of undifferentiated 3T3-L1 cells and there is no clearly visible lipid droplets in 3T3-L1 cells. Figure 3B-E respectively show the staining of differentiated 3T3-L1 cells treated with different concentration of the PCCP (0, 50, 100 and 200 μg mL⁻¹) and there are clearly visible lipid droplets in 3T3-L1 cells. The polysaccharides isolated from Chinese herbs and mushrooms have attracted a great deal of public attention because of various bioactivities (Guo et al., 2004; Xu et al., 2009; Yamada, 1994). In the present study, it was found that the PCCP inhibited to some extent the proliferation of 3T3-L1 cells 48 h after treatment. But the difference is not significant (p>0.05). It is similar with the fucoidan (a sulfated polysaccharide) (Kim et al., 2010).

Preadipocyte differentiation is a transformation from a fibroblast-like cell to a lipid-filled cell. A failure in adipocyte differentiation has been suggested as one of the many causes of type 2 diabetes (Anand and Chada, 2000; Danforth, 2000).

Previous reports indicate that adipocytes of type 2 diabetes patients are insulin-resistant and are not capable of accumulating lipids to their full capacity (Anand and Chada, 2000; Okuno et al., 1998; Yang et al., 2004). Accordingly, adipocyte differentiation in cell culture has been used as a model of insulin insensitivity to study novel antidiabetic drugs (Maeda et al., 2001; Staels and Frucht, 2005). *Poría cocos* alone or in combination with
other herbs is often used to treat diabetes. Pachymic acid, as one of components of *Porvia cocos* has been shown to induce triglyceride accumulation in differentiated adipocytes (Huang et al., 2010). But the effect of polysaccharides isolated from *Porvia cocos* on adipocyte differentiation has not been researched so far. In present study, researchers evaluated the effect of the PCCP on lipid accumulation of the 3T3-L1 preadipocytes.

The results showed that low concentration of the PCCP (50,100 µg mL−1) promoted lipid accumulation and high concentration of the PCCP (200 µg mL−1) mildly inhibited lipid accumulation. The reason for this effect was probably due to influence the expression of hallmark genes such as PPARγ and C/EBPα. But the exact mechanism of the effect on differentiation need further research.

**CONCLUSION**

The study shows that PCCP was involved in regulation of adipogenesis and lipogenesis. It may be used for control of type 2 diabetes.

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