

Effects of Crude Polysaccharides from *Catathelasma ventricosum* On the Proliferation and Differentiation of 3T3-L1 Cells

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Abstract: To study the effects of Crude Polysaccharides from *Catathelasma ventricosum* (CVCP) on proliferation and differentiation of 3T3-L1 preadipocyte. 3T3-L1 cells were treated with culture media contained different concentrations of CVCP (0, 150, 300 and 600 $\mu\text{g mL}^{-1}$) for 24 h and subsequently cell proliferation was assayed by MTT Method. The extent of the differentiation was determined by fluorescence spectrophotometry and Nile Red staining on days 8 after treatment with culture media supplemented with different concentration CVCP (0, 150, 300 and 600 $\mu\text{g mL}^{-1}$). As the results, the different concentration CVCP (150, 300 and 600 $\mu\text{g mL}^{-1}$) promoted proliferation of 3T3-L1 preadipocytes when compared with the control ($p < 0.05$). And it stimulated cell differentiation into adipocyte at low concentration (150 $\mu\text{g mL}^{-1}$) while it inhibited cell differentiation into adipocyte at higher concentration (300 and 600 $\mu\text{g mL}^{-1}$). But the difference was not significant ($p > 0.05$). Besides, lipid content in 3T3-L1 cells treated with 150 $\mu\text{g mL}^{-1}$ was significantly higher than treated with 600 $\mu\text{g mL}^{-1}$ ($p < 0.05$). It can be concluded from the results that the CVCP promoted proliferation of the 3T3-L1 cells 24 h after treatment. Meanwhile, it was involved to some extent in regulation of adipogenesis and lipogenesis.

Key words: *Catathelasma ventricosum*, polysaccharides, 3T3-L1, proliferation, differentiation, China

INTRODUCTION

Adipose tissue, an important endocrine organ, plays critical roles in energy regulation and homeostasis (Rosen and Spiegelman, 2006). These functions are mediated by the actions of number of hormones produced in adipocytes (Havel, 2004). During the process of adipocyte differentiation, fibroblast-like preadipocytes differentiate into lipid-loaded adipocytes. The process is regulated by nutritional, hormonal and growth factor signals (Bengoechea-Alonso and Ericsson, 2010). Dysregulation of this differentiation may lead to metabolic diseases. Edible mushrooms are welcomed by people for low calorific value and a high content of proteins, vitamins, iron, fibre and minerals.

Besides their nutritional value, numerous reports indicate that extract of edible mushrooms have antitumor, cardiovascular and antibacterial activities in biological systems (Guillamon *et al.*, 2010; Hideyasu *et al.*, 2003; Ramesh and Pattar, 2010). Additional researches have shown that mushrooms have effect on adipocyte differentiation. For instance, extract of *Inonotus obliquus* can stimulate 3T3-L1 adipocyte differentiation while extract of *Ganoderma lucidum* can inhibit adipocyte

differentiation (Joo *et al.*, 2010; Thyagarajan-Sahu *et al.*, 2011). Polysaccharides as one of the most important components of mushroom, exhibit various bioactivities such as antitumor, antibacterial and immunological activities (Kim *et al.*, 2008; Mizuno *et al.*, 1996; Zhu *et al.*, 2011). Bioactivities of the crude polysaccharides from *Catathelasma ventricosum* has not been reported so far. To deeply explore the therapeutic value of the *Catathelasma ventricosum*, in the present study we evaluated the effect of the CVCP on the proliferation and differentiation in 3T3-L1 preadipocytes. Understanding of the effects of the CVCP on proliferation and differentiation of 3T3-L1 preadipocyte would allow for manipulation of adipocyte cell number and control of certain animal diseases.

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. The 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 Isobutylmethylxanthine (IBMX, Sigma) for 2 days (day 2). The cells were then cultured in the culture medium supplemented with 170 nM insulin (In, Sigma) for another 2 days (day 4). At the beginning of the 5th day, cells were continuously cultured in DMEM supplemented with 10% NBS and medium was changed every other day for 4 days. Undifferentiation control was only treated with DMEM supplemented with 10% NBS and medium was changed every other day for 8 days. To investigate the effects of the CVCP on the proliferation and adipocyte differentiation, the CVCP was prepared by water extraction and ethanol precipitation from *Catathelasma ventricosum*. The content of the CVCP is 47.4% and the CVCP were added to the culture media at different concentrations (0, 150, 300 and 600 µg mL⁻¹).

MTT cell viability assay: MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) Method was used to detect the effects of the CVCP on 3T3-L1 preadipocyte proliferation. The test was performed in 96 well plates. 3T3-L1 preadipocytes were seeded at a density of 2000 cells well⁻¹.

The culture media contained CVCP (0, 150, 300 and 600 µg mL⁻¹) were added at 24 h after cell seeding. Cells were incubated for 24 h. The medium was then changed and replaced with 100 µL of fresh 10% NBS and 10 µL of MTT solution (5 mg mL⁻¹). Cells were then returned to the incubator for an additional 4 h. And then the medium replaced with Dimethyl Sulfoxide (DMSO, 100 µL) to dissolve the formazan crystals. The absorbance was measured at 490 nm using a microplate reader (Thermo Varioskan, USA).

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 contained different concentration of the CVCP (0, 150, 300 and 600 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.

Nile Red staining: To visualize intracellular lipids, cells treated with culture media contained different concentration CVCP for 8 days were stained with Nile Red dye (AdipoRed, Lonza). In brief, culture media was removed and each well was washed with 200 µL of PBS (pH 7.4). About 5 µL of the Nile Red dye was added to each well. After 10 min, fluorescent images were captured with an inverted fluorescence microscope (Olympus IX71) 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 crude CVP on cell viability: To evaluate the effect of the CVCP on the viability of 3T3 L1 preadipocytes, MTT was carried out at 24 h (Fig. 1). The 3T3 L1 preadipocytes were treated with various concentrations of the CVCP (0, 150, 300, 600 µg mL⁻¹) for 24 h. The CVCP showed significant increase in cell viability. For example, 150, 300 and 600 µg mL⁻¹ CVCP increased cell viability by 14.3, 13.5 and 8.3%, respectively when compared to the control group (p<0.05). And this effect becomes weaker at very high concentrations (600 µg mL⁻¹).

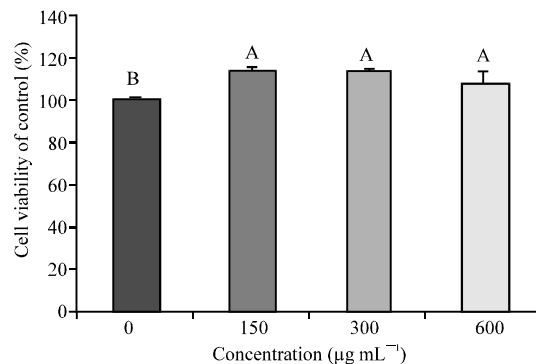


Fig. 1: Effects of the CVCP on cell viability for 3T3-L1 cells. 3T3-L1 preadipocytes were treated with culture media contained different concentrations of the CVCP for 24 h and their viability was determined by MTT assay. Means with the same letter are not significantly different (p>0.05, GLM, Duncan's test)

The effect of crude CVP on lipid accumulation: To evaluate the effect of the CVCP on lipid accumulation, 3T3-L1 cells were treated with different concentration CVCP (0, 150, 300 and 600 $\mu\text{g mL}^{-1}$) during differentiation. Lipid content was measured on day 8 by AdipoRed assay. As shown in Fig. 2, the CVCP promoted lipid accumulation at low concentration (150 $\mu\text{g mL}^{-1}$) in 3T3 L1 cells while it inhibited lipid accumulation at high concentration in 3T3-L1 cells. For example, 150 $\mu\text{g mL}^{-1}$ of the CVCP increased lipid content by 24.9% when compared to the control group but the difference was not significant ($p>0.05$).

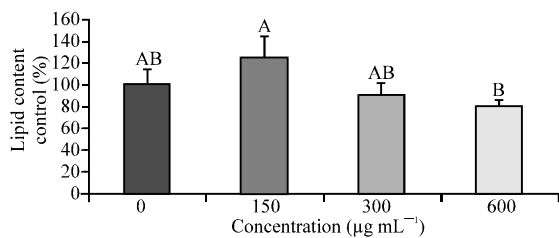


Fig. 2: Effect of the CVCP on lipid accumulation (control%) in 3T3-L1 cells. Cells were treated with different concentration of the CVCP during differentiation. Lipid content was measured on day 8 by AdipoRed assay. Means with the same letter are not significantly different ($p>0.05$, GLM, Duncan's test)

In contrast, 300 and 600 $\mu\text{g mL}^{-1}$ of the CVCP inhibited lipid content by 8.6 and 19.1%, respectively when compared to the control group and the difference was not significant ($p>0.05$). Moreover, Lipid content in 3T3-L1 cells treated with 150 $\mu\text{g mL}^{-1}$ was significantly higher than treated with 600 $\mu\text{g mL}^{-1}$ ($p<0.05$).

Nile Red staining: Nile Red is a fluorescent dye which has also been shown to be highly selective and specific for neutral lipids (Greenspan *et al.*, 1985). Figure 3A shows the staining of undifferentiated 3T3-L1 cells and there is no clearly visible lipid droplets in 3T3-L1 cells. Figure 3 (B-E), respectively show the staining of differentiated 3T3-L1 cells treated with different concentration of the CVCP (0, 150, 300 and 600 $\mu\text{g mL}^{-1}$) and there are clearly visible lipid droplets in 3T3-L1 cells. The polysaccharides extracted from the mushrooms have attracted a great deal of public attention because of various bioactivities (Guo *et al.*, 2004; Xu *et al.*, 2009). In the present study, it was found that the CVCP has a promotional effect on the proliferation of 3T3-L1 cells 24 h after treatment. It is consistent with the polysaccharides from Astragalus (Liu *et al.*, 2007). Furthermore, the effect of the CVCP on cell proliferation at low concentration (150 $\mu\text{g mL}^{-1}$) was stronger than at high concentration (600 $\mu\text{g mL}^{-1}$). It is consistent with the extract from Chaga mushroom (Joo *et al.*, 2010). Preadipocyte differentiation is a transformation from a

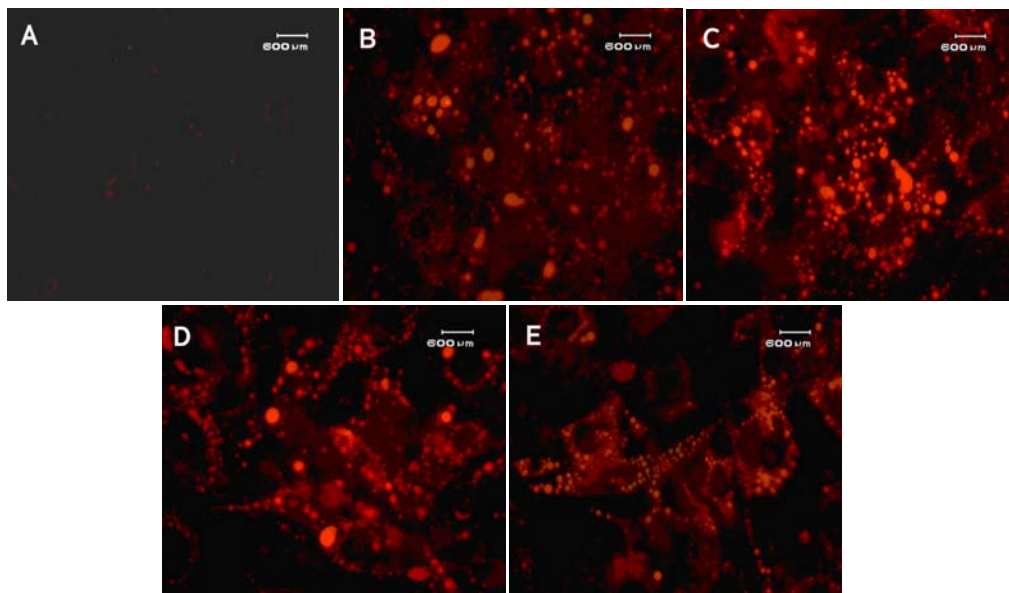


Fig. 3: Nile Red staining of 3T3-L1 cells treated with culture media contained different concentrations of the CVCP. 3T3-L1 cells were treated with CVCP (0, 150, 300 and 600 $\mu\text{g mL}^{-1}$) in culture media from days 0-8 of adipogenesis. Nile Red stains were done; A) Undifferentiation control; B) CVCP 0 $\mu\text{g mL}^{-1}$; C) CVCP 150 $\mu\text{g mL}^{-1}$; D) CVCP 300 $\mu\text{g mL}^{-1}$ and E) CVCP 600 $\mu\text{g mL}^{-1}$

fibroblast-like cell to a lipid-filled cell. The intracellular lipid accumulation is commonly monitored as a general marker to indicate the extent of adipogenesis in 3T3-L1 cells (Yeo *et al.*, 2011). Previous research showed that many factors such as vitamins, extracts from chinese traditional herbs and some hormones affect lipid accumulation in 3T3 L1 preadipocytes. In this study, researchers evaluated the effect of the CVCP on lipid accumulation of the 3T3-L1 preadipocytes. The results showed that low concentration of the CVCP (150 $\mu\text{g mL}^{-1}$) promoted lipid accumulation and high concentration of the CVCP (300 and 600 $\mu\text{g mL}^{-1}$) inhibited lipid accumulation. The reason for this effect was probably due to influence the expression of hallmark genes such as *PPAR γ* and *C/EBP α* . But the exact mechanism of the effect on differentiation need further research.

CONCLUSION

In this study, the CVCP promoted proliferation of the 3T3-L1 cell 24 h after treatment. Meanwhile, it was involved to some extent in regulation of adipogenesis and lipogenesis.

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