Research Journal of Biological Sciences 6 (11): 597-601, 2011
ISSN: 1815-8846

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

[1]Xun Wang, [1]Haifeng Liu, [2]Ling Zhao, [1]Han Dong, [1]Chunyou Ning,
[1]Xuewei Li and [2]Hengmin Cui
[1]College of Animal Science and Technology,
[2]College of Veterinary Medicine, Sichuan Agricultural University, Yaan, China

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 fluescence 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 ressitance 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 antiemetic 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-pachyman, a polysaccharide composed of β-pachymarose, pachymic acid and poricoic acid (Lu *et al*., 2010; Taia *et al*., 1995). Polysaccharides as one of the most important components of *Poria cocos* exhibit various bioactivies. 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 differentation 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% CO_2 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

Corresponding Author: Hengmin Cui, Department of Animal Science, College of Animal Science and Technology, Sichuan Agricultural University, #46 Xinkang Road, Yucheng District, 625014 Ya'an, P.R. China

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. Undifferentiaion 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 PCCP on the proliferation and adipocyte differentiation, the PCCP was kindly provided by pharmocology lab of Sichuan Agricultural University. The PCCP were added to the culture media at different concentrations (0, 50, 100 and 200 μg mL^{-1}).

MTT cell viability assay: MTT (3-(4, 5-dimethylthiazol-2y-l)-2, 5-diphenyltetrazolium bromide) Method was used to detect the effects of the PCCP on 3T3-L1 preadipocyte proliferation. The test was performed in 96-well plates. About 3T3-L1 preadipocytes were seeded at a density of 2000 cells/well. The culture media contained PCCP (0, 50, 100 and 200 μg mL^{-1}) were added at 24 h after cell seeding. Cells were incubated for 48 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^{-1}) 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 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 AdiopoRed assay. Cells were washed with PBS (pH 7.4) and 200 μL^{-1} 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) equiped 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 viablility: To evaluate the effect of the PCCP on the viabiliy 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

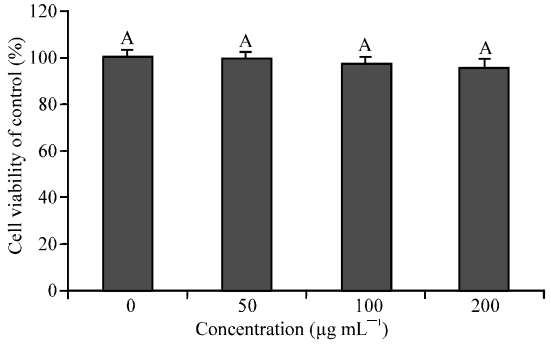

Fig. 1: Effects of the PCCP on cell viability for 3T3-L1 cells; 3T3-L1 preadipocytes were treated with culture media contained different concentrations of the PCCP for 48 h and their viability was determined by MTT assay; means with the same letter are not significantly different; ($p>0.05$, GLM, Duncan's test)

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 ($p>0.05$).

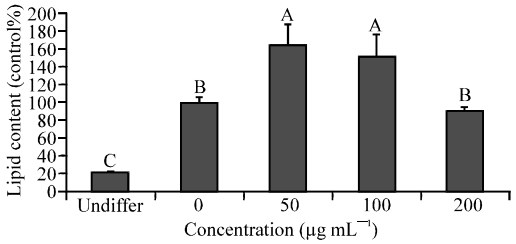

Fig. 2: Effect of the PCCP on lipid accumulation (control%) in 3T3-L1 cells. Cells were treated with culture medium contained with different concentration of the PCCP during differentiation; undifferentiation control (undiffer) was only treated with DMEM supplemented with 10% NBS. Lipid content was measured on day 8 by AdipoRed assay. Means with the same letter are not significantly different ($p>0.05$, GLM, Duncan's test)

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 differented 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 bioactivites (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 differertiation 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 antidaibetic drugs (Maeda *et al*., 2001; Staels and Fruchart, 2005). *Poria cocos* alone or in combination with

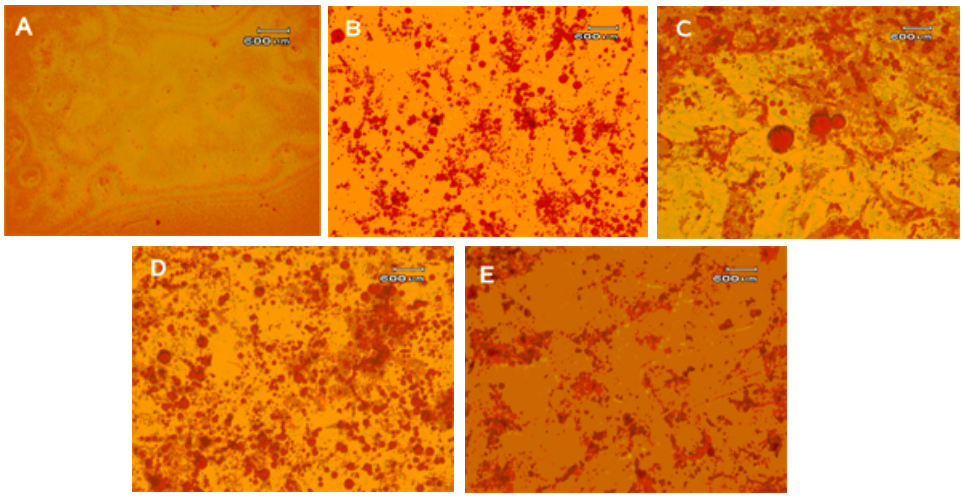

Fig. 3: Oil Red O staining of 3T3-L1 cells treated with culture medium contained different concentrations of the PCCP. 3T3-L1 cells were treated with PCCP (0, 50, 100 and 200 μg mL^{-1}) in culture media from days 0-8 of adipogennesis; undifferentiation control was only treated with DMEM supplemented with 10% NBS; Oil Red O stains were done; A) Undifferentation control; B) PCCP 0 μg mL^{-1}; C) PCCP 50 μg mL^{-1}; D) PCCP 100 μg mL^{-1} and E) PCCP 200 μg mL^{-1}

other herbs is often used to treat diabetes. Pachymic acid, as one of components of *Poria cocos* has been shown to induce triglyceride accumulation in differentiated adipocytes (Huang *et al*., 2010). But the effect of polysccharides isolated from *Poria 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.

ACKNOWLEDGEMENTS

This study was supported by grants from the National Special Foundation for Transgenic Species of China (2009ZX08009-155B and 2008ZX08006-003), the International Science and Technology Cooperation Program of China (2011DFB30340), the National Natural Science Foundation of China (30901024) and Chongqing Funds for Distinguished Young Scientists (CSTC2010BA1007).

REFERENCES

Anand, A. and K. Chada, 2000. *In vivo* modulation of *Hmgic* reduces obesity. Nat. Genet., 24: 377-380.

Danforth, E., 2000. Failure of adipocyte differentiation causes type II diabetes mellitus?. Nat. Genet., 26: 13-13.

Goossens, G.H., 2008. The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance. Physiol. Behav., 94: 206-218.

Guo, F.C., B.A. Williams, R.P. Kwakkel, H.S. Li and X.P. Li *et al*., 2004. Effects of mushroom and herb polysaccharides, as alternatives for an antibiotic, on the cecal microbial ecosystem in broiler chickens. Poult. Sci., 83: 175-182.

Huang, Q., Y. Jin, L. Zhang, P.C.K. Cheung and J.F. Kennedy, 2007. Structure, molecular size and antitumor activities of polysaccharides from *Poria cocos* mycelia produced in fermenter. Carbohydr. Polym., 70: 324-333.

Huang, Y.C., W.L. Chang, S.F. Huang, C.Y. Lin, H.C. Lin and T.C. Chang, 2010. Pachymic acid stimulates glucose uptake through enhanced GLUT4 expression and translocation. Eur. J. Pharmacol., 648: 39-49.

Jia, W., W. Gao and L. Tang, 2003. Antidiabetic herbal drugs officially approved in China. Phytother. Res., 17: 1127-1134.

Kim, K.J., O.H. Lee and B.Y. Lee, 2010. Fucoidan, a sulfated polysaccharide, inhibits adipogenesis through the mitogen-activated protein kinase pathway in 3T3-L1 preadipocytes. Life Sci., 86: 791-797.

Lau, C.H., C.M. Chan, Y.W. Chan, K.M. Lau and T.W. Lau *et al*., 2008. *In vitro* antidiabetic activities of five medicinal herbs used in Chinese medicinal formulae. Phytother. Res., 22: 1384-1388.

Lee, K.Y., H.J. You, H.G. Jeong, J.S. Kang, H.M. Kim, S.D. Rhee and Y.J. Jeon, 2004. Polysaccharide isolated from *Poria cocos* sclerotium induces NF-êB/Rel activation and iNOS expression through the activation of p38 kinase in murine macrophages. Int. Immunopharmacol., 4: 1029-1038.

Li, T.H., C.C. Hou, C.L. Chang and W.C. Yang, 2011. Anti-hyperglycemic properties of crude extract and triterpenes from *Poria cocos*. Evidence-Based Complementary Altern. Med., 10.1155/2011/128402

Lu, M.K., J.J. Cheng, C.Y. Lin and C.C. Chang, 2010. Purification, structural elucidation and anti-inflammatory effect of a water-soluble 1,6-branched 1,3-α-d-galactan from cultured mycelia of *Poria cocos*. Food Chem., 118: 349-356.

Maeda, N., M. Takahashi, T. Funahashi, S. Kihara and H. Nishizawa *et al*., 2001. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes, 50: 2094-2099.

Okuno, A., H. Tamemoto, K. Tobe, K. Ueki and Y. Mori *et al*., 1998. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J. Clin. Invest., 101: 1354-1361.

Rosen, E.D. and B.M. Spiegelman, 2006. Adipocytes as regulators of energy balance and glucose homeostasis. Nature, 444: 847-853.

Savage, D.B., K.F. Petersen and G.I. Shulman, 2007. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol. Rev., 87: 507-520.

Staels, B. and J.C. Fruchart, 2005. Therapeutic roles of peroxisome proliferator-activated receptor agonists. Diabetes, 54: 2460-2470.

Tai, T., Y. Akita, K. Kinoshita, K. Koyama, K. Takahashi and K. Watanabe, 1995. Anti-emetic principles of *Poria cocos*. Planta Med., 61: 527-530.

Taia, T., T. Shingu, T. Kikuchi, Y. Tezuka and A. Akahori, 1995. Triterpenes from the surface layer of *Poria cocos*. Phytochemistry, 39: 1165-1169.

Wu, S.J., L.T. Ng and C.C. Lin, 2004. Antioxidant activities of some common ingredients of traditional chinese medicine, *Angelica sinensis, Lycium barbarum* and *Poria cocos*. Phytother. Res., 18: 1008-1012.

Xu, W., F. Zhang, Y. Luo, L. Ma, X. Kou and K. Huang, 2009. Antioxidant activity of a water-soluble polysaccharide purified from *Pteridium aquilinum*. Carbohydr. Res., 344: 217-222.

Yamada, H., 1994. Pectic polysaccharides from Chinese herbs: Structure and biological activity. Carbohydr. Polym., 25: 269-276.

Yang, X., P.A. Jansson, I. Nagaev, M.M. Jack and E. Carvalho *et al*., 2004. Evidence of impaired adipogenesis in insulin resistance. Biochem. Biophys. Res. Commun., 317: 1045-1051.

Zhang, L., L. Chen, X. Xu, F. Zeng and P.C.K. Cheung, 2005. Effect of molecular mass on antitumor activity of heteropolysaccharide from *Poria cocos*. Biosci. Biotechnol. Biochem., 69: 631-634.