

Effects of *Dendrobium officinale* polysaccharide on adipogenic differentiation of rat bone marrow mesenchymal stem cells

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Abstract

This study investigated the effect of *Dendrobium officinale* polysaccharide (DOP) on the adipogenic differentiation of rat bone marrow mesenchymal stem cells (BMSCs). DOP was extracted from fresh *Dendrobium officinale*. Rat BMSCs were prepared, and then were treated with 0 (control), 50, 100, 200, 400, 800 µg/mL DOP, respectively. The cell viability was determined by MTT assay. The adipogenic differentiation was quantitatively analyzed by oil red O staining assay. The mRNA expressions of adipogenic differentiation related gene peroxisome proliferator-activated receptor gamma (PPARG), lipoprotein lipase (LPL) and fatty acid binding protein 4 (FABP4) were detected by RT-PCR. Results showed that, DOP with 0-800 µg/mL concentration had no significant toxicity to BMSCs. 200-800 µg/mL DOP could obviously inhibit the adipogenic differentiation of BMSCs. Compared with control group, the expression levels of PPARG, LPL and FABP4 mRNA 200, 400 and 800 µg/mL DOP groups were significantly decreased ($P < 0.05$ or $P < 0.01$). DOP can inhibit the adipogenic differentiation of BMSCs, which may be related with its down-regulation of PPARG, LPL and FABP4 expressions in BMSCs.

Keywords: *Dendrobium officinale*; polysaccharide; bone marrow mesenchymal stem cells; adipogenic differentiation.

Practical Application: *Dendrobium officinale* polysaccharide has an application prospect in treating the osteoporosis.

1 Introduction

Bone marrow mesenchymal stem cells (BMSCs) are the pluripotent adult stem cells, which can differentiate into osteoblasts, adipocytes, nerve cells, cartilage cells and muscle cells under certain induction conditions (Kopen et al., 1999; Tagami et al., 2003; Baksh et al., 2007; Izadpanah et al., 2008). In recent years, BMSCs are closely related to the occurrence and development of osteoporosis. The decrease of bone mass is always accompanied with the increase of adipose cells in bone marrow cavity (Carbonare et al., 2009). Therefore, seeking an effective way to inhibit the differentiation of BMSCs into adipogenic cells has become a research target for prevention and treatment of osteoporosis.

Dendrobium officinale is a precious medicinal plant recorded in the Chinese pharmacopeia. It is an epiphytic and perennial herb, and is mainly distributed in the south areas of China. *Dendrobium officinale* has many kinds of main active ingredient in which polysaccharide is the main ingredient (Chen et al., 2012). Previous studies demonstrate that *Dendrobium officinale* polysaccharide (DOP) possesses antioxidant, immunomodulatory and anti-hypoglycaemic activities (Pan et al., 2014; Cai et al., 2015). It is found that, some polysaccharides from herb can prevent the osteoporosis (Yang et al., 2016) and bone loss (MengYong et al., 2008) in animals. Until now the role of DOP in the differentiation of BMSCs into adipogenic cells has not been reported. This study investigated the effect of DOP on the adipogenic differentiation of rat BMSCs and the expressions of adipogenic differentiation

related gene peroxisome proliferator-activated receptor gamma (PPARG), lipoprotein lipase (LPL) and fatty acid binding protein 4 (FABP4). The objective was to provide a theory basis for application of DOP to treating the osteoporosis.

2 Materials and methods

2.1 Materials

Fresh *Dendrobium officinale* was purchased from Zhejiang Taizhou *Dendrobium officinale* Planting Base (Taizhou, China), and was identified by School of Life Sciences of Zhejiang University (Hangzhou, China). 4-6 week Sprague-Dawley rats were provided by the Laboratory Animal Center, Zhejiang University (Hangzhou, China). a-MEM and oil red O dye were purchased from Gibco Inc. (TX, USA). Trizol was purchased from Invitrogen Corp. (MD, USA). REVER Tra Ace[®] qPCR RT Kit was provided by Toyobo (Shanghai) Biotechnology Co. Ltd. (Shanghai, China). Other reagents were provided by Sigma-Aldrich Corp. (MO, USA).

2.2 Extraction of DOP

Fresh *Dendrobium officinale* was washed with water. After removing the impurities, it was sterilized at 100 °C, and dried at 60 °C. After smashing and sieving with 60 mesh sieve, the final powder was obtained. The DOP polysaccharide was

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extracted using hot water, and purified by repeated ethanol precipitation. The Sevage method was to remove the protein, and the phenol-sulfuric acid method was used to determine the DOP content of extract. Finally the content of DOP in extract was 85% m/m.

2.3 Preparation of BMSCs

SD rat was killed by cervical dislocation, followed by soaking in 75% ethanol for 10-15 min. The femur and tibia were taken under aseptic condition. After removing the muscle tissue, the bones were washed with PBS for 3 times. The metaphysis was removed, and the bone marrow cavity was exposed. 5 mL a-MEM was injected to bone marrow cavity to flush out the bone marrow. The process was repeated for several times. The bone marrow cell suspension was collected in 15 mL centrifuge tubes, and the fat and periosteum were removed by centrifugation at 1500 r/min for 5 min. The cells were suspended with a-MEM, and then were inoculated to the culture dish. After 72 h the culture medium was renewed, followed by renewing once every 2 days. When cell confluence was 80%-90%, the cells are digested with 0.25% trypsin (including 0.02% EDTA), followed by passage for 3 generations.

2.4 Detection of cell viability

BMSCs were inoculated to 96-well plate, with the density of 5×10^3 cells/well. After 24 h of culture, the culture medium was exchanged with a-MEM containing 0 (control), 50, 100, 200, 400, 800 $\mu\text{g/mL}$ DOP, respectively, 6 wells for each concentration. After culture for 24, 48 and 72 h, the MTT staining was performed to detect the cell viability (Yang et al., 2014). The optical density (OD) value of each well was determined at 570 nm using Multiskan FC microplate reader (Thermo Fisher Scientific, Inc., MA, USA).

2.5 Adipogenic differentiation of BMSCs

BMSCs were inoculated to 96-well plate, with the density of 5×10^5 cells/well. After 24 h of culture, the culture medium was exchanged with a-MEM containing 0, 50, 100, 200, 400, 800 $\mu\text{g/mL}$ DOP plus adipogenic induction agents (1 $\mu\text{mol/L}$ dexamethasone, 0.5 $\mu\text{g/mL}$ insulin, 200 $\mu\text{mol/L}$ indomethacin), respectively, 6 wells for each concentration, followed by continued culture. The culture medium was exchanged every other day.

2.6 Oil red O staining and quantitative analysis

On the 20th day of adipogenic differentiation, the culture medium was removed. The cells were washed with PBS for 3 times, and were fixed with 10% neutral formaldehyde for 30 min, followed by staining with oil red O dye for 30 min. After washing with PBS for 3 times, the cells were observed and photographed under DVM6 optical microscope (Leica Science Lab, Leica Camera AG Berlin, Germany). According to the reported method (Fu et al., 2014), isopropanol was used to extract the oil red O dye, the OD value of cell solution was measured by at 540 nm using microplate.

2.7 Detection of mRNA expression of adipogenic differentiation related genes

The mRNA expression of adipogenic differentiation related genes including PPAR γ , LPL and FABP4 were detected using RT-PCR method. On the 20th day of adipogenic differentiation, the cells were collected. The total RNA was extracted with Trizol, followed by reverse transcription to cDNA with Rever Tra Ace[®] qPCR RT Kit. β -actin was used as internal reference. The primer sequences were as follows: PPAR γ : sense 5'-TGC TTG TGA AGG ATG CAA GGG T-3' and anti-sense 5'-AGCAAGGCACTTCTGAAACCGA-3'; LPL: sense 5'-TGG ATG GAC GGT GAC AGG AAT GTA-3' and anti-sense 5'-CGG CAG ACA CTG GAT AAT GTT GCT-3'; FABP4: sense 5'-ATC AGC GTA GAA GGG GAC TTG GT-3' and anti-sense 5'-TGG TCG ACT TTC CAT CCC ACT TCT-3'. The PCR amplification conditions were as follows: 95 °C for 5 min; 40 cycles of 50 °C for 2 min, 95 °C for 2 min and 60 °C for 1 min, followed by 72 °C for 1 min. The relative expression level was determined using the $2^{-\Delta\Delta\text{Ct}}$ analysis method (Livak & Schmittgen, 2001).

2.8 Statistical analysis

All statistical analysis was carried out using SPSS17.0 software (SPSS Inc., Chicago, IL, USA). Data were presented as standard deviation. Comparisons between two groups were performed using t test. $P < 0.05$ was considered as statistically significant.

3 Results

3.1 Effects of DOP on survival rate of BMSCs

When the concentration of DOP was in 0-800 $\mu\text{g/mL}$ range, there was no significant difference of OD value of cell solution, which represented the survival rate of BMSCs. This indicated that, DOP had no significant toxicity to BMSCs in this concentration range (Figure 1).

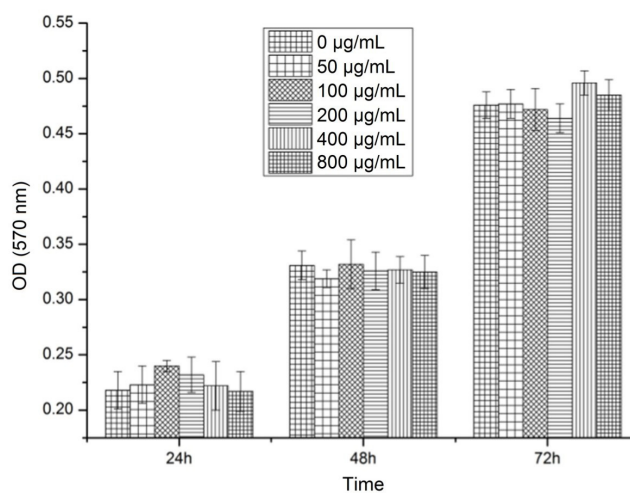


Figure 1. Effects of different concentrations of DOP on survival rate of BMSCs. DOP, *Dendrobium officinale* polysaccharide; BMSCs, bone marrow mesenchymal stem cells; OD, optical density.

3.2 Effects of DOP on adipogenic differentiation of BMSCs

Oil red O staining assay showed that, 0, 50 and 100 $\mu\text{g/mL}$ DOP had no significant difference on the OD value of cell solution. When the concentration of DOP was 200, 400 and 800 $\mu\text{g/mL}$, the OD value was significantly decreased compared with 0, 50 and 100 $\mu\text{g/mL}$ DOP groups ($P < 0.05$ or $P < 0.01$). There was no significant difference between 400 and 800 $\mu\text{g/mL}$ DOP groups ($P > 0.05$) (Figure 2). Figure 3 showed that, the

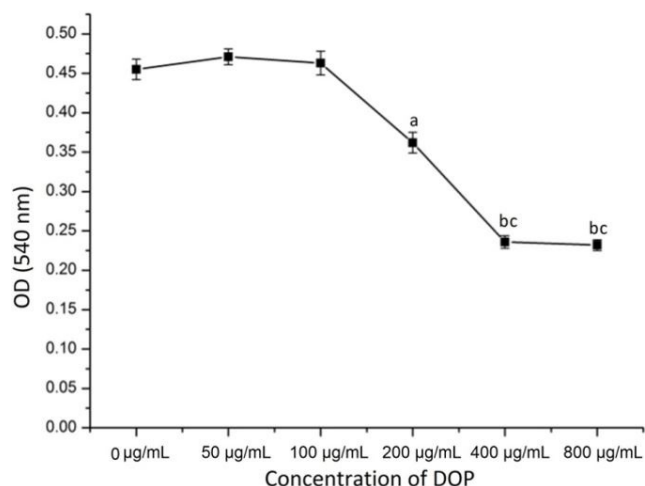


Figure 2. Effects of DOP on adipogenic differentiation of BMSCs (OD value). ^a $P < 0.05$ compared with 0, 50 and 100 $\mu\text{g/mL}$ DOP groups; ^b $P < 0.01$ compared with 0, 50 and 100 $\mu\text{g/mL}$ DOP groups; ^c $P < 0.05$ compared with 200 $\mu\text{g/mL}$ DOP group. DOP, *Dendrobium officinale* polysaccharide; BMSCs, bone marrow mesenchymal stem cells; OD, optical density.

numbers of stained lipid droplets in 200, 400 and 800 $\mu\text{g/mL}$ DOP groups were obviously less than 0, 50 and 100 $\mu\text{g/mL}$ DOP groups, especially for the 800 $\mu\text{g/mL}$ DOP group. This indicated that, DOP with concentration of 200-800 $\mu\text{g/mL}$ could obviously inhibit the adipogenic differentiation of BMSCs.

3.3 Effect of DOP on expressions of PPARG, LPL and FABP4 mRNA

As shown in Figure 4, the expression level of PPARG mRNA in 100, 200, 400 and 800 $\mu\text{g/mL}$ DOP groups was significantly lower than 0 and 50 $\mu\text{g/mL}$ DOP groups ($P < 0.05$ or $P < 0.01$), and that in 400 and 800 $\mu\text{g/mL}$ DOP groups was significantly lower than 100 $\mu\text{g/mL}$ DOP group ($P < 0.01$). Figure 5 showed that, the expression level of PPARG mRNA in 50, 100, 200, 400 and 800 $\mu\text{g/mL}$ DOP groups was significantly lower than 0 $\mu\text{g/mL}$ DOP group ($P < 0.05$), and that in 800 $\mu\text{g/mL}$ DOP group was significantly lower than 50 $\mu\text{g/mL}$ DOP groups ($P < 0.05$). As shown in Figure 6, the expression level of FABP4 mRNA in 200, 400 and 800 $\mu\text{g/mL}$ DOP groups was significantly lower than 0, 50 and 100 $\mu\text{g/mL}$ DOP groups ($P < 0.05$ or $P < 0.01$), and that in 400 and 800 $\mu\text{g/mL}$ DOP groups was significantly lower than 200 $\mu\text{g/mL}$ DOP group ($P < 0.05$).

4 Discussion

Osteoporosis is a systemic bone disease characterized by bone mass decrease and bone microstructure degeneration. In this disease, the bone fragility increases, leading to occurrence of fracture (Sandhu & Hampson, 2011). With the improvement of living standards and aging aggravation in more and more countries, the harm caused by osteoporosis is becoming more and more serious. However, the pathogenesis of osteoporosis is

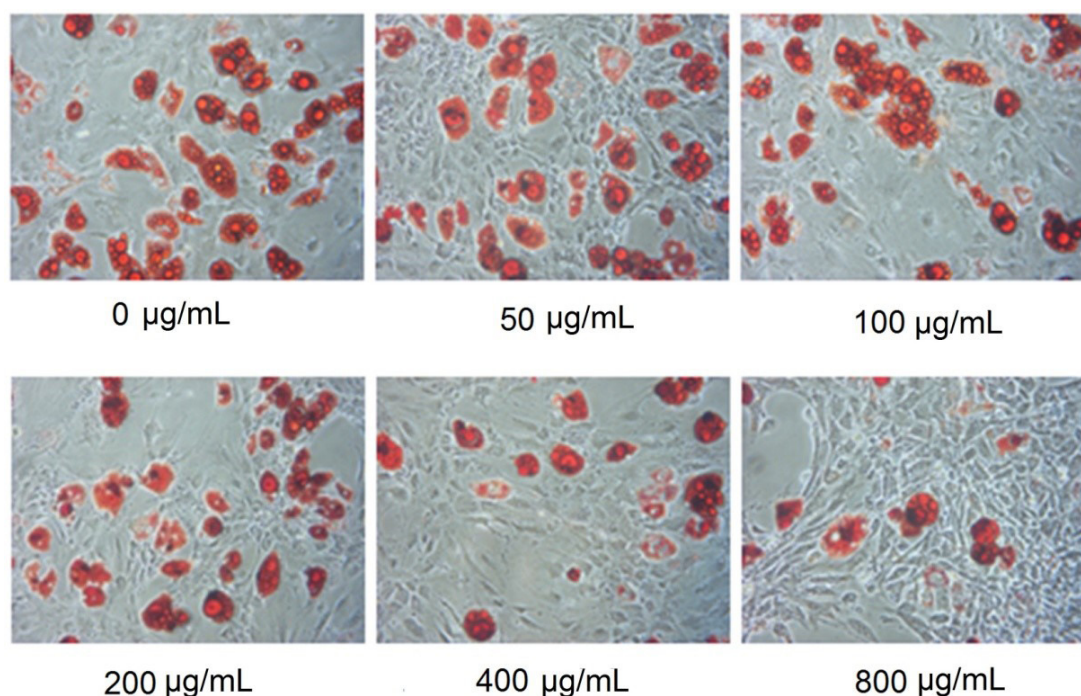


Figure 3. Results of oil red O staining assay with different DOP concentrations. DOP, *Dendrobium officinale* polysaccharide.

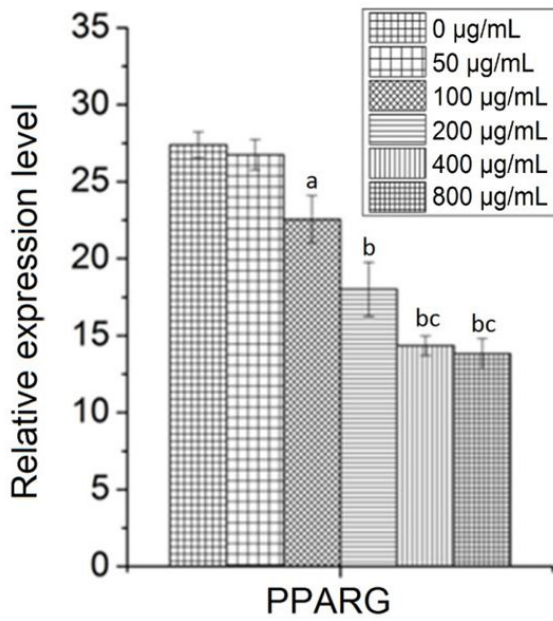


Figure 4. Effect of DOP on expressions of PPARG mRNA. ^aP < 0.05 compared with 0 and 50 µg/mL DOP groups; ^bP < 0.01 compared with 0 and 50 µg/mL DOP groups; ^cP < 0.01 compared with 100 µg/mL DOP group. DOP, *Dendrobium officinale* polysaccharide; PPARG, peroxisome proliferator-activated receptor gamma.

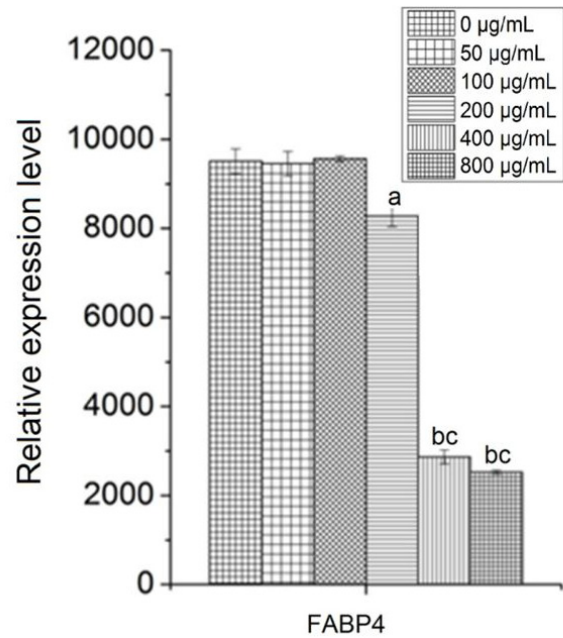


Figure 6. Effect of DOP on expressions of FABP4 mRNA. ^aP < 0.05 compared with 0, 50 and 100 µg/mL DOP groups; ^bP < 0.01 compared with 0, 50 and 100 µg/mL DOP groups; ^cP < 0.01 compared with 200 µg/mL DOP group. DOP, *Dendrobium officinale* polysaccharide; FABP4, fatty acid binding protein 4.

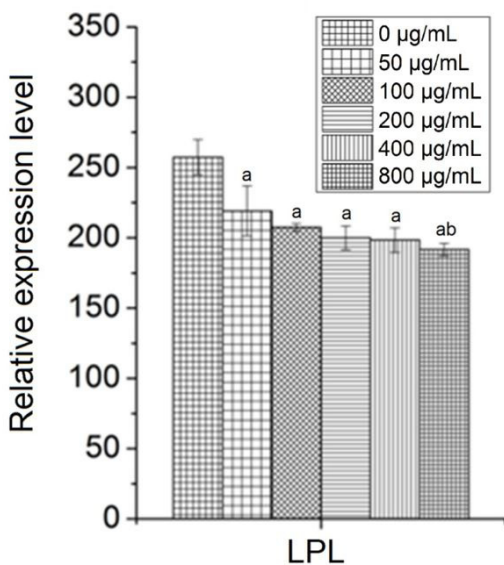


Figure 5. Effect of DOP on expressions of LPL mRNA. ^aP < 0.05 compared with 0 µg/mL DOP group; ^bP < 0.05 compared with 50 µg/mL DOP group. DOP, *Dendrobium officinale* polysaccharide; LPL, lipoprotein lipase.

still not clear. In recent years, it is found that there is an important relationship between the osteogenesis-adipogenesis imbalance of BMSCs and the occurrence of osteoporosis. The decrease of bone mass is often accompanied with the increase of the bone marrow adipose tissue. Once too much BMSCs differentiate into the adipogenic cells, the number of BMSCs differentiating

into osteoblasts will be reduced accordingly (Ye et al., 2014). This has provided a new direction for preventing and treatment of osteoporosis by promoting BMSCs differentiating into osteoblasts and inhibiting the differentiation into adipogenic cells.

DOP is one of the main components of *Dendrobium officinale* polysaccharide, and also the secondary metabolite. The content and quality of DOP are considered to the key indicators for evaluating the quality of *Dendrobium officinale* (Jin et al., 2016). DOP is the multi-hydroxyl polymer composed of glucose, mannose, galactose, glucose acid, glucose acid, xylose, Arabia sugar, galactose acid and other monosaccharide (Xia et al., 2012). This study observed the effect of DOP on the adipogenic differentiation of BMSCs. Result showed, DOP had no significant toxicity to BMSCs, and that with concentration of 200-800 µg/mL could obviously inhibit the adipogenic differentiation of BMSCs.

PPARG belongs to the peroxisome proliferator-activated receptors family, and is a key transcription factor for the activation and transcription of the adipogenic differentiation. It is highly expressed in adipose tissue. It is the major regulatory factor in the process of adipogenic differentiation, and is a major regulator of adipocyte related genes expression (Jäger & Neuman, 2011). LPL can bind the protein polysaccharide of extracellular matrix, thus inhibit the osteogenic differentiation and mineralization, and promote the adipogenic differentiation (Linehan et al., 2012). FABP4 belongs to the fatty acid binding protein family. It is a marker gene of adipocyte differentiation and maturation, and is widely involved in the transmembrane transport and metabolism of fatty acids (Lee et al., 2014). Results of this study showed that, after treatment with DOP, the expressions of PPARG, LPL and FABP4 mRNA decreased. This indicates that,

the inhibition of DOP on adipogenic differentiation of BMSCs may be related with its down-regulation of PPARG, LPL and FABP4 expressions in BMSCs.

In conclusion, DOP can inhibit the adipogenic differentiation of BMSCs. The mechanism may be related with its down-regulation of PPARG, LPL and FABP4 expressions in BMSCs. This study has provided a theory basis for application of DOP to treating the osteoporosis. However, there may be other mechanisms for the role of DOP in inhibiting the adipogenic differentiation of BMSCs. This needs to be further investigated in next studies.

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