# Effect of emodin on proliferation and differentiation of 3T3-L1 preadipocyte and FAS activity

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**Objective** To study the effects of emodin on proliferation and differentiation of 3T3-L1 preadipocyte and the possible mechanism.

**Methods** Cell proliferation was determined by MTT spectrophotometry, cell differentiation was determined by Oil Red O staining, and fatty acid synthase (FAS) activity was determined by spectrophotometry.

**Results** Emodin promoted proliferation of 3T3-L1 preadipocyte at low concentration and inhibited the proliferation at high concentration in a dose-related manner. In contrast, it inhibited cell differentiation into adipocyte at low concentration in a dose-related manner. *In vitro* emodin inhibited the activity of FAS in a dose-related manner.

**Conclusions** The effects of emodin on 3T3-L1 cell's proliferation and differentiation are dose dependent. Emodin inhibits the activity of FAS. Our results suggest that emodin should have a potential to serve as a fat-reducing drug.

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The mass of fat is relevant to the number of adipocytes, and to the volume of the adipocyte as well.[1] The number of the adipocyte is determined by the number of preadipocytes, and also determined by the differentiation rate from preadipocyte into adipocyte. The differentiation rate and the volume of adipocyte are affected by lipid metabolism.[1,2] Therefore, knowing the proliferation and differentiation of adipocyte and the regulation of lipid metabolism in the light of the mechanism of obesity development will be useful in screening of obesity-reducing drugs.

Emodin widely exists in Chinese medical herbs such as Rhubarb, Aloe and so on. It is an anthraquinone, named 1, 3, 8-trihydroxy-6-methylanthraquinone. It has been reported that emodin has a regulatory effect on the proliferation of human primary T lymphocyte[3] and immune responses in human mesangial cells,[4] inhibits the growth of neuroectodermal cancer[5] and breast cancer,[6] promotes the repair of nucleiotide excision to the DNA damage of human cells caused by UV and cislatin induction,[7] and finally competedly blocks the activity of casein kinase Ⅱ.[8]

Obesity is a chronic disease that threatens human health. Most new fat-reducing drugs are targeted to the proliferation and differentiation of adipocyte and the fatty acid synthase.[9,10] This is the first report in the literature that emodin has effects either on the inhibition or promotion of mouse 3T3 - L1 preadipocyte, and on the inhibition of FAS, in a dose dependent manner.

## METHODS

3T3-L1 cell line was purchased from ATCC. Emodin was purchased from Sigma Co., USA. Proliferation medium was prepared by DME/Ham's F-12 supplimented with 10% of fetal calf serum (FCS), and differentiation medium, prepared by DME/Ham's F-12 supplimented with 5% of FCS, 0.5 μmol/L of human insulin, 33 μmol/L of biotin and 0.2 nmol/L of triiodothronine.

### Cell culture

Cells were seeded in 96-well plates at a density of 6.0 ×

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$10^3$/ml. Different concentrations of emodin were added to the medium for the test of differentiation and proliferation. Cells were cultured for 72 hours, after culture cells in all plates were detected with MTT spectrophotometry to determine cell numbers by the standard curve previously established. For differentiation test the medium was changed to differentiation medium at the cell confluence, then the cells were cultured in the presence of different concentrations of emodin for 6 days. Oil Red O staining was used to detect cell differentiation. The control group was the cells cultured without emodin for 6 days.

### MTT spectrophotometry

MTT (5.0 mg/ml) was dissolved in PBS. Twenty μl of the solution was added to each well, and incubated for 4 hours at 37℃. All liquid in each well was tipped out and 100 μl of alcohol was added. The OD at 570 nm was determined by ELISA spectrometry.

### Oil Red O staining

Oil Red O (0.1 mg/ml) was dissolved in isopropyl alcohol. Cells were fixed with 10% of formaldehyde for 1 hour and then stained with 0.1 mg/ml Oil Red O solution for 2 hours at room temperature. All liquid in each well was tipped out and 100 μl of isopropyl alcohol was added to dissolve the precipitation. The OD at 510 nm was determined by ELISA spectrometry.

### Detection of FAS activity

All the components including malonyl-CoA were incubated in a microcuvette at 37℃, and the rate of decrease of absorbance at 340 nm was recorded and its slope was calculated. Slope at 0 μmol/L of emodin concentration was taken as 1 (100%) relative activity of FAS. Then the inhibition rates of emodin at different concentrations were carried out as above when different amounts of emodin solution were added.

### Statistical method

Each concentration of emodin was set up with three parallels in the experiments for proliferation and differentiation, as well as in the experiments for activity assay of fatty acid synthase. All values were expressed as $\bar{x} \pm s$ and analyzed by Excel 97. The value of correlation coefficient ($r$) was determined by linear regression analysis with Excel 97.

## RESULTS

### Effect of emodin on proliferation of 3T3-L1 preadipocyte

The number of 3T3-L1 preadipocyte was increased from $0.6 \times 10^4$/ml to $5.05 \times 10^4$/ml in 72 hours in the absence of emodin. It was increased by 8.4 fold comparing to the initial cell number. Meanwhile the cell number went up to $6.1 \times 10^4$/ml while the concentration of emodin reached to 5 μmol/L, which has a 10.2 fold increase. The cell number reached to $8.96 \times 10^4$/ml when the emodin concentration was increased to 10 μmol/L during the period of time, which had a 15 fold increase. It was shown that the effect of emodin on growth was inhibition instead of promotion when the concentration of emodin went up to 20 μmol/L. The increase of cell number is only 27% at concentration of emodin of 20 μmol/L, 14% at 40 μmol/L. The value of increase turned to be negative while the concentration of emodin went up to 80 μmol/L. The results demonstrated that the emodin had dual effect on 3T3-L1 preadipocyte. It promoted the proliferation at low concentration and inhibited the proliferation at high concentration. It also induced cell death when the concentration went up to 80 μmol/L (Table 1).

### Effect of emodin on proliferation of 3T3-L1 preadipocyte in time-course manner

As shown in Fig. 1, the effect of emodin on cell proliferation was dependent on the concentration used in the experiments. The low concentration promoted the proliferation with time and high concentration inhibited the proliferation completely in 18 hours.

### Effect of emodin on differentiation of 3T3-L1 preadipocyte

The fat content in the differentiating 3T3-L1 cells decreased with the increase of emodin concentrations in medium as shown in Table 2. It demonstrated that the inhibition of 3T3-L1 differentiation by emodin was dose dependent ($r = 0.9572$).

**Table 1.** Effect of emodin of different concentrations on proliferation of 3T3-L1 preadipocyte

| | Concentrations of emodin (μmol/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 (control) | 5 | 10 | 20 | 40 | 80 | 160 | 320 |
| $OD_{570}$ | | | | | | | | |
| 1 | 0.405 | 0.515 | 0.606 | 0.177 | 0.181 | 0.138 | 0.045 | 0.020 |
| 2 | 0.510 | 0.579 | 0.733 | 0.199 | 0.133 | 0.061 | 0.058 | 0.039 |
| 3 | 0.474 | 0.469 | 0.535 | 0.301 | 0.109 | 0.098 | 0.068 | 0.004 |
| $\bar{x} \pm s$ | 0.463 ± 0.0534 | 0.547 ± 0.0453** | 0.627 ± 0.1003* | 0.226 ± 0.0662* | 0.141 ± 0.0367* | 0.099 ± 0.0385* | 0.057 ± 0.0115* | 0.021 ± 0.0175* |
| Cell | | 6.10 | 8.96 | 1.36 | 0.72 | 0.38 | 0.19 | 0.08 |

For each emodin concentration, 3 parallel wells were taken, and the final values were expressed as $\bar{x} \pm s$. * $P < 0.01$, ** $P < 0.05$, vs control group.

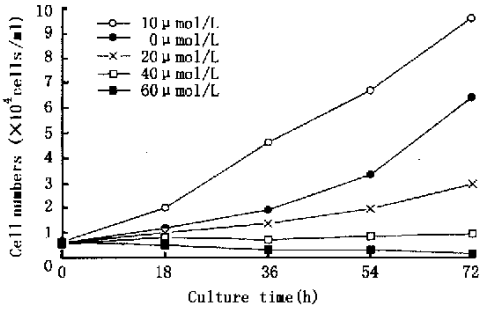


**Fig. 1.** Effect of emodin of different concentrations on proliferaton of 3T3-L1 preadipocyte in manner of time-course. For each emodin concentration and culture period, 3 parallel wells were taken, and the final values were expressed as $\bar{x} \pm s$.

**Table 2.** Effect of emodin of different concentrations on differentiation of 3T3-L1 preadipocyte

| | Concentrations of emodin (μmol/L) | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | 80 |
| $OD_{510}$ | | | | | |
| 1 | 0.319 | 0.107 | 0.052 | 0.057 | 0.003 |
| 2 | 0.305 | 0.116 | 0.068 | 0.064 | 0.010 |
| 3 | 0.292 | 0.130 | 0.077 | 0.047 | 0.012 |
| $\bar{x} \pm s$ | 0.30±0.01 | 0.11±0.01* | 0.06±0.01* | 0.05±0.001* | 0.008±0.01* |

For each emodin concentration, 3 parallel wells were taken, and the final values were expressed as $\bar{x} \pm s$. * $P < 0.01$, vs the control group.

## Cell morphology and lipid accumulation in the cell after the treatment of emodin

The morphology of proliferating 3T3-L1 preadipocytes looked the same as fibroblasts (Fig. 2A). There was no lipid accumulation in the cell tested by Oil Red O staining (Fig. 2A'). The morphology of the cells had changed after 6 days' culture in a differentiation medium in the presence of emodin at the concentration of 40 μmol/L (Fig. 2B). It shows the accumulation of lipid (Fig. 2B'). Most of the cells become round (Fig. 2C) and accumulate lipid (Fig. 2C') after 6 days culture in a differentiation medium without emodin.

## Inhibition of emodin on fatty acid synthase activity

The relative activities of FAS were 90, 64, 45, 15 and 0%, respectively at the concentration of emodin of 2, 5, 10, 20 and 40 μmol/L as the FAS activity was considered as 100% in the condition without emodin. It showed that the inhibition of FAS by emodin was dose dependent as shown in Table 3.

**Table 3.** Inhibition of emodin on FAS activity

| | Concentration of emodin (μmol/L) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 5 | 10 | 20 | 40 |
| Slopes (average of 3 values) | 17.0 | 15.3 | 10.9 | 7.7 | 2.6 | 0 |
| FAS relative activities (%) | 100 | 90 | 64 | 45 | 15 | 0 |

The slope at 0 μmol/L of emodin was taken as 1 (100%) relative activity of FAS. Then the inhibition rates of emodin at different concentrations were carried out as above when different amounts of emodin solution were added. Averages of each three values were taken to get each slope.

## DISCUSSION

Emodin promoted the proliferation of 3T3-L1 preadipocyte at

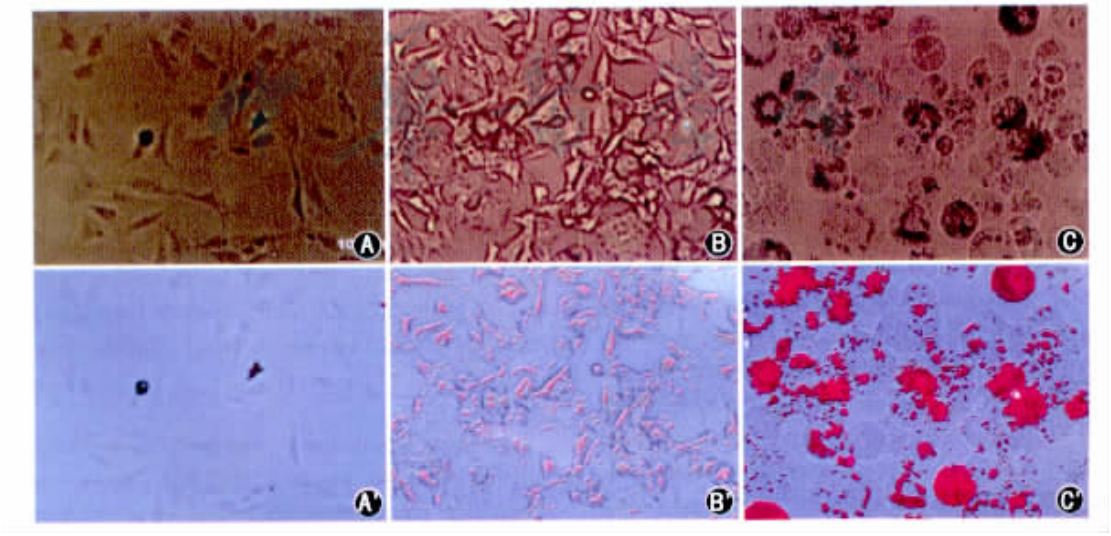


**Fig. 2.** Shapes and lipid accumulation of the cells in proliferating and differentiating. The proliferating 3T3-L1 preadipocytes take the shape of fibroblasts (A), and no lipid were observed when stained by Oil Red O (A'); the cells' shape changed when cultured in differentiation medium with emodin of 40 μmol/L for 6 days (B), and lipid accumulation were observed when stained by Oil Red O (B'); most of the cells became round when cultured in differentiation medium without emodin for 6 days (C), and accumulated large amount of lipid (C') (original magnification × 400).

low concentration and inhibited the proliferation while its concentration was above 40 μmol/L. It remarkably inhibited the differentiation of 3T3-L1 preadipocyte at low concentration. Emodin showed little effect on the differentiation while the concentration used was higher.

Emodin has an inhibition effect on fatty acid synthase (FAS), which is dose dependent. The inhibition of differentiation of 3T3-L1 by emodin might be related to the block of FAS activity, because the lipid synthesis is a very important biochemical event in the cause of differentiation of preadipocyte into adipocyte.

It is the first time in the world, as we know, that we report that emodin has an effect on the differentiation and proliferation of preadipocyte, as well as on the lipid metabolism. The results described here may be useful in the development of emodin as a medicine to reduce fat.

REFERENCES

1. Spiegelman BM, Flier JS. Adipogenesis and obesity: rounding out the big picture. Cell 1996; 87: 377-389.
2. Prins JB, O'Rahilly S. Regulation of adipose cell number in man. Clin Sci (Colch) 1997; 92: 3-11.
3. Kuo YC, Meng HC, Tsai WJ, et al. Regulation of cell proliferation, inflammatory cytokine production and calcium mobilization in primary human T lymphocytes by emodin from Polygonum hypoleucum Ohwi. Inflamm Res 2001; 50: 73-82.
4. Kuo YC, Tsai WJ, Meng HC, et al. Immune responses in human mesangialcells regulated by emodin from Polygonum hypoleucum Ohwi. Life Sci 2001; 68: 1271-1286.
5. Pecere T, Gazzola MV, Mucignat C. Aloe-emodin is a new type of anticancer agent with selective activity against neuroectodermal tumors. Cancer Res 2000; 60: 2800-2804.
6. Zhang L, Lau YK, Xia W, et al. Tyrosine kinase inhibitor emodinsupresses growth of HER-2/neu-overexpressing breast cancer cells in athymic mice and sensitizes these cells to the inhibitory effect of paclitaxel. Clin Cancer Res 1999; 5: 343-353.
7. Chang LC, Sheu HM, Huang YS, et al. A novel function of emodin: enhancement of the nucleotide excision repair of UV-and cislatin-induced DNA damage in human cells. Biochem Pharmacol 1999; 58: 49-57.
8. Yim H, Lee YH, Lee SK. Emodin, an anthraquinone derivative isolated from the rhizomes of Rheum paimatum, selectively inhibits the activity of casein kinase Ⅱ as a competitive inhibitor. Planta Med 1999; 65: 9-13.
9. Zhou YT, Wang ZW, Higa M, et al. Reversing adipocyte differentiation: implications for treatment of obesity. Proc Natl Acad Sci U S A 1999; 96: 2391-2395.
10. Thomas ML, Donna EJ, Gojeb LF, et al. Reduced food intake and body weight in mice treated with fatty acid synthase inhibitor. Science 2000; 288: 2379-2381.

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