Effect of puerarin on osteoblast proliferation in vitro and Wnt/β-catenin signaling pathway expression

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Objective: To study the effect of puerarin on osteoblast proliferation in vitro and Wnt/β-catenin signaling pathway expression. Methods: Osteoblasts MC3T3-E1 were cultured and divided into control group, 10⁻⁶ mol/L PUE group, 10⁻⁷ mol/L PUE group and 10⁻⁸ mol/L PUE group, and after 24 h of treatment, mRNA expression of osteoblast marker genes, apoptosis genes and Wnt/β-catenin pathway genes were determined. Results: ALP, Runx2, OC, Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin mRNA expression in 10⁻⁶ mol/L PUE group, 10⁻⁷ mol/L PUE group and 10⁻⁸ mol/L PUE group were significantly higher than those in control group while caspase-3, caspase-8, caspase-9 and caspase-12 mRNA expression were significantly lower than those in control group; the higher the puerarin dose, the higher the ALP, Runx2, OC, Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin mRNA expression in cells while the lower the caspase-3, caspase-8, casapase-9 and caspase-12 mRNA expression in cells. Conclusion: Puerarin can effectively promote the proliferation of osteoblasts and the activation of Wnt/β-catenin pathway.

1. Introduction

Osteoporosis is a systemic skeletal disease that is mainly characterized by bone loss and bone microstructure degradation, and patients are with increased bone fragility and risk of bone fractures[1]. During the progress of osteoporosis, the bone remodeling process is significantly abnormal, and the balance between osteoblasts and osteoclasts changes significantly. The disruption of bone formation mediated by osteoblasts can directly affect bone remodeling, leading to bone loss and osteoporosis[2,3]. Thus, enhancing osteoblast activity is an ideal target for osteoporosis. Puerarin is the effective component in Chinese herbal medicine kudzu vine root, which is a kind of plant isoflavone, has significant cytoprotective effect, and can promote cell proliferation and inhibit apoptosis[4]. In the following study, the effect of puerarin on osteoblast proliferation in vitro and Wnt/β-catenin signaling pathway expression was analyzed.

2. Experimental materials and methods

2.1 Experimental materials

Osteoblasts MC3T3-E1 were bought in ATCC cell company, cell culture medium DMEM, fetal bovine serum and trypsin were bought in Hyclone company, and the puerarin was bought in Nanjing Dilger company and configured to 10⁻³ mol/L solution with absolute ethyl alcohol, which was preserved in -20 °C refrigerator. RNA extraction kits, cDNA synthesis kits and fluorescent quantitative PCR kits were purchased in Promega company.
2.2 Experimental methods

2.2.1 Cell culture methods

MC3T3-E1 cells were recovered and then cultured with DMEM containing 10% fetal bovine serum and 20 mmol/HEPES, the culture medium was replaced once every 3 d, the cells in culture bottle were digested and sub-cultured with 0.125% trypsin after the density reached 90%, the density of sub-cultured cells was adjusted to 10^4/L, and then the cells were inoculated in 12-well culture plate, 1ml in each well.

2.2.2 Cell grouping and treatment methods

The cells that were inoculated in 12-well culture plate were treated after the density reached 80%-90%, they were divided into control group and 10^-6 mol/L PUE group, 10^-7 mol/L PUE group and 10^-8 mol/L PUE group according to the different treatment conditions, and the treatment methods were as follows: the control group were treated with serum-free DMEM, and 10^6 mol/L PUE group, 10^-7 mol/L PUE group and 10^-8 mol/L PUE group were treated with serum-free DMEM containing 10^6 mol/L, 10^-7 mol/L and 10^-8 mol/L puerarin respectively.

2.2.3 Gene expression detection methods

24 h after treatment with different conditions, the cell culture medium was abandoned, the cells were kept and washed with PBS buffer for 1 to 2 times, then cell RNA extraction kits and cDNA synthesis kits were used to extract the RNA in cells and reversely transcribe it into cDNA, and then fluorescence quantitative PCR kits were used to determine ALP, Runx2, OC, caspase-3, caspase-8, caspase-9, caspase-12, Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin mRNA expression.

2.3 Statistical methods

SPSS 20.0 software was used to input gene expression data, comparison of gene expression among groups was by variance analysis and SPSS 20.0 software was used to input gene expression data, comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis and comparison of gene expression among groups was by variance analysis.

3. Results

3.1 Osteoblast marker gene expression

Analysis of the effect of puerarin treatment on osteoblast marker genes ALP, Runx2 and OC mRNA expression in osteoblasts was as follows: ALP, Runx2 and OC mRNA expression in 10^-6 mol/L PUE group, 10^-7 mol/L PUE group and 10^-8 mol/L PUE group were significantly higher than those in control group (P<0.05); the higher the puerarin dose, the higher the ALP, Runx2 and OC mRNA expression in cells (P<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>ALP</th>
<th>Runx2</th>
<th>OC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>1.03±0.15</td>
<td>0.99±0.13</td>
<td>1.05±0.17</td>
</tr>
<tr>
<td>10^-6 mol/L PUE group</td>
<td>5</td>
<td>1.33±0.187</td>
<td>1.37±0.207</td>
<td>1.29±0.17</td>
</tr>
<tr>
<td>10^-7 mol/L PUE group</td>
<td>5</td>
<td>1.78±0.25*</td>
<td>1.83±0.24*</td>
<td>1.65±0.19*</td>
</tr>
<tr>
<td>10^-8 mol/L PUE group</td>
<td>5</td>
<td>2.31±0.36*</td>
<td>2.44±0.38*</td>
<td>2.18±0.34*</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05;  ; compared with 10^-6 mol/L PUE group, P<0.05; : compared with 10^-7 mol/L PUE group, P<0.05.

3.2 Apoptosis gene expression

Analysis of the effect of puerarin treatment on apoptosis genes caspase-3, caspase-8, caspase-9 and caspase-12 expression in osteoblasts was as follows: caspase-3, caspase-8, caspase-9 and caspase-12 mRNA expression in 10^-6 mol/L PUE group, 10^-7 mol/L PUE group and 10^-8 mol/L PUE group were significantly lower than those in control group (P<0.05); the higher the puerarin dose, the lower the caspase-3, caspase-8, caspase-9 and caspase-12 mRNA expression in cells (P<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Caspase-3</th>
<th>Caspase-8</th>
<th>Caspase-9</th>
<th>Caspase-12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>1.04±0.17</td>
<td>0.97±0.12</td>
<td>1.02±0.15</td>
<td>1.09±0.20</td>
</tr>
<tr>
<td>10^-6 mol/L PUE group</td>
<td>5</td>
<td>0.83±0.12*</td>
<td>0.76±0.10*</td>
<td>0.79±0.08*</td>
<td>0.71±0.06*</td>
</tr>
<tr>
<td>10^-7 mol/L PUE group</td>
<td>5</td>
<td>0.71±0.11*</td>
<td>0.65±0.08*</td>
<td>0.67±0.09*</td>
<td>0.60±0.08*</td>
</tr>
<tr>
<td>10^-8 mol/L PUE group</td>
<td>5</td>
<td>0.45±0.08*</td>
<td>0.40±0.06*</td>
<td>0.38±0.05*</td>
<td>0.32±0.05*</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05;  ; compared with 10^-6 mol/L PUE group, P<0.05; : compared with 10^-7 mol/L PUE group, P<0.05.

3.3 Wnt signaling pathway gene expression

Analysis of the effect of puerarin treatment on Wnt signaling pathway genes Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin expression in osteoblasts was as follows: Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin mRNA expression in 10^-6 mol/L PUE group, 10^-7 mol/L PUE group and 10^-8 mol/L PUE group were significantly higher than those in control group (P<0.05); the higher the puerarin dose, the higher the Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin mRNA expression in cells (P<0.05).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Wnt1</th>
<th>Wnt2</th>
<th>Wnt3a</th>
<th>Wnt10b</th>
<th>β-catenin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>5</td>
<td>1.05±0.12</td>
<td>1.02±0.14</td>
<td>0.98±0.11</td>
<td>0.96±0.13</td>
<td>1.06±0.15</td>
</tr>
<tr>
<td>10^-6 mol/L PUE group</td>
<td>5</td>
<td>1.44±0.10*</td>
<td>1.35±0.16*</td>
<td>1.38±0.18*</td>
<td>1.48±0.22*</td>
<td>1.62±0.18*</td>
</tr>
<tr>
<td>10^-7 mol/L PUE group</td>
<td>5</td>
<td>1.89±0.25*</td>
<td>1.72±0.22*</td>
<td>1.83±0.25*</td>
<td>1.71±0.22*</td>
<td>1.79±0.25*</td>
</tr>
<tr>
<td>10^-8 mol/L PUE group</td>
<td>5</td>
<td>2.64±0.35*</td>
<td>2.42±0.33*</td>
<td>2.77±0.32*</td>
<td>2.57±0.37*</td>
<td>2.49±0.34*</td>
</tr>
</tbody>
</table>

*: compared with control group, P<0.05;  ; compared with 10^-6 mol/L PUE group, P<0.05; : compared with 10^-7 mol/L PUE group, P<0.05.
4. Discussion

The bone formation disorder mediated by osteoblasts is closely related to the occurrence of osteoporosis, and enhancing the activity of osteoblasts is ideal expression for the treatment of osteoporosis[5]. Studies have shown that puerarin has significant promoting effect on osteoblast proliferation[6,7], but the puerarin effects on osteoblast activity have not yet been clarified in gene expression levels. In the proliferation of osteoblasts, the synthesis and secretion of ALP, Runx2, OC and a variety of active molecules increase. ALP is an important catalytic enzyme in bone formation, which hydrolyzes pyrophosphate and inorganic phosphates and promotes the formation of the mineralization mechanism protein[8]; Runx2 is a key transcription factor that promotes the differentiation of bone marrow stromal stem cells into osteoblasts, and it is closely related to the differentiation and maturation of osteoblasts[9]; OC is the non-collagen abundant in the bone, which has the role of promoting bone mineralization, and is directly related to the activity of osteoblasts[10]. In the study, analysis of the effect of puerarin treatment on the reactive molecule expression in osteoblasts showed that different doses of puerarin could increase the expression of ALP, Runx2 and OC, and the higher the puerarin dose, the higher the expression of ALP, Runx2 and OC. This suggests that puerarin can promote the differentiation and proliferation of osteoblasts.

The process of cell proliferation is closely related to the process of apoptosis, and the inhibition of apoptosis can effectively promote cell proliferation. The main regulatory mechanisms of apoptosis include endogenous pathway, exogenous pathway, and endoplasmic reticulum pathway[11]. Endogenous apoptotic pathway specifically refers to the mitochondrial apoptotic pathway, which activates caspase-9 molecules in cytoplasm by the release of mitochondrial cytochrome C, and thus initiates apoptosis cascade amplification[12]; exogenous apoptotic pathway specifically refers to death receptor apoptotic pathway, which activates downstream caspase-8 molecules through the combination of Fas and FasL, and thus initiates apoptosis cascade amplification[13]; endoplasmic reticulum apoptotic pathway is mainly closely related to the endoplasmic reticulum stress, and the high expression of a variety of endoplasmic reticulum stress proteins can activate caspase-12, and then start the apoptosis cascade amplification[14,15]. Caspase-3 is the common downstream molecule of apoptotic cascade, caspase-8, caspase-9 and caspase-12 can activate apoptotic cascade amplification and ultimately mediate cell apoptosis through active caspase-3. In the study, analysis of the effect of puerarin treatment on apoptosis gene expression in osteoblasts showed that different doses of puerarin could all inhibit the expression of caspase-3, caspase-8, caspase-9 and caspase-12, and the higher the puerarin dose, the lower the caspase-3, caspase-8, caspase-9 and caspase-12 expression. This suggests that puerarin can promote the differentiation and proliferation of osteoblasts.

Wnt/β-catenin pathway is an important signaling pathway to regulate the bone remodeling and bone formation process, the pathway is highly conservative in evolution, and it includes a variety of Wnt molecules, β-catenin as well as LRP5/6, Frizzled, TCF/LEF and other members. Wnt molecule is the upstream signal molecule in Wnt/β-catenin pathway, which can be activated and then be combined with LRP5/6 and Frizzled on cell membrane to inhibit the degradation of β-catenin and promote the accumulation of β-catenin in the cytoplasm; when the β-catenin accumulation reaches a certain amount, it can transfer into the nucleus and work together with transcription factors TCF/LEF to regulate target gene expression and promote osteoblast differentiation[16,17]. Wnt1, Wnt2 and Wnt3a in Wnt molecules can significantly enhance the activity of osteoblasts, while Wnt10b can significantly induce osteoblast differentiation. In the study, analysis of the effect of puerarin treatment on Wnt pathway gene expression in osteoblasts showed that different doses of puerarin could all increase the Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin expression, and the higher the puerarin dose, the higher the Wnt1, Wnt2, Wnt3a, Wnt10b and β-catenin expression. This suggests that puerarin can promote the activation of the Wnt pathway and thus help the differentiation and proliferation of osteoblasts.

To sum up, it is believed that puerarin has significant promoting effect on the activity of osteoblast proliferation in vitro, and at the same time, it can promote the activation of Wnt/β-catenin pathway and then promote the differentiation and proliferation of osteoblasts through the activation of the signaling pathway.

References

[6] Zhang Ying-ying, Zhou Jian-bin, Zeng Xiang-wei, Zhao Feng-ming,


