High concentration of tacrolimus inhibits proliferation and osteoblastic differentiation of human mesenchymal stem cells

WEI Hong-yan[1], PAN Wei[2], QIU Ni[2], HUANG Li[2], ZHOU Hong-hao[2], XIAO Zhou-sheng[2]

(*1. Department of Clinical Pharmacy, Hunan Provincial People's Hospital, Changsha 410005, China;*
2. Institute of Clinical Pharmacology, Central South University, Changsha 410078, China)

Abstract: OBJECTIVE To investigate the effect of tacrolimus on cell proliferation and osteoblastic differentiation of primary human bone marrow-derived mesenchymal stem cells (hBMSCs). **METHODS** hBMSCs were cultured with tacrolimus 0.001 – 5 $\mu mol \cdot L^{-1}$. BrdU incorporation was used to assess the cell proliferation while cellular alkaline phosphatase (ALP) activity and calcium deposition were measured to evaluate the osteoblastic differentiation of hBMSCs cultures. The calcineurin (CaN) activity was also examined using commercial CaN assay kit, and core binding factor 1 alpha subunit (Cbfα1) protein level was determined by Western blotting. **RESULTS** Tacrolimus 0.001 – 0.1 $\mu mol \cdot L^{-1}$ promoted BrdU incorporation but had no effect on ALP activity and calcium deposition, whereas tacrolimus 0.5 – 5 $\mu mol \cdot L^{-1}$ resulted in significant decrease in both cell proliferation and osteoblastic maturation, by reducing BrdU incorporation, ALP activity, and calcium deposition of hBMSCs cultures in a concentration-dependent manner. In addition, tacrolimus 0.5 – 5 $\mu mol \cdot L^{-1}$ led to concentration-dependent decrement in CaN activity, which was consistent with down-regulated Cbfα1 protein in the tacrolimus treated cells. **CONCLUSION** High concentration of tacrolimus might inhibit the cell proliferation and osteoblastic differentiation of hBMSCs cultures through a CaN/Cbfα1 pathway.

Key words: tacrolimus; human bone marrow-derived mesenchymal stem cells; calcineurin; core binding factor alpha1 subunits

CLC number: R979.5 **Document code**: A **Article ID**: 1000-3002(2011)03-0223-06

DOI: 10.3867/j.issn.1000-3002.2011.03.001

In T-cell activation, calcineurin (CaN) transduces Ca^{2+} signals through dephosphorylation of a family of transcription factors, such as the nuclear factor of activated T cells (NFATs). Then the dephosphorylated NFATs translocate to the nucleus and induce the transcription of cytokines including interleukin-2 (IL-2) to enhance T-cell function[1]. Recently, activated T cells have been reported to promote differentiation of mesenchymal stem cells (MSCs) into osteoblasts. The interactive influence between MSCs and immune cells can be mediated through some cytokine[2]. CaN/NFATs pathway which is abundantly distributed in the brain and lymphocytes[3] may be also involved in regulating bone formation and bone mass[4]. CaN A knock out mice (CaN $A^{-/-}$) displayed severe osteoporosis, markedly reduced mineral apposition rates, and attenuated colony formation in *ex vivo* stromal cell cultures, indicating a role of CaN in osteoblast-mediated bone formation[4]. Therefore, the suppression of CaN activity by its inhibitors may weaken osteoblast function and exert clinical effects on the skeleton.

Previous rodent experiments also revealed that both tacrolimus(FK506) and ciclosporin A (CsA) induced high-turnover bone loss or osteoporosis[5-6]. In T-cells, either CsA binds to immunophilin or FK506 binds to FK506-binding protein (FKBP) in the cytoplasm to forms a complex to suppress CaN activity, leading to the inhibition of dephosphorylation and nuclear translocation of NFATs, and inactivation of T-cell function. However, the impacts of CsA and FK506 on osteoblasts[7-8] and osteoclasts[9-10]

Foundation item: The project supported by grants of Teaching and Research Award Program for Outstanding Young Teachers (TRAPOYT) in Higher Education Institutions of MOE, PRC (30040002); National Natural Science Foundation of China (N30171085); and by National Institutes of Health, USA (RO1-AR049712)

Biography: WEI Hong-yan(1977 –), female, Postgraduate of Medicine, main research field is clinical pharmacy, E-mail: weihy2001@126.com

Corresponding author: XIAO Zhou-sheng, Tel: (0731) 82278323, E-mail: xiaozs64@yahoo.com

function are controversial, as whether administration of FK506 or CsA results in bone loss through the similar molecular mechanism in T-cells remains unclear.

The human bone marrow-derived mesenchymal stem cells (hBMSCs), which were capable of recapitulating the temporal sequence of osteoblast maturation in culture[11], were used to investigate the effects of FK506 on cell proliferation and osteoblastic differentiation in hBMSC cultures, and explore the role of CaN/NFATs signaling pathway in FK506-mediated cell responses.

1 MATERIALS AND METHODS

1.1 Reagents and chemicals

Alpha minimum essential medium (α-MEM), fetal bovine serum (FBS), penicillin-streptomycin solution, and sodium dodecyl sulfate (SDS) were obtained from Gibco BRL (Grand Island, NY, USA). Pronase E, ascorbic acid, β-glycerophosphate, p-nitrophenol, diethanolamine, p-nitrophenol phosphate, tacrolimus (FK506), dextran-charcoal and histopaques solution were purchased from Sigma (St. Louis, MO, USA). BrdU Cell Proliferation Assay was purchased from Chemicon (Temecula, CA, USA). Calcium kit for calcium content was purchased from Jiancheng Biotech (Nanjing, China). The CaN Cellular Assay Kit PLUS is a product in the Biomol (Plymouth Meeting, PA, USA). Bio-Rad reagent for protein assay was obtained from Bio-Rad Laboratories (Hercules, CA, USA). The RUNX2 antibody (sc-10758) was bought from Santa Cruz Biotech (CA, USA), the horseradish peroxidase conjugated goat anti-rabbit antibody and ECM of Western blotting from Boste Biotech (Wuhan, China). Nuclear extracts were bought from Beyotime Biotech Reagents (Shanghai, China). All other chemicals were of analytical grade and purchased from Shanghai Biotech (Shanghai, China).

1.2 Experimental equipment

A carbon dioxide (CO_2) incubator was purchased from Sheldon Manufacturing (Cornelius, USA). A super clean bench was obtained from Jiangsu Purification Group (Suzhou, China). An inverted microscope was purchased from Nikon (Tokyo, Japan). Electronic balance and super-speed refrigerated centrifuge were purchased from Sigma (USA). An ultrasonic cell disrupter and UV-visible spectrophotometer were purchased from Shimadzu (Kyoto, Japan). A multiskan ascent microplate reader was purchased from Thermo Labsystems (Helsinki, Finland). High-temperature oven, the level of shaking, the regulator steady flow electrophoresis and electrophoresis apparatus were purchased from Beijing Liuyi Instrument Factory.

1.3 Cell culture and treatment

Primary hBMSCs were obtained from the limb bones of a 5-month-old aborted fetus (Hunan Maternal and Child Health Hospital, Changsha, China) as previously described[14]. Briefly, the mononuclear cells were isolated by density gradient centrifugation using histopaques solution and subsequently seeded in α-MEM, 10% FBS (inactivated), and maintained in a humidified incubator with 5% CO_2 and 95% air at a temperature of 37℃. The 3rd to the 5th passages of hBMSCs were used in this study. At subconfluence, hBMSCs 5×10^7 L^{-1} were detached with 0.25% trypsin, seeded in a 12-well plate and were cultured in osteogenic differentiation medium, which consisted of phenol red-free α-MEM plus 10% FBS (dextran-coated charcoal stripped, DCS) supplemented with dexamethasone 10 nmol·L^{-1}, ascorbic acid 50 mg·L^{-1} and β-glycerophosphate 10 mmol·L^{-1} to induce osteoblastic differentiation. The cells were treated with FK506 0.001, 0.01, 0.1, 0.5, 1 and 5 μmol·L^{-1}, 0.1% DMSO (vehicle) or 17β-estrodiol (E_2) 0.01 μmol·L^{-1} or caffeine 100 μmol·L^{-1} (positive control), respectively. The medium was replaced every 3 d thereafter.

1.4 BrdU incorporation assay for detecting hBMSCs proliferation

The measurements were performed according to the kit instructions. A total of 2×10^4 hBMSCs were seeded into 96-well culture plates. Cells were brought to 90% confluence and then fasted overnight in serum-free media

before addition of BrdU 10 mmol·L^{-1} in α-MEM with 7.5% FBS (DCS). Two hours later, the cells were treated with FK506, 0.1% DMSO, or E_2 as described in **1.3**, for 24 h, washed free of BrdU and harvested. The cells were fixed with 4% paraformaldehyde for 30 min, blocked with 1% BSA in phosphate-buffered saline containing 0.2% Triton X-100 for 30 min, incubated with a HRP-BrdU antibody (1∶200) for 2 h at 37℃, and washed. A TMB substrate solution was added to the wells and color developed in proportion to the amount of BrdU bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm with a spectrophotometer. Cell proliferation was calculated by BrdU incorporation rate. Cell proliferation (%) = $A_{\text{Test group}}/A_{\text{DMSO group}} \times 100\%$.

1.5 Colorimetric method for detecting alkaline phosphatase (ALP) activity

Cells were washed 3 times with PBS, and cellular proteins were solubilized with 1% TritonX-100 in 0.9% NaCl and centrifuged. Supernatants were assayed for ALP activity on the basis of the method of Bessey *et al*[12]. Enzyme activity was determined colorimetrically using p-nitrophenylphosphate (p-NPP) as the substrate at pH 10.3 after incubation at 37℃ for 30 min and the optical density was read at 405 nm. ALP activity was normalized to the protein content and expressed as mmol·h^{-1}·g^{-1} protein.

1.6 *o*-Cresolphthalein complexone method for quantitation of calcium deposition

Calcification was assessed by a modification of the method described by Jono *et al*[13]. Briefly, the cultures were decalcified with HCl 0.6 mol·L^{-1} for 24 h. The calcium content was determined by measuring the concentrations of calcium in the HCl supernatant by the *o*-cresolphthalein complexone method. After decalcification, the cultures were washed with PBS and solubilized with 0.1% SDS containing NaOH 0.1 mol·L^{-1}. Total protein content was measured with a Bio-Rad protein assay kit. The calcium content of the cell layer was normalized to the protein content and expressed as μg·g^{-1} protein.

1.7 Colorimetric method for detecting CaN activity

The total cell lysates were used to detect the CaN activity after the treatment with various concentrations of FK506. 0.1% DMSO was used as vehicle control group while caffeine 100 μmol·L^{-1} which can activate CaN via releasing intracellular calcium was positive group. CaN phosphatase activity was measured using the Biomol calcineurin assay kit. RⅡ-phosphopeptide was used as a highly specific substrate for CaN. The detection of free phosphate released from RⅡ peptide by CaN was determined photometrically using the Biomol Green reagent. CaN activity was calculated as the total phosphatase activity minus activity in the presence of EGTA.

1.8 Western blotting for core binding factor alpha1 subunit (Cbfα1) protein expression

Nuclear extracts were prepared using Beyotime Biotech Reagents. Protein concentrations were determined with a Bio-Rad protein assay kit. Extracted proteins were separated by 10% PAGE, followed by transfer to PVDF membranes. Membranes were blocked with 10% BSA in TBST and then incubated with the primary antibodies against Cbfα1 (rabbit polyclonal IgG, dilution in TBST 1∶200). Anti-rabbit (diluted 1∶5000 in TBST) horseradish-peroxidase-conjugated second antibodies were applied after washing the blots three times in TBST. Chemiluminescent signal was developed using an ECL kit according to the manufacturer's instructions. Band densitometry was quantified using Quantity One Analysis software (version 4.5.2, Bio-Rad Laboratories, CA, USA) and Cbfα1 signaling was expressed as relative values when normalized to β-actin bands.

1.9 Statistical analysis

Values are expressed as $\bar{x} \pm s$ for the indicated number of separate experiments. Equality examinations were performed before variance analysis. Statistical significance was evaluated by one-way ANOVA and a P value of less than 0.05 was considered statistically significant.

2 RESULTS

2.1 Effect of FK506 on hBMSCs proliferation and osteoblastic differentiation

FK506 0.001 – 5 μmol·L^{-1} had concentraion-dependent biphasic effects on cell proliferation (Tab 1). FK506 0.001 – 0.01 μmol·L^{-1} promoted cell proliferation, with FK506 0.01 μmol·L^{-1} resulting in the most profound enhancement ($P < 0.05$). However, FK506 0.5 – 5 μmol·L^{-1} impaired cell proliferation when compared with that of the cells treated with 0.1% DMSO ($P < 0.05$) (Tab. 1). In contrast, FK506 0.001 – 0.1 μmol·L^{-1} had no effect on osteoblastic differentiation in hBMSCs cultures, demonstrated by the absence of obvious changes in the ALP activity at d 8 (Tab. 2) and the calcium deposition at d12 compared with 0.1% DMSO group (Tab. 3). However, FK506 0.5 – 5 μmol · L^{-1} markedly suppressed both ALP activity and calcium deposition.

Tab. 1 Effect of FK506 on primary human bone marrow-derived mesenchymal stem cells (hBMSCs) proliferation

Drug/μmol·L^{-1}	Cell proliferation/%
0.1% DMSO (control)	100 ± 15
FK506 0.001	108 ± 14
0.01	133 ± 18 *
0.1	90 ± 4
0.5	75 ± 8 *
1	66 ± 5 *
5	40 ± 9 *
Estradiol 0.01	135 ± 13 *

The hBMSCs were cultured for 24 h. Cell proliferation was calculated by BrdU incorporation rate. Cell proliferation (%) = $A_{\text{Test group}}/A_{\text{DMSO group}} \times 100\%$. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, compared with 0.1% DMSO group.

2.2 Effect of FK506 on CaN activity and Cbfα1 expression

As shown in Tab. 4, compared with 0.1% DMSO group, FK506 0.1 – 5 μmol·L^{-1} exhibited a concentration-dependent and significant attenuation of CaN activity. The highest inhibition was achieved in FK506 5 μmol·L^{-1} group, and caffeine 100 μmol · L^{-1} caused 2-fold increase in CaN activity of these cell culture.

Tab. 2 Effect of FK506 on alkaline phosphatase (ALP) activity of hBMSCs

Drug/μmol·L^{-1}	ALP activity/mmol·h^{-1}·g^{-1} protein
0.1% DMSO	2.13 ± 0.17
FK506 0.001	2.06 ± 0.12
0.01	1.96 ± 0.23
0.1	1.85 ± 0.09
0.5	1.54 ± 0.11 *
1	1.30 ± 0.13 *
5	0.87 ± 0.08 *
Estradiol 0.01	2.76 ± 0.21 *

See Tab 1 for the legend. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, compared with 0.1% DMSO group.

Tab. 3 Effect of FK506 on calcium deposition of hBMSCs

Drug/μmol·L^{-1}	Calcium deposition/μg·g^{-1} protein
0.1% DMSO	120.5 ± 4.1
FK506 0.001	118.4 ± 1.5
0.01	116.4 ± 3.6
0.1	111.9 ± 5.07
0.5	92.6 ± 7.2 *
1	81.5 ± 7.3 *
5	73.1 ± 2.6 *
Estradiol 0.01	146.4 ± 2.4 *

See Tab. 1 for the legend. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, compared with 0.1% DMSO.

Tab. 4 Effect of FK506 on calcineurin (CaN) activity of hBMSCs

Drug/μmol·L^{-1}	CaN activity/μmol·h^{-1}·g^{-1} protein
0.1% DMSO	0.85 ± 0.09
FK506 0.1	0.76 ± 0.12 *
0.5	0.41 ± 0.09 *
1	0.26 ± 0.02 *
5	0.18 ± 0.02 *
Caffeine 100	1.57 ± 0.02 *

See Tab. 1 for the legend. $\bar{x} \pm s$, $n = 6$. * $P < 0.05$, compared with 0.1% DMSO control group.

As shown in Fig. 1, FK506 0.5 – 5 μmol·L^{-1} induced a concentration-dependent reduction in Cbfα1 protein. Compared with 0.1% DMSO group, FK506 5 μmol · L^{-1} reached maximum inhibition effect, leading to approximately 3-fold reduction of Cbfα1 protein.

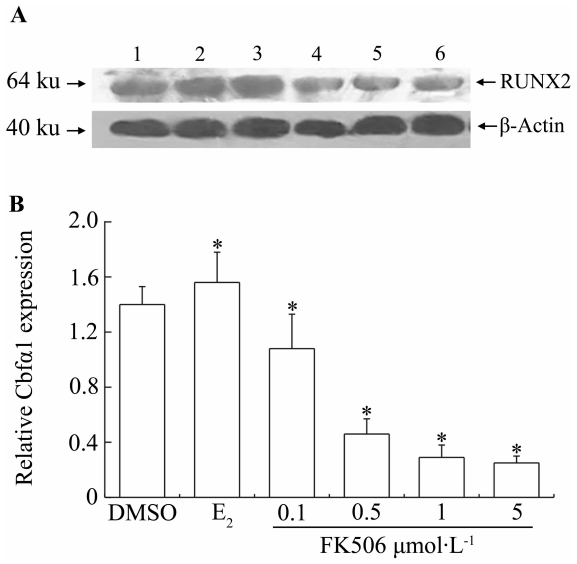

Fig. 1 Effect of FK506 on core binding factor alpha 1 subunit (Cbfα1) protein expression by Western blotting. The cells were treated with FK506 or 17β-estrodiol (E_2) 0.01 μmol·L^{-1} for 24 h. Lane 1: 0.1% DMSO; lane 2: E_2 group; lanes 3 – 6: FK506 0.1, 1 and 5 μmol·L^{-1} groups, respectively. $\bar{x} \pm s$, $n = 6$. $^*P < 0.05$, compared with 0.1% DMSO group.

3 DISCUSSION

FK506 exerted concentration-dependent biphasic effects on cell proliferation, while FK506 0.1 – 5 μmol·L^{-1} inhibited hBMSCs proliferation. These results are inconsistent with previous observations that FK506 was shown to increase both osteoblastic activity and ossification markers of mouse and rat bone marrow cell cultures[7]. FK506 was also reported to inhibit cell proliferation and osteopontin mRNA expression but induce ALP activity in rat osteoblast-like cell line, ROS17/2.8 cells[14]. Moreover, CsA was found to elicit dose-dependent biphasic anabolic or catabolic effect on bone formation, osteoblast differentiation, and bone mass[15], which suggested that these contradictory reports and paradoxical results, including ours, stem, in part, from the wide variation in the conditions (*e.g.* different cell types and culture media) for these studies and also in the concentrations of the immunosuppressants used.

It has repeatedly been demonstrated that bone loss is the highest within the first 6 to 12 months after renal transplantation and persists, albeit at a lower rate, for many years[16]. Meanwhile, it has been verified that the ratio of FK506 concentration in the second or third post-transplantation month was higher than in the following month[17]. Such clinical evidences means that high dosage of FK506 may result in osteopenia or osteoporosis after organ transplantation. It has been also reported that overexpression of CaN resulted in a profound increase of osteoblastic differentiation markers including Cbfα1[4], so inhibition of CaN would lead to down-regulation of Cbfα1 expression. In this regard and with clinical evidence, we investigated if higher concentrations of FK506 0.5 – 5 μmol·L^{-1} had any impact on the CaN activity in hBMSC cultures. FK506 0.1 – 5 μmol·L^{-1} exhibited a concentration-dependent attenuation of CaN activity in line with the decrement of Cbfα1 protein in the hBMSC cultures. Thus, it can be speculated that CaN restraint may interpret, at least partly, the inhibitory effect of FK506 on the osteoblastic differentiation in hBMSC cultures at high concentrations, which however, requires further investigations. In addition to CaN/Cbfα1 pathway, FK506 was found to affect other signaling pathways such as ERK and PI-3K/Akt signaling in other cultured cells[18], which could be other potential mechanisms for FK506-mediated suppression effect in hBMSC cultures.

In summary, FK506 < 0.1 μmol·L^{-1} promoted the proliferation of the hBMSCs, while FK506 > 0.1 μmol·L^{-1} elicited inhibited proliferation and osteoblastic differentiation, and CaN/Cbfα1 pathway might be responsible for FK506-mediated suppression effect in hBMSC cultures.

REFERENCES:

[1] Aramburu J, Heitman J, Crabtree GR. Calcineurin: a central controller of signalling in eukaryotes[J]. *EMBO Rep*, 2004, **5**(4):343-348.

[2] Liu YL, Jiang XX, Su YF, Huo SW, Zhu H, Wu Y, *et al*. Activated T cells promote differentiation of mesenchymal stem cells into osteoblasts[J]. *J Exp Hematol*, 2009, **17**(4):974-976.

[3] Kung L, Batiuk TD, Palomo-Pinon S, Noujaim J, Helms LM, Halloran PF. Tissue distribution of calcineurin and its sensitivity to inhibition by cyclosporine[J]. *Am J Transplant*, 2001, **1**(4):325 – 333.

[4] Sun L, Blair HC, Peng Y, Zaidi N, Adebanjo OA, Wu XB, *et al*. Calcineurin regulates bone formation by the

osteoblast[J]. *Proc Natl Acad Sci USA*, 2005, **102**(47):17130-17135.
[5] Wada C, Kataoka M, Seto H, Hayashi N, Kido J, Shinohara Y, *et al*. High-turnover osteoporosis is induced by cyclosporin A in rats[J]. *J Bone Miner Metab*, 2006, **24**(3):199-205.
[6] Kirino S, Fukunaga J, Ikegami S, Tsuboi H, Kimata M, Nakata N, *et al*. Regulation of bone metabolism in immunosuppressant (FK506)-treated rats[J]. *J Bone Miner Metab*, 2004, **22**(6):554-560.
[7] Tang L, Ebara S, Kawasaki S, Wakabayashi S, Nikaido T, Takaoka K. FK506 enhanced osteoblastic differentiation in mesenchymal cells[J]. *Cell Biol Int*, 2002, **26**(1):75-84.
[8] Song LH, Xiao ZS, Pan W, Yu YH, Zhou HH. Effect of cyclosporin A on the osteoblastic differentiation[J]. *Chin J Organ Transplant*, 2003, **24**(4):236-238.
[9] Igarashi K, Hirotani H, Woo JT, Stern PH. Cyclosporine A and FK506 induce osteoclast apoptosis in mouse bone marrow cell cultures[J]. *Bone*, 2004, **35**(1):47-56.
[10] Sun L, Peng Y, Zaidi N, Zhu LL, Iqbal J, Yamoah K, *et al*. Evidence that calcineurin is required for the genesis of bone-resorbing osteoclasts[J]. *Am J Physiol Renal Physiol*, 2007, **292**(1):F285-F291.
[11] Dai Z, Li Y, Quarles LD, Song T, Pan W, Zhou H, *et al*. Resveratrol enhances proliferation and osteoblastic differentiation in human mesenchymal stem cells via ER-dependent ERK1/2 activation[J]. *Phytomedicine*, 2007, **14**(12):806-814.
[12] Bessey OA, Lowry OH, Brock MJ. A method for the rapid determination of alkaline phosphates with five cubic millimeters of serum[J]. *J Biol Chem*, 1946, **164**:321-329.
[13] Jono S, Nishizawa Y, Shioi A, Morii H. Parathyroid hormone-related peptide as a local regulator of vascular calcification. Its inhibitory action on *in vitro* calcification by bovine vascular smooth muscle cells[J]. *Arterioscler Thromb Vasc Biol*, 1997, **17**(6):1135-1142.
[14] Ogawa T, Tokuda M, Tomizawa K, Matsui H, Itano T, Konishi R, *et al*. Osteoblastic differentiation is enhanced by Rapamycin in rat osteoblast-like osteosarcoma (ROS 17/2.8) cells[J]. *Biochem Biophys Res Commun*, 1998, **249**(1):226-230.
[15] Yeo H, Beck LH, McDonald JM, Zayzafoon M. Cyclosporin A elicits dose-dependent biphasic effects on osteoblast differentiation and bone formation[J]. *Bone*, 2007, **40**(6):1502-1516.
[16] Weisinger JR, Carlini RG, Rojas E, Bellorin-Font E. Bone disease after renal transplantation[J]. *Clin J Am Soc Nephrol*, 2006, **1**(6):1300-1313.
[17] Xiu DR, Zhang TL, Yuan J, Song SB. Relationship between oral doses of tacrolimus and changes in blood concentration after liver transplantation[J]. *Chin J Organ Transplant*, 2004, **25**(3):135-137.
[18] Zawadzka M, Kaminska B. Immunosuppressant FK506 affects multiple signaling pathways and modulates gene expression in astrocytes[J]. *Mol Cell Neurosci*, 2003, **22**(2):202-209.

高浓度他克莫司抑制人骨髓间质干细胞增殖及向成骨细胞分化

韦鸿雁[1], 潘 伟[2], 邱 霓[2], 黄 丽[2], 周宏灏[2], 肖洲生[2]
(1. 湖南省人民医院临床药学室, 湖南 长沙 410005; 2. 中南大学临床药理研究所, 湖南 长沙 410078)

摘要: 目的 探讨他克莫司(FK506)对人骨髓间质干细胞(hBMSCs)增殖及向成骨细胞分化的影响。**方法** FK506 0.001 ~ 5 μmol·L^{-1}处理 hBMSCs 细胞中,雌二醇 0.01 μmol·L^{-1}或咖啡因 100 μmol·L^{-1}为阳性对照组,作用 24 h 后用 BrdU 掺入法检测细胞增殖,在促成骨细胞分化液中作用 8 d 后用比色法检测碱性磷酸酶(ALP)活性,作用 12 d 后用邻甲酚酞络合法检测钙沉积量;通过检测磷酸盐释放量间接反映钙调神经磷酸酶(CaN)活性,Western 印迹法检测核心结合因子 α1 亚基(Cbfα1)表达。**结果** 与 DMSO 对照组相比, FK506 0.001 ~ 0.01 μmol·L^{-1}促进细胞增殖,但对 ALP 活性及钙沉积量无影响; FK506 0.5 ~ 5 μmol·L^{-1}则呈浓度依赖性地抑制细胞增殖,显著抑制 ALP 活性及减少钙沉积量($P < 0.05$)。此外, FK506 0.1 ~ 5 μmol·L^{-1}呈浓度依赖性地降低 CaN 活性,与相同浓度 FK506 呈浓度依赖性地下调 Cbfα1 的表达效应相一致。**结论** 高浓度 FK506 可通过 CaN/Cbfα1 通路抑制 hBMSCs 增殖及向成骨细胞成骨分化。

关键词: 他克莫司; 人骨髓间质干细胞; 钙调神经磷酸酶; 核心结合因子 α1 亚基

基金项目: 教育部高等学校优秀青年教师教学科研奖励计划(TRAPOYT)资助项目(30040002); 国家自然科学基金资助项目(N30171085); 美国国立卫生研究院资助项目(RO1-AR049712)

通讯作者: 肖洲生, Tel: (0731)82278323, E-mail: xiaozs64@yahoo.com

(收稿日期: 2010-08-16 接受日期: 2011-03-21)
(本文编辑: 乔 虹)