第 25 卷第 10 期
2009 年 10 月

无 机 化 学 学 报
CHINESE JOURNAL OF INORGANIC CHEMISTRY

Vol.25 No.10
1835~1841

Fe^{3+}和 Fe^{2+}对原代培养的成骨细胞增殖、分化和矿化功能的影响

张金超*,[1,2] 李亚平[1] 刘翠莲[1] 孙 静[3] 赵燕燕[4]

([1]河北大学化学与环境科学学院化学生物学实验室,保定 071002)

([2]药物化学与分子诊断省部共建重点实验室,保定 071002)

([3]河北大学附属医院 B 超室,保定 071000)

([4]河北大学实验中心,保定 071000)

摘要: 采用噻唑蓝(MTT)法、碱性磷酸酶(ALP)比活性测定、油红 O 染色和茜素红染色及定量分析,研究了不同浓度的 Fe^{3+}和 Fe^{2+}对原代培养的成骨细胞增殖、分化及矿化功能的影响。结果表明:浓度为 1×10^{-9}~1×10^{-4} mol·L^{-1} 的 Fe^{3+}和 Fe^{2+}促进成骨细胞增殖,但是在较高浓度 1×10^{-3} mol·L^{-1} 时,它们则抑制成骨细胞增殖。与成骨细胞作用 48 h,浓度为 1×10^{-8}~1×10^{-4} mol·L^{-1} 的 Fe^{3+}和 Fe^{2+}抑制其分化,但在较低的浓度 1×10^{-9} mol·L^{-1} 时则对其分化没有影响;进一步延长作用时间为 72 h,Fe^{3+}对成骨细胞分化没有影响,除 1×10^{-6} mol·L^{-1} 浓度的 Fe^{2+}促进成骨细胞分化外,其他浓度的 Fe^{2+}则抑制其分化;测试浓度下的 Fe^{3+}对成骨细胞向脂肪细胞的横向分化表现为抑制或没有影响,而 Fe^{2+}的影响则依赖于浓度和作用时间。在 1×10^{-8}~1×10^{-5} mol·L^{-1} 浓度范围内,Fe^{3+}和 Fe^{2+}对矿化结节的影响表现出相反的效应。在较高浓度(1×10^{-4} mol·L^{-1})下,它们促进矿化节结的形成,而在较低浓度(1×10^{-9} mol·L^{-1})下,Fe^{3+}抑制矿化节结的形成,Fe^{2+}则没有影响。结果提示:浓度,作用时间和铁离子的价态都是影响 Fe^{3+}和 Fe^{2+}生物效应(从毒性到活性,从损伤到保护,从上调到下调)转变的关键因素。

关键词: 铁离子; 成骨细胞; 增殖; 分化; 矿化

中图分类号: O614.81+1 **文献标识码**: A **文章编号**: 1001-4861(2009)10-1835-07

Effects of Fe^{3+} and Fe^{2+} on Proliferation, Differentiation and Mineralization Function of Primary Osteoblasts *in vitro*

ZHANG Jin-Chao*,[1,2] LI Ya-Ping[1] LIU Cui-Lian[1] SUN Jing[3] ZHAO Yan-Yan[4]

([1]*College of Chemistry & Environmental Science, Chemical Biology Laboratory, Hebei University, Baoding, Hebei* 071002)

([2]*The Key Laboratory of Medicinal Chemistry and Molecular Diagnosis, Ministry of Education, China, Hebei University, Baoding, Hebei* 07100)

([3]*B-Ultrasound Lab., Affiliated Hospital of Hebei University, Baoding, Hebei* 071000)

([4]*The Experimental Center, Hebei University, Baoding, Hebei* 071000)

Abstract: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromid (MTT), alkaline phosphatase (ALP) activity, oil red O assays and alizarin red-S (ARS) stain were used to evaluate the effects of Fe^{3+} and Fe^{2+} on proliferation, differentiation and mineralization function of primary osteoblasts (OBs) in vitro. The results indicate that both Fe^{3+} and Fe^{2+}(1×10^{-9}~1×10^{-4} mol·L^{-1}) promote the proliferation of OBs, but turn to inhibit at a higher concentration of 1×10^{-3} mol·L^{-1}. Both Fe^{3+} and Fe^{2+}(1×10^{-8}~1×10^{-4} mol·L^{-1}) inhibit differentiation of OBs, but have no effect on differentiation at a lower concentration of 1×10^{-9} mol·L^{-1} for 48 h. Whereas, Fe^{3+} shows no effect on differentiation of OBs, Fe^{2+} begins to promote differentiation of OBs at concentration of 1×10^{-6} mol·L^{-1}, but Fe^{2+}

收稿日期:2009-04-13。收修改稿日期:2009-07-16。

河北省自然科学基金(No.B2009000161), 教育部科学技术研究重点项目(No.208018), 河北省教育厅科学研究计划课题(No.2008311),河北省应用基础研究计划重点基础研究项目(No.08966415D),河北省留学人员科技活动项目和河北大学自然科学基金资助项目。

*通讯联系人。E-mail:jczhang6970@yahoo.com.cn

第一作者:张金超, 男, 40 岁, 博士, 教授;研究方向:无机药物化学和细胞生物无机化学。

inhibits differentiation of OBs at other concentrations by prolonging the incubation time. Fe^{3+} inhibits adipocytic trans-differentiation of OBs or has no effects on adipocytic trans-differentiation of OBs at tested concentrations. The promotion or inhibition effect of Fe^{2+} on adipocytic trans-differentiation of OBs depends on concentration and incubation time. Fe^{3+} and Fe^{2+}(1×10^{-8}~1×10^{-5} mol·L^{-1}) inhibit and promote the formation of mineralized matrix nodules of OBs, respectively. Fe^{3+} and Fe^{2+} promote the formation of mineralized matrix nodules at a higher concentration of 1×10^{-4} mol·L^{-1}, but Fe^{3+} inhibits the formation of mineralized matrix nodules, Fe^{2+} shows no effect at a lower concentration of 1×10^{-9} mol·L^{-1}. The results suggest that concentration, culture time and valence state of iron ion are key factors for switching the biological effects of Fe^{3+} and Fe^{2+} from toxicity to activity, from damage to protection, or from down-regulation to up-regulation.

Key words: iron ion; osteoblasts; proliferation; differentiation; mineralization

0 Introduction

As a common metabolic bone disease, osteoporosis (OP) is characterized by low bone mass, a disturbance of bone micro-architecture, the increasing incidence of bone fragility and fracture[1]. With the coming of aging society, osteoporosis occurs more and more frequently, severely impairs the quality of life, increases mortality and care costs[2~5]. It has been recognized as a major public health problem and much attention has been focused on searching powerful medicine and therapeutic strategy.

In the whole life, bone remodeling occurs continuously to maintain the normal bone mass, in which osteoclasts (OCs) and osteoblasts (OBs) are two major cells involved[6,7]. The abnormality in the balance between OB and OC activities results in osteoporosis. A great deal of evidence suggests that osteoporosis is the result of a reciprocal interaction between genetic susceptibility and environmental factors[8]. Among them, certain essential trace elements were reported to be involved in the pathogenesis of osteoporosis[9~14]. As an essential trace element, adequate iron plays irreplaceable roles in body activities. Iron loaded persons are at increased risk for infection, neoplasia, arthropathy, cardiomyopathy, an array of endocrine, neurodegenerative diseases and osteoporosis[15~17]. Animal experiment has shown that the iron content is significantly lower than control group in rats with resection of ovarian osteoporosis model[18]. Low molecular mass iron chelators as well as a natural protein iron chelator, lactoferrin, may be useful in prevention of osteoporosis[19]. It has been suggested that iron-deficient rats have lower bone mass than iron-replete animals [20]. However, the effects of Fe^{3+} and Fe^{2+} on bone-forming function of primary OBs in vitro were not reported so far. In order to elucidate the effects of Fe^{3+} and Fe^{2+} on bone metabolism at cell levels, the effects of Fe^{3+} and Fe^{2+} on the proliferation, differentiation and mineralization function of primary OBs in vitro were studied in this paper.

1 Materials and methods

1.1 Materials and reagents

Institute of Cancer Research (ICR) mice were obtained from Agricultural University of Hebei. Dulbeccos modified Eagles medium (DMEM) and trypsin were purchased from GIBCO, USA. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT), penicillin and streptomycin, β-glycerphosphate, dexamethasone, ascorbic acid, collagenase type II, insulin, oil red O stain, alizarin red-S(ARS) and cetylpyridium chloride were from Sigma, USA. Neonatal bovine serum (NBS) was from Hangzhou Sijiqing Organism Engineering Institute. Alkaline phosphatase (ALP) activity kit was obtained from Nanjing Jiancheng Biological Engineering Institute (Nanjing, China), and micro-protein assay kit was purchased from Beyotime Biotechnology (Haimen, China). Ferric chloride and Ferrous chloride (Purity>99.9%) were analytical reagent

(AR).

1.2 Methods

1.2.1 Isolation and culture of primary OBs

OBs were isolated mechanically from newborn ICR mouse skull by sequential digestion as reported previously with minor modification[21]. Briefly, skull was dissected, endosteum and periosteum were stripped off, and the bone was cut into approximately 1 ~2 mm^2 pieces and digested with trypsin(2.5 $g \cdot L^{-1}$) for 30 min and the digestion was discarded. Then the bone was digested with collagenase Ⅱ (1.0 $g \cdot L^{-1}$) twice with 1 h for each, and the cells were collected and cultured. After incubating overnight at 37 ℃, in a 5% CO_2 humidified incubator, the DMEM was removed. Then the medium was replaced every 3 d in all experiments.

1.2.2 Cell proliferation assay

The protocol described by Mosmann was followed with some modifications[22]. Briefly, OBs(2×10^4 cells per well) were plated in 96-well culture plates and cultured overnight at 37 ℃, in a 5% CO_2 humidified incubator. Fe^{3+} or Fe^{2+} was added to the wells at various concentrations. Control wells were prepared by addition of DMEM. Wells containing DMEM without cells were used as blanks. Wells containing NaF (1×10^{-6} $mol \cdot L^{-1}$) without Fe^{3+} or Fe^{2+} were used as positive control. Upon completion of the incubation, MTT dye solution (20 μL, 5 $mg \cdot mL^{-1}$) was added to each well. After 4 h incubation, the supernatant was removed and DMSO (100 μL) was added to solubilize the MTT. The optical density(OD) of each well was measured on a microplate spectrophotometer (BioRad Model 3550, USA) at a wavelength of 570 nm. The proliferation rate(%) was calculated according to the formula: $[(OD_{treated}-OD_{control})/OD_{control}]\times100\%$.

1.2.3 ALP activity assay

OBs(2×10^4 cells per well) were plated in 48-well culture plates, and treated with Fe^{3+} or Fe^{2+} for 48 h and 72 h, respectively. The plates were washed thrice with ice-cold PBS and lysed by two cycles of freezing and thawing. Aliquots of supernatants were subjected to ALP activity and protein measurement using an ALP kit and a micro-protein assay kit, respectively. All results were normalized by protein content.

1.2.4 Oil red O stain and measurement

The OBs(3×10^4 cells per well) were plated in 48-well culture plates, after being induced by adipogenic supplement(10 $\mu g \cdot mL^{-1}$ insulin, 10^{-7} $mol \cdot L^{-1}$ dexthamethone) and treated with Fe^{3+} or Fe^{2+}, and fat droplets within differentiated adipocytes from OBs were stained using the oil red O described by Ichiro et al.[23] with some modifications. Briefly, cell monolayers were fixed in 4% formaldehyde, washed in water and stained with a 0.6%(*W*/*V*) oil red O solution(60% isopropanol, 40% water) for 15 min at room temperature. For quantification, cell monolayers were then washed extensively with water to remove unbound dye, and recorded by inverted phase contrast microscopy (Olympus IX 51), then 1 mL of isopropyl alcohol was added to the culture plates. After 5 min, the absorbance of the extract was measured by a spectrophotometer at 510 nm. The adipocytic trans-differentiation promoting rate(%) was calculated according to the formula: $[(OD_{treated}-OD_{control})/OD_{control}]\times100\%$.

1.2.5 Mineralized matrix formation assay

OBs(3×10^4 cells per well) were plated in 24-well tissue culture plates and cultured overnight at 37 ℃, in a 5% CO_2 humidified incubator. The medium was then changed to differentiation medium containing 10 $mmol \cdot L^{-1}$ β-glycerophosphate and 50 $\mu g \cdot mL^{-1}$ ascorbic acid in the presence or absence of Fe^{3+} or Fe^{2+} for 21 d. The formation of mineralized matrix nodules was determined by ARS stain. Briefly, Cell monolayers were fixed in 95% ethanol for 10 min at room temperature, then washed by PBS twice and stained with 0.1% alizarin red S for 30 min also at room temperature. Quantitation of ARS staining was performed by elution with 10%(*W*/*V*) cetylpyridium chloride for 10 min at room temperature and the absorbance was measured at 570 nm[24]. Results were expressed as moles of ARS/per milligram of total cellular protein and the mineralized matrix promoting rate(%) was further calculated.

1.2.6 Statistical analysis

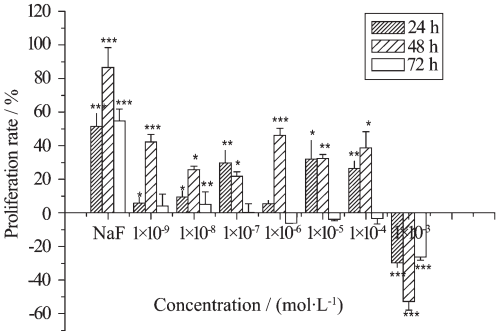
Data were collected from at least four separate experiments. The results were expressed as means ± standard deviation(SD). The statistical differences were analyzed using SPSS′ *t*-test. *P* values less than 0.05

were considered to be statistically different.

2 Results

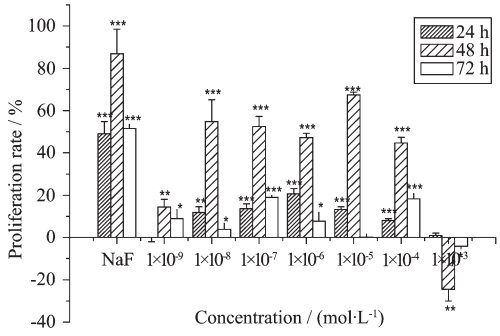
2.1 Effects of Fe^{3+} and Fe^{2+} on the proliferation of OBs

As shown in Fig.1 and Fig.2, Fe^{3+}(1×10^{-9}~1×10^{-4} mol·L^{-1}) promotes the proliferation of OBs for 24 h and 48 h, but the influence of Fe^{3+} on the proliferation disappears for 72 h. Fe^{2+}(1×10^{-9}~1×10^{-4} mol·L^{-1}) promotes the proliferation of OBs for 24 h, 48 h and 72 h. Fe^{3+} and Fe^{2+} inhibit the proliferation of OBs at a higher concentration of 1×10^{-3} mol·L^{-1}.



*P<0.05, **P<0.01, ***P<0.001 vs control group, n=5

Fig.1 Effect of Fe^{3+} on the proliferation of OBs

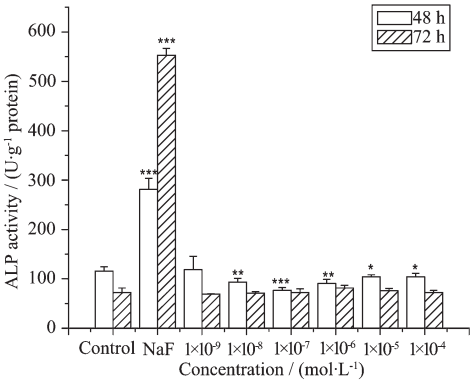


*P<0.05, **P<0.01, ***P<0.001 vs control group, n=5

Fig.2 Effect of Fe^{2+} on the proliferation of OBs

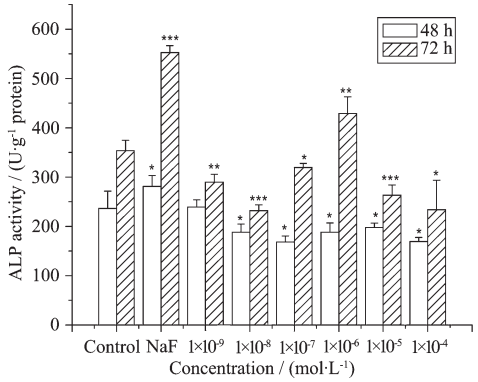
2.2 Effects of Fe^{3+} and Fe^{2+} on the differentiation of OBs

As shown in Fig.3 and 4, after incubating 48 h, both Fe^{3+} and Fe^{2+}(1×10^{-8}~1×10^{-4} mol·L^{-1}) inhibit differentiation of OBs, but show no effect on differentiation at a lower concentration of 1×10^{-9} mol·L^{-1}. Whereas, Fe^{3+} has no effect on differentiation of OBs, Fe^{2+} turns to promote differentiation of OBs at concentration of 1×10^{-6} mol·L^{-1}, but inhibits differentiation of



*P<0.05, **P<0.01, ***P<0.001 vs control group, n=5

Fig.3 Effect of Fe^{3+} on the differentiation of OBs



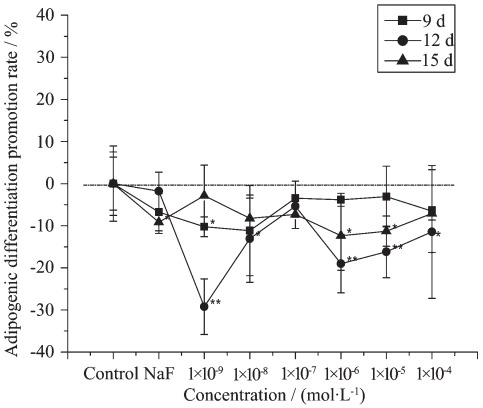
*P<0.05, **P<0.01, ***P<0.001 vs control group, n=5

Fig.4 Effect of Fe^{2+} on the differentiation of OBs

OBs at other concentrations by prolonging the incubation time.

2.3 Effects of Fe^{3+} and Fe^{2+} on the adipocytic trans-differentiation of OBs

As shown in Fig.5, Fe^{3+} inhibits adipocytic trans-differentiation of OBs at concentrations of 1×10^{-8} and

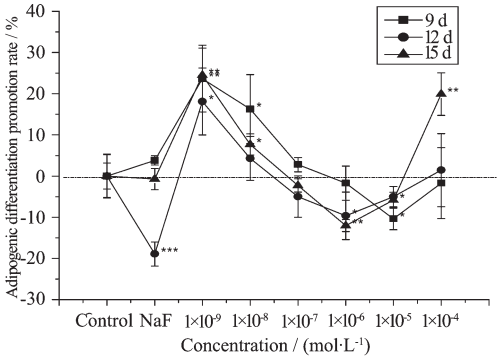


*P<0.05, **P<0.01, ***P<0.001 vs control group, n=5

Fig.5 Effect of Fe^{3+} on the adipocytic trans-differentiation of OBs

1×10^{-9} mol·L^{-1}, while it shows no effect at other tested concentrations for 9 d. Fe^{3+} inhibits adipocytic trans-differentiation of OBs at all tested concentrations except 1×10^{-7} mol·L^{-1} for 12 d. Fe^{3+} inhibits adipocytic trans-differentiation of OBs at concentrations of 1×10^{-6}, 1×10^{-5} mol·L^{-1}, while has no obvious effect at other tested concentrations for 15 d.

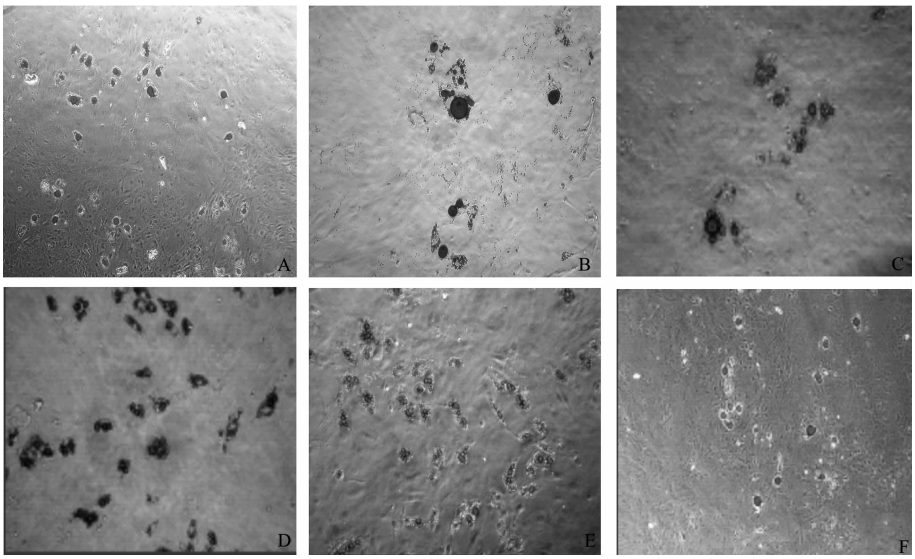
As shown in Fig.6, Fe^{2+} promotes adipocytic trans-differentiation of OBs at concentrations of 1×10^{-9} and 1×10^{-8} mol·L^{-1}, turns to inhibit at concentration of 1×10^{-5} mol·L^{-1}, then has no effect at a higher concentration of 1×10^{-4} mol·L^{-1} for 9 d. Fe^{2+} promotes adipocytic trans-differentiation of OBs at concentration of 1×10^{-9} mol·L^{-1}, turns to inhibit at concentrations of 1×10^{-6} and 1×10^{-5} mol·L^{-1}, then shows no effect at a higher concentration of 1×10^{-4} mol·L^{-1} for 12 d. Fe^{2+} promotes adipocytic trans-differentiation of OBs at concentrations of 1×10^{-9} and 1×10^{-8} mol·L^{-1}, inhibits



$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs control group, n=5

Fig.6　Effect of Fe^{2+} on the adipocytic trans-differentiation of OBs

adipocytic trans-differentiation of OBs at concentrations of 1×10^{-6} and 1×10^{-5} mol·L^{-1}, then turns to promote adipocytic trans-differentiation at a higher concentration of 1×10^{-4} mol·L^{-1} for 15 d. The experimental results are in accordance with morphological observations(Fig.7).



(A) Control group; (B) 1×10^{-6} mol·L^{-1} NaF; (C) 1×10^{-8} mol·L^{-1} Fe^{3+}; (D) 1×10^{-7} mol·L^{-1} Fe^{3+}; (E) 1×10^{-8} mol·L^{-1} Fe^{2+}; (F) 1×10^{-7} mol·L^{-1} Fe^{2+}

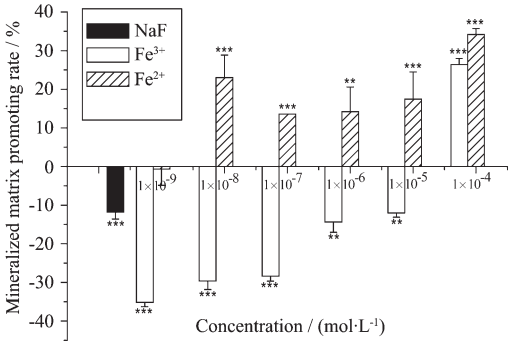
Fig.7　Adipocytes stained by oil red O when OBs being cultured in the presence of adipogenic supplement for 12 d

2.4　Effects of Fe^{3+} and Fe^{2+} on the formation of mineralized matrix nodules

As shown in Fig.8, Fe^{3+}(1×10^{-9}~1×10^{-5} mol·L^{-1}) inhibits the formation of mineralized matrix nodules of OBs, but promotes the formation of mineralized matrix nodules at a higher concentration 1×10^{-4} mol·L^{-1}. Fe^{2+} (1×10^{-8}~1×10^{-4} mol·L^{-1}) promotes the formation of mineralized matrix nodules, but shows no effect at a lower concentration 1×10^{-9} mol·L^{-1}.

3　Discussion

In present study, an *in vitro* model was used to examine the effects of Fe^{3+} and Fe^{2+} on the proliferation, differentiation and mineralization function of primary OBs. The results indicate that the effects of Fe^{3+} and Fe^{2+} on proliferation, differentiation and mineralization



*P<0.05, **P<0.01, ***P<0.001 vs control group, n=5

Fig.8 Effects of Fe^{3+} and Fe^{2+} on the mineralized nodule formation of OBs

function of primary OBs depend on concentration and incubation time, but are not dose-dependent. Many trace elements have been reported to be involved in the pathogenesis of OP. Cen et al.[25,26] have reported that Zn^{2+} could promote proliferation and differentiation of the rat OBs *in vitro*. Zn^{2+} could promote the transition of cell cycle from phases G0/G1 to S phase, thereby promote DNA synthesis and cell proliferation. It could activate the phosphoinositol signal transduction system in rat OBs *in vitro*, and elevate triphosphate inositol (IP3) content and protein kinase C (PKC) activity. The mechanism includes not only the membrane receptor-mediated pathway but also possibly the direct Zn^{2+} action. Fu et al.[27] reported Gd^{3+}(2~60 μmol·L^{-1}) exerted proliferation-promoting effects in a concentration-dependent manner. Gd^{3+} had remarkable effect on cell cycle distribution. Gd^{3+}(60 μmol·L^{-1}) drove the synchronized cells to enter S phase at 12 h and G2/M phase at 18 h by increasing the levels of cyclins D, E, A and phosphorylated retinoblastoma protein and decreasing the expressions of $p21^{Cipl}$ and $p27^{Kipl}$. Gd^{3+} drove cells through G1/S transition point and promoted cell cycle progression via activation of both ERK and PI3K signaling pathways. It was reported that H_2O_2 promoted osteoblastic differentiation of vascular smooth muscle cells by inducing oxidative stress in vascular smooth muscle cells and subsequently activating MAPK pathways. Shi et al.[28] reported that La^{3+} suppressed the effect of H_2O_2 probably by meliorate cell redox status through MAPK pathway. So we deduce that Fe^{3+} and Fe^{2+} promote proliferation of OBs probably by promoting the transition of cell cycle from phases G0/G1 to S phase thereby promoting DNA synthesis and cell proliferation. The difference between Fe^{3+} and Fe^{2+} might be related to reactive oxygen species.

Medullary adipocytes are secretory cells that may influence hematopoiesis and osteogenesis. Adipocytic and osteogenic cells are believed to be derived from multipotential stromal cells in the marrow, and *in vitro* studies have shown an inverse relationship between the differentiation of adipocytic and osteogenic cells[29]. It was reported that preadipocytes isolated from mouse marrow might regulate the activity and final differentiation of OBs. The condition medium harvested from mouse stromal preadipocytes decreased the ALP activity of a mouse stromal osteoblastic cell line[30]. Some investigators suggested that adipocytes might be involved in hematopoietic and osteogenic process by supplying the necessary soluble cell surface factors for OC differentiation and function *in vitro* [31]. Sakaguchi et al.[32] demonstrated that adipocyte-enriched stromal cells supported OC formation. Benayahu et al. [33] reported that preadipocytes also had the potential to stimulate OC differentiation. Adipocytes synthesized and released a variety of peptide and nonpeptide compounds or secreted cytokines such as tumor necrosis factor-α(TNF-α) and interleukin(IL-6), and the main effect of these cytokines is a stimulation of bone resorption[34]. So a reversal of adipogenesis will provide an important therapeutic approach to prevent aged-related and steroids-induced osteoporosis. Our experimental results indicate that the effect of Fe^{3+} and Fe^{2+} on adipocytic trans-differentiation of OBs also depends on concentration and incubation time. Fe^{3+} and Fe^{2+} may have protective effect on bone and this protective effect on bone may be mediated by inhibiting adipocytic trans-differentiation of OBs which may promote differentiation and mineralization of OBs.

In conclusion, the effects of Fe^{3+} and Fe^{2+} on the proliferation, differentiation and mineralization function of primary OBs *in vitro* are very complicated, but concentration, culture time and valence states of iron ion are key factors for switching the biological effects of Fe^{3+} and Fe^{2+} from toxicity to activity, from damage to

protection, or from down-regulation to up-regulation. The mechanism of the effects of Fe^{3+} and Fe^{2+} on the proliferation, differentiation, adipocytic transdifferentiation and mineralization function of primary OBs remains to be further studied.

References:

[1] Tuck S P, Francis R M. *Postgrad. Med. J.*, **2002**,**78**(923):526~532

[2] Freitas S S, Barrett-Connor E, Ensrud K E. *Osteoporos Int.*, **2008**,**19**(5):615~623

[3] Kirke P N, Sutton M, Burke H, et al. *Injury*, **2002**,**33**(5):387~391

[4] Papaioannou A, Kennedy C C, Ioannidis G, et al. *Osteoporos Int.*, **2008**,**19**(4):581~587

[5] Pongchaiyakul C, Nguyen N D, Jones G, et al. *J. Bone Miner. Res.*, **2005**,**20**(8):1349~1355

[6] Pittenger M F, Mackay A M, Beck S C, et al. *Science*, **1999**,**5411**(284):143~147

[7] Dewar A L, Farrugia A N, Condina M R, et al. *Blood*, **2006**, **107**(11):4334~4337

[8] Wang T, Zhang J C, Chen Y, et al. *J. Trace Elem. Med. Biol.*, **2007**,**21**(2):84~91

[9] Saltman P D, Strause L G. *J. Am. Coll. Nutr.*, **1993**,**12**(4):384~389

[10]Moonga B S, Dempster D W. *J. Bone Miner. Res.*, **1995**,**10**(3):453~457

[11]Zhang J C, Huang J, Xu S J, et al. *Prog. Nat. Sci.*, **2003**,**13**(4):266~270

[12]Marie P J, Hott M. *Metabolism*, **1986**,**35**(6):547~551

[13]JING Ling(井　玲), SUN Bo(孙　波), HU Xiu-Li(胡秀丽), et al. *Chin. J. Gerontol.*(*Zhongguo Laonianxue Zazhi*), **1995**, **15**(4):238~239

[14]LI He-Cheng(李贺成), SONG Xiang-Fang(宋祥芳), LI Guang-Yuan(李广元). *Foreign Med. Sci.* (*Section of Medgeography*)(*Guowai Yixue*: *Yixue Dili Fence*), **1995**,**16**(4):145~147

[15]Weinberg E D, Weinberg G A. *Curr. Opin. Infect. Dis.*, **1995**,**8**(3):164~169

[16]Weinberg E D. *Eur. J. Cancer Prev.*, **1996**,**5**(1):19~36

[17]Connor J R, Beard J L. *Nutrition Today*, **1997**,**32**(3):102~109

[18]LU Bao-Min(路宝民), GUO Qing-Sheng(郭庆生), LU Sheng-Man(路生满). *Chin. J. Osteoporosis*(*Zhongguo Guzhishusong Zazhi*), **2007**,**13**(5):313~314

[19]Weinberg E D. *Bio. Metals*, **2006**,***19***(6):633~635

[20]D′Amelio P, Cristofaro M A, Tamone C. *Bone*, **2008**,**43**(6):1010~1015

[21]Thomas O C, Kathleen C M, Bruce E, et al. *Endocrinology*, **1998**,**139**(1):36~43

[22]Mosmann T. *J. Immunol. Methods*, **1983**,**65**(1~2):55~63

[23]Sekiya I, Larson B L, Vuoristo J T et al. *J. Bone Miner. Res.*, **2004**,**19**(2):256~264

[24]Gori F, Divieti P, Demay M B. *J. Biol. Chem.*, **2001**,**276**(49):46515~46522

[25]CEN Xiao-Bo(岑小波), WANG Rui-Shu(王瑞淑), WU Zhao-Feng(吴兆锋). *Chin. J. Prev. Med.*(*Zhonghua Yufang Yixue Zazhi*), **1999**,**33**(4):221~223

[26]CEN Xiao-Bo(岑小波), WANG Rui-Shu(王瑞淑), WU Zhao-Feng(吴兆锋). *Acta Nutrimenta Sinica*(*Yingyang Xuebao*), **1999**,**21**(3):263~268

[27]Fu L J, Li J X, Yang X G, et al. *J. Biol. Inorg. Chem.*, **2009**,**14**(2):219~227

[28]Shi Y, Gou B D, Shi Y L, et al. *Biometals*, **2009**,**22**(2):317~327

[29]Beresford J N, Bennett J H, Devlin C, et al. *J. Cell Sci.*, **1992**, **102**(2):341~351

[30]Benayahu D, Zipori D, Wientroub S. *Biochem. Biophys. Res. Commun.*, **1993**,**197**(3):1245~1252

[31]Kelly K A, Tanaka S, Baron R, et al. *Endocrinology*, **1998**, **139**(4):2092~2101

[32]Sakaguchi K, Morita I, Murota S. *Prostag. Leukot. Essent. Fatty Acids*, **2000**,**62**(5):319~327

[33]Benayahu D, Peled A, Zipori D. *J. Cell Biochem.*, **1994**,**56**(3):374~384

[34]Ailhaud G, Grimaldi P, Negrel R. *Annu. Rev. Nutr.*, **1992**, **12**:207~233