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Effects of La^{3+} on osteogenic and adipogenic differentiation of primary mouse bone marrow stromal cells

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Abstract: In order to elucidate the action of La^{3+} on bone metabolism, effects of La^{3+} on the osteogenic and adipogenic differentiation of primary mouse bone marrow stromal cells (BMSCs) were studied by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test, alkaline phosphatase (ALP) activity measurement, mineralized function, oil red O stain and measurement. The results showed that La^{3+} promoted the proliferation of BMSCs except at 1×10^{-10} and 1×10^{-6} mol/L. The effect of La^{3+} on the osteogenic differentiation depended on concentrations at the 7th day, but the osteogenic differentiation was inhibited at any concentration at the 14th day. La^{3+} promoted the formation of mineralized matrix nodules except at 1×10^{-8} and 1×10^{-5} mol/L. La^{3+} inhibited adipogenic differentiation except at 1×10^{-10} and 1×10^{-7} mol/L at the 10th day, and inhibited adipogenic differentiation except at 1×10^{-9} mol/L at the 16th day. These findings suggested that La^{3+} might have protective effect on bone at appropriate dose and time. This would be valuable for better understanding the mechanism of the effect of La^{3+} on bone metabolism.

Keywords: La^{3+}; bone marrow stromal cells; osteogenic differentiation; adipogenic differentiation; mineralization; rare earths

The biological properties of the lanthanides have been the basis for research into potential therapeutic applications since the early part of the twentieth century. Now, lanthanum carbonate (Fosrenol) as a phosphate binder for the treatment of hyperphosphatemia in renal dialysis patients has been approved in both USA and Europe. It was reported that La^{3+} was rapidly cleared from the blood and redistributed to tissues, primarily the bone after intravenous administration[1]. Is La^{3+} beneficial or harmful to bone? This essential question deserves an urgent answering.

Owing to the similarity of La^{3+} and Ca^{2+} in the physical and chemical properties, La^{3+} has been reported to be involved in the pathogenesis of osteoporosis. Li et al. reported that long-term oral $La(NO_3)_3$ supplementation at a low dose to rats caused lanthanum accumulation in the bone tissue, reduced Ca/P ratio, decreased bone density, changed microstructure of bone and increased bone crystallinity[2]. Huang et al. reported that $La(NO_3)_3$ retarded bone maturation of male Wistar rats by gavage at the dose of 2.0 mg $La(NO_3)_3$·kg/day over a 6-month period[3]. We previously reported that the effects of La^{3+} on the proliferation and differentiation of osteoclasts (OCs) and UMR 106 cell line depended on concentrations and culture time[4,5].

BMSCs are pluripotent cells which have the capacity to become OBs and adipocytes[6]. There is more and more evidence that suggests a great degree of plasticity exists between OBs and adipocytes and this transdifferentiation is reciprocal[7]. It was found that an increase in the number of adipocytes occurred at the expense of OBs in osteopenic disorders. It was reported that there was a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis with a concomitant increase in osteoblastogenesis[8]. We previously reported the effect of Dy^{3+} on the osteogenic and adipogenic differentiation of primary mouse BMSCs[9]. Until now, although the OCs and OBs as cell models have been used to study the effects of La^{3+} on the bone metabolism, the effects of La^{3+} on the osteogenic and adipogenic differentiation of BMSCs were not reported. In this paper, the effects of La^{3+} on the osteogenic and adipogenic differentiation of BMSCs were studied in order to further elucidate the effect of La^{3+} on bone metabolism.

1 Materials and methods

1.1 Materials and reagents

Kunming (KM) mice (4–6 weeks) were obtained from Experimental Animal Center of Hebei Medical University.

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Dulbecco's modified eagle's medium (DMEM) and fetal bovine serum (FBS) were from Gibco. Benzylpenicillin, streptomycin, MTT, β-glycerophosphate, trypsin, dexamethasone, ascorbic acid, insulin, Alizarin red S (ARS), oil red O stain and cetylpyridium chloride were obtained from Sigma. An ALP activity kit was obtained from Nanjing Jiancheng Biological Engineering Institute, and a micro-protein assay kit was from Beyotime Biotechnology. Lanthanum chloride (Purity>99.9%) were purchased from Beijing Institute of Rare Earth Sci. & Tech. Co., Ltd.

1.2 Methods

Isolation and culture of primary BMSCs: The mouse BMSCs were obtained from adult KM mice by a method previously reported[8]. Briefly, mice were sacrificed, femora and tibiae were aseptically harvested, and the whole bone marrow was flushed using DMEM. The cells were collected and cultured in a culture flask. After incubating at 37 ºC for 3 d, in a 5% CO_2 humidified incubator, the nonadherent cells were removed and the medium was replaced with fresh DMEM. Then the medium was changed every 3 d in all experiments.

Cell proliferation assay: The protocol described by Mosmann was followed by some modifications[10]. Briefly, BMSCs (3×10^6 cells per well) were plated in 96-well culture plates and cultured overnight at 37 ºC, in a 5% CO_2 humidified incubator. La^{3+} was added at final concentrations of 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} mol/L. Control wells were prepared by addition of DMEM. Wells containing DMEM without cells were used as blanks. The plates were incubated for 44 h, then MTT dye solution (20 μl, 5 mg/ml) was added. After 4 h incubation, the supernatant was removed and DMSO (100 μl) was added. The optical density $D(\lambda)$ was measured on a microplate spectrophotometer (MD VersaMax, USA) at a wavelength of 570 nm. The proliferation rate (%) was calculated according to the formula: $(D(\lambda)_{treated}/D(\lambda)_{control}-1)\times100\%$.

Measurement of ALP activity: BMSCs (3×10^6 cells per well) were plated in 48-well culture plates, after being induced by osteogenic supplement (10^{-7} mol/L dexamethasone, 5.0 mmol/L β-glycerophosphate, 50 μg/ml ascorbic acid) and treated with La^{3+} at final concentrations of 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} mol/L for 7 d and 14 d, 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 by an ALP kit and a micro-protein assay kit respectively. The osteogenic differentiation promotion rate (%) was calculated according to the formula: $(a_{ALP\text{-}treated}/a_{ALP\text{-}control}-1)\times100\%$[11].

Mineralized matrix formation assay: BMSCs (2×10^5 cells per well) were plated in 48-well culture plates and cultured overnight. The medium was changed to differentiation medium containing 10 mmol/L β-glycerophosphate and 50 μg/ml ascorbic acid in the presence or absence of the concentrations of 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} mol/L La^{3+} for 21 d. The cells were fixed in 70% ethanol for 1 h at room temperature. The fixed cells were washed with PBS and stained with 40 mmol/L ARS, pH 4.2, for 30 min 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 $D(\lambda)$ was measured at 570 nm[12]. Results were expressed as moles of ARS/permilligram of total cellular protein.

Oil red O stain and measurement: BMSCs (3×10^6 cells per well) were plated in 48-well culture plates, after being induced by adipogenic supplement (10 μg/ml insulin, 10^{-7} mol/L dexthamethone) and treated with La^{3+} at final concentrations of 1×10^{-10}, 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, 1×10^{-6}, and 1×10^{-5} mol/L, and fat droplets within differentiated adipocytes from BMSCs were stained by the oil red O[13]. 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. Then 1 ml of isopropyl alcohol was added, after 5 min, the $D(\lambda)$ was measured at 510 nm. The adipogenic differentiation inhibition rate (%) was calculated according to the formula: $(1-D(\lambda)_{treated}/D(\lambda)_{control})\times100\%$.

Statistical analysis: Data were collected from at least three 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 indicate statistical differences.

2 Results

2.1 Effect of La^{3+} on the BMSC proliferation

As shown in Fig. 1, La^{3+} had no effect on the BMSC proliferation at a concentration of 1×10^{-10} mol/L, promoted the BMSC proliferation at concentrations of 1×10^{-9}, 1×10^{-8}, 1×10^{-7}, and 1×10^{-5} mol/L, but inhibited the BMSC proliferation at a concentration of 1×10^{-6} mol/L.

2.2 Effect of La^{3+} on the osteogenic differentiation

As shown in Fig. 2, La^{3+} had no effect on the osteogenic differentiation of BMSCs at concentrations of 1×10^{-10} and 1×10^{-6} mol/L, promoted osteogenic differentiation at con-

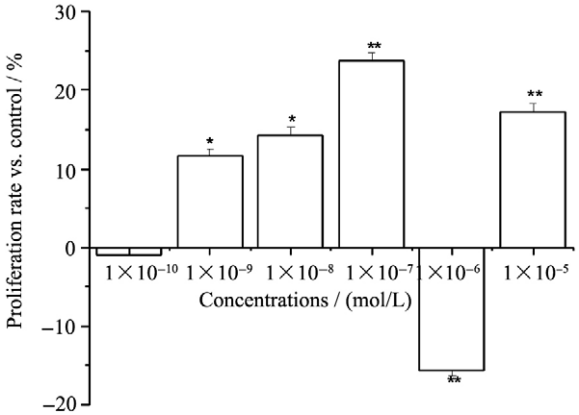

Fig. 1 Effect of La^{3+} on proliferation of BMSCs ($^*P<0.05$, $^{**}P<0.01$ compared with control group, $n=6$)