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Ytterbium ion promotes apoptosis of primary mouse bone marrow stromal cells

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Abstract: One of the main target organs for the lanthanides (Ln) is bone. Previous studies revealed that ytterbium (Yb) produced damage to the skeletal system *in vivo*. But the effects of Yb^{3+} on bone marrow stromal cells (BMSCs) *in vitro* had not been reported. In this paper, cell viability, apoptosis, mitochondrial membrane potential (MMP), reactive oxygen species (ROS) and lactate dehydrogenase (LDH) were measured in order to study the effects of Yb^{3+} on BMSCs. The results indicated that Yb^{3+} displayed a slight positive effect on the BMSCs viability at concentrations of 1×10^{-6}, 1×10^{-5}, and 1×10^{-4} mol/L, but turned to decrease the viability of BMSCs at the highest concentration of 1×10^{-3} mol/L for 24, 48 and 72 h. Yb^{3+} at 1×10^{-3} mol/L promoted apoptosis of BMSCs, increased the levels of ROS and LDH, and decreased MMP in BMSCs. It suggested that the precipitate of $YbPO_4$ might decrease the viability of BMSCs. Yb^{3+} induced the apoptosis of BMSCs via mitochondrial pathway. The results might be useful for more rational application of Yb-based compounds in the future.

Keywords: ytterbium; bone marrow stromal cells; precipitation; apoptosis; rare earths

Ytterbium is one of the lanthanide (Ln) elements, with an atomic number of 70[1]. Because of the excellent spectral characteristics, ytterbium was widely used in optical fiber-based telecommunications[2–4] and laser technology[5–8]. Ytterbium-based complexes as Lewis acid catalysts were also widely utilized for organic reactions[9,10]. The isotopic distribution pattern of ytterbium is the most similar to that of gadolinium among the lanthanides[11]. The animal experimental studies suggest that gadolinium and ytterbium are suitable contrast media for dynamic CT investigations[12]. It was reported that ytterbium-based nanomaterials could be potentially used as contrast agents in bioimaging because of their low toxicity[13], high-resolution[14], good specificity[15] and NIR-to-visible up-conversion properties[16,17]. For example, Er^{3+}-doped Yb_2O_3 up-conversion nanoparticles showed the excellent biocompatibility *in vitro*. Their biosafety was further confirmed by pharmacokinetics, biodistribution, as well as clearance of nanoparticles after intravenous injection in a mouse model. Thus, long-circulating Er^{3+}-doped Yb_2O_3 up-conversion nanoparticles might be an *in vivo* X-ray CT imaging contrast agent[18]. Liu et al. reported that long-circulating Gd_2O_3:Yb^{3+},Er^{3+} up-conversion nanoprobes might be used as high-performance contrast agents for multi-modality imaging[19]. Yb(III)-based complexes were also used as contrast agents in magnetic resonance (MR) and fluorescent molecular probes. For example, Yb-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethyl-1,4,7,10-tetraacetate (Yb-DOTMA) can act both as a contrast agent in magnetic resonance imaging (MRI) and as a reporter of physicochemical environment in magnetic resonance spectroscopy (MRS)[20]. Near-infrared (NIR) emissive complexes had several advantages over common luminescent probes[21]. Multiple NIR emissive complexes containing ytterbium were also reported[21–25]. For example, Yb(III) complex with amphiphilic bisporphyrin can be used as a bifunctional photodynamic therapeutic and NIR tumor-imaging agent[26]. However, these extensive applications increased the chance of human exposure to Yb and its compounds and thus raised deep concerns regarding their riskiness.

Bone is one of the main target organs of Ln[27]. It was reported that the Ln were rapidly redistributed to the liver, spleen and bone after intravenous administration[27]. Gale[1] reported that ytterbium chloride produced damage to the skeletal system in golden hamsters. Thus it is likely that Yb intervenes in bone-remodeling process and affects bone cell function. However, the potential effect

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and the mechanism of Yb on bone metabolism are not well-understood. Bone mesenchymal stem cells (BMSCs) are pluripotent cells which can differentiate to osteoblast, adipocyte, chondrocyte and myoblast lineages[28,29]. They can be readily isolated and expanded *ex vivo* and induced either *in vitro* or *in vivo* to terminally differentiate[30,31]. So far, whether Yb^{3+} has the potential effect on BMSCs has not been reported. In this work, we investigated the effect of Yb^{3+} on mouse primary BMSCs for the first time. The results provided novel evidence to explain the mechanism of bone metabolism by Yb^{3+} and might be helpful for more rational application of Yb-based compounds in the future.

1 Materials and methods

1.1 Materials and reagents

Kunming (KM) specific pathogen free (SPF) female mice (4–6 weeks) were obtained from Experimental Animal Center of Hebei Medical University. Ytterbium(III) chloride hexahydrate (purity>99.9%) was obtained from Beijing Institute of Rare Earth Sci. & Tech. Co. (Beijing, China). Dulbecco's modified eagle's medium (DMEM) was obtained from Gibco-BRL (Grand Island, NY, USA). Neonatal bovine serum (NBS) was purchased from Sijiqing Biological Engineering Materials Co., Ltd. (Hangzhou, China). Benzylpenicillin, streptomycin, trypsin, 3-[4,5-dimetylthiazole-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), RNase A, propidium iodide (PI), Rhodamine-123 (Rh123), and 2′,7′-dichlorodihydrofluorescein-diacetate (DCFH-DA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4′, 6-diamidino-2-phenylindole (DAPI), and annexin V-FITC apoptosis detection kit was purchased from Beyotime (Haimen, China). All other reagents were of analytical grade.

1.2 Method

1.2.1 Isolation and culture of BMSCs

The mouse BMSCs were obtained from KM female mice using the method as described previously[30]. Briefly, the mice were executed by cervical dislocation. Femora and tibiae were aseptically harvested, and the bone marrow was flushed using supplemented DMEM. The whole bone marrow was suspended by DMEM. Then the cells were collected and cultured in DMEM with 10% heat-inactivated NBS, 100 U/mL penicillin and 100 μg/ml streptomycin in a 5% CO_2 humidified atmosphere at 37 ºC, the medium was changed after 4 d. After that, the cell culture medium was changed every 3 d in all the experiments.

1.2.2 Cell viability assay

The cell viability was assessed using MTT assay as described previously[30]. BMSCs were seeded in 96-well culture plates at the density of 5×10^5 cells per well. Yb^{3+} was added at final concentrations of 1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}, and 1×10^{-3} mol/L. Wells containing cells without Yb^{3+} treatment were used as control. Wells containing DMEM without cells were used as blanks. After 24, 48 and 72 h further incubations, the adherent cells were used for MTT assay. In brief, MTT (10 μL, 5.0 mg/mL) was added and incubated at 37 °C for another 4 h. The supernatant was removed and 100 μL dimethyl sulfoxide (DMSO) was added to each well to solubilize formazan crystals. The optical density (OD) was measured at 570 nm by a microplate spectrophotometer (MD VersaMax, USA). The cell viability (%) was calculated as a percentage of $[OD_{sample}–OD_{blank}]/[OD_{control}–OD_{blank}]\times 100$.

Furthermore, the effect of the supernatant of Yb^{3+} in complete cell culture medium on cell viability was also evaluated by MTT assay. Briefly, the supernatant was prepared by adding $YbCl_3\cdot6H_2O$ to DMEM supplemented with 10% NBS, and the mixture was centrifugated at 10000 r/min for 10 min. BMSCs were incubated with or without the centrifugated and the uncentrifugated media for 24 h, respectively. The effect of the supernatant on cell viability was evaluated by MTT assay as above.

1.2.3 Cell apoptosis assay

Cells falling in the sub-G1 region are considered as apoptotic cells[32]. In brief, BMSCs were seeded in 6-well culture plates at the density of 2.5×10^7 cells per well. After 1×10^{-3} mol/L Yb^{3+} treatment for 24 h, cells were fixed in 70% ice-cold ethanol at 4 ºC overnight and then washed twice with phosphate buffered saline (PBS). Then, cells were incubated in 10 μg/mL PI and 100 μg/mL DNase- free RNase at 37 ºC for 30 min. Samples were analyzed (20,000 cells per sample) by FACScalibur flow cytometer (BD Biosciences, USA). Data were analyzed with Modfit 3.2 software. Cells falling in the subdiploid DNA contents (sub-G1) region are considered as apoptosis[33].

The apoptosis of cells was detected using FACScalibur flow cytometer with the annexin V-FITC/PI double labeling method[33]. In brief, BMSCs were harvested after treatment as described above. Then the cells were washed with PBS and resuspended in 195 μL binding buffer. 5 μL annexin V-FITC was added, cells were incubated at room temperature in the dark for 10 min. Then the cells were washed with PBS and resuspended in 190 μL binding buffer, 10 μL PI was added to each sample. Cells (20,000 cells per sample) were analyzed using an FACSCalibur flow cytometer (Becton Dickinson, FACSCalibur™, USA).

Furthermore, the apoptosis was also studied by cell morphology. Briefly, BMSCs were seeded in glass coverslips and cultured. After treatment with Yb^{3+} at final concentrations of 1×10^{-4} and 1×10^{-3} mol/L for 24 h, cells were stained with DAPI for 10 min. Then cells were washed with cold PBS three times. The nuclei undergo-

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