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High extracellular magnesium inhibits mineralized matrix deposition and modulates intracellular calcium signaling in human bone marrow-derived mesenchymal stem cells

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ABSTRACT

Mesenchymal stem cells (MSCs) have the potential to differentiate into several cell types and provide an attractive source of autologous cells for regenerative medicine. However, their cellular biology is not fully understood. Similar to Ca^{2+}, extracellular Mg^{2+} plays an important role in the functions of the skeletal system. Here, we examined the effects of extracellular Mg^{2+} on the deposition of calcium phosphate matrix and Ca^{2+} signaling with or without ATP stimulation in human bone marrow-derived mesenchymal stem cells (hBMSCs). We found that high extracellular Mg^{2+} concentration ($[Mg^{2+}]_e$) inhibited extracellular matrix mineralization in hBMSCs in vitro. hBMSCs also produced a dose-dependent decrease in the frequency of calcium oscillations during $[Mg^{2+}]_e$ elevation with a slight suppression on oscillation amplitude. In addition, spontaneous ATP release was inhibited under high $[Mg^{2+}]_e$ levels and exogenous ATP addition stimulated oscillation reappear. Taken together, our results indicate that high $[Mg^{2+}]_e$ modulates calcium oscillations via suppression of spontaneous ATP release and inactivates purinergic receptors, resulting in decreased extracellular mineralized matrix deposition in hBMSCs. Therefore, the high magnesium environment created by the rapid corrosion of Mg alloys may result in the dysfunction of calcium-dependent physiology processes and be disadvantageous to hBMSCs physiology.

1. Introduction

Mesenchymal stem cells (MSCs) have the potential to differentiate into several cell types and provide an attractive alternative source of cells for regenerative medicine [1,2]. As they have become more available in recent years, various studies are providing new information about physiological processes of MSCs, including proliferation, differentiation and mineralization. However, their cellular biology is not fully understood, especially those cellular activities regulated by the cytosolic Ca^{2+}.

Calcium is a highly versatile intracellular messenger responsible for controlling numerous cellular functions [3]. The calcium signaling pattern of human MSCs occurs as repetitive spontaneous calcium oscillations. These oscillations in MSCs are initiated by autocrine/paracrine ATP via the activation of the inositol trisphosphate receptors (IP_3Rs) mediated Ca^{2+} release from intracellular stores and sustained by Ca^{2+} influx/extrusion through cell membrane. The role of the Ca^{2+} oscillation is still unknown but they have been conclusively shown to regulate cellular processes. Cells have frequency or amplitude coding and decoding properties [4,5]. The evidence for calcium oscillation involvement in cellular physiology is that Ca^{2+} spikes can initiate gene expression more effectively than a steadily maintained level of the same average intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Consistently, some recent studies revealed manipulating Ca^{2+} oscillations in hBMSCs can indeed regulate their proliferation and differentiation and mineralization [6–8].

Magnesium and calcium are crucial components of bone and play important roles in skeletal health. An appropriate balance between $[Mg^{2+}]$ and extracellular Ca^{2+} concentration ($[Ca^{2+}]_e$) is associated with normal cerebellar, cardiovascular and skeletal functions. Significant fluctuations in $[Mg^{2+}]_e$ can result in changes in $[Ca^{2+}]_i$ as well as a variety of cellular process dependent on it [9–11]. Epidemiology has linked insufficient magnesium intake with osteoporosis [12] while the effect of high magnesium on

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skeletal system still remains to be clarified. During the fast degrading process, local magnesium concentration on the surface of a biodegradable magnesium implant may be higher than physiological concentration in intercellular space in bone tissue. In addition, some puzzling clinic data have shown that high magnesium status leads to mineralization defects [13] and osteopenia [14], leading to a question on excess magnesium's detrimental effect on skeletal system. In this study, we aimed to investigate the effect of high $[Mg^{2+}]_e$ on extracellular mineralized matrix deposition in hBMSCs and the mechanism involved in its calcium signaling modulation. These findings provide a new perspective on the molecular mechanisms involved in hBMSCs physiology and novel information regarding the use of magnesium-based alloys as biomaterials.

2. Materials and methods

2.1. hBMSCs isolation and expansion

All experimental protocols involving bone marrow collection were approved by the Ethics Committee of Shanghai Tongji University School of Medicine, China. Human bone marrow-derived mesenchymal stem cells (hBMSCs) were isolated and expanded according to the methods reported by Pittenger [15] with some modifications. The bone marrow aspirates were placed on tissue culture dishes in α-MEM containing 10% fetal bovine serum and 1% penicillin/streptomycin solution (Invitrogen, Carlsbad, CA, USA) in a 37 °C, 5% CO_2 environment. At the end of the second passage, the hBMSCs were cryopreserved until use. We used hBMSCs with less than five passages in the following experiments.

2.2. Alizarin Red staining

hBMSCs were grown in a 24-well plate (six wells per group) in control medium (0.8 mM Mg^{2+}), osteoinductive medium (OM, with 0.8 mM Mg^{2+}) or osteoinductive medium supplemented with different magnesium (1.05 mM Mg^{2+}, 1.3 mM Mg^{2+}, 1.8 mM Mg^{2+} or 3.8 mM Mg^{2+}) for 21 days. For all osteoinductive medium was supplemented with a cocktail of 100 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μM ascorbic acid-2-phosphate (Sigma–Aldrich, St. Louis, MO, USA). The medium was replaced every 3 days. After 21 days, Alizarin Red staining was performed as described elsewhere [16] to quantify the mineralization. Cells were observed and recorded with an inverted epifluorescence microscope (Olympus 1 × 2-ILL 100).

2.3. Calcium dye loading and fluorescence imaging

Ca^{2+} imaging was conducted using confocal microscopy scanning technique as described previously [8]. Briefly, Cells were loaded with 10 μM fluo-4 acetoxymethyl ester (AM) (Molecular Probes, Invitrogen) dissolved in HBSS (HEPES-buffered saline) solution for 30 min at 37 °C in the dark. HBSS solution contained (in mM): 121 NaCl, 5.4 KCl, 0.8 $MgCl_2$, 25 HEPES, 1.8 $CaCl_2$, and 6.0 $NaHCO_3$ (pH = 7.3). The concentration of magnesium in α-MEM culture medium and standard bath solution is 0.8 mM. Thereafter, cells were rinsed with HBSS twice and the loaded dye was allowed to de-esterify for 20 min at 37 °C in the dark. Coverslips were then mounted on a temperature-controlled perfusion chamber mounted on an inverted microscope (Leica, Wetzlar, Germany) and fluorescence data were recorded through a 40× quartz objective lens. Fluorescence images of $[Ca^{2+}]_i$ of calcium oscillations and after ATP stimulation were acquired in real time at 10 s and 2 s interval, respectively. In some studies, HBSS with different magnesium (1.3 mM Mg^{2+}, 1.8 mM Mg^{2+}, 3.8 mM Mg^{2+}, 5.8 mM Mg^{2+} and 10.8 mM Mg^{2+}) was achieved by magnesium addition.

2.3. Analysis of Ca^{2+} responses

We used pseudo-ratio $\delta F/F0$: $\delta F/F0 = (F - F_{base})/F_{base}$ to analysis calcium response in hBMSCs. F: measured fluorescence intensity of Fluo-4, F_{base}: the lowest level of fluorescence intensity in the cell. Ca^{2+} responses elicited by ATP showed characteristic biphasic decays with rapid and slow components. The amplitude of this secondary component was the distance from the well-defined intersection point between the rapid and slow phases of $[Ca^{2+}]_i$ and baseline level.

2.4. Adenosine 5′-triphosphate assay

The concentrations of ATP were measured using a luciferase-based ATPlite-M kit (Perkin–Elmer) as reported previously [17]. The ATP values were determined by an ATP standard calibration curve calculated on the basis of corresponding HBSS (i.e., matched $[Mg^{2+}]$ and $[Ca^{2+}]_e$) and expressed in absolute values (nM).

2.5. Statistical analysis

All statistical data are expressed as the mean ± one standard deviation (SD). Paired and/or unpaired Student's t-tests were used as appropriate to evaluate the statistical significance of differences between two group means (using SPSS 18.0 software). Significant differences were defined as $^{*}p < 0.05$, $^{**}p < 0.01$ versus control.

3. Results

3.1. High magnesium inhibit mineralization in vitro

To evaluate the effect of high magnesium on the matrix mineralization in vitro, hBMSCs were treated with different concentrations of $[Mg^{2+}]_e$ while osteoinduction. Extracellular calcium deposition was quantified after 21 days, mineralization was significantly inhibited when cultured in the presence of the OM with $\geqslant$1.3 mM $[Mg^{2+}]_e$ in a dose-dependent manner as compared to that observed in OM. But there is no significance between the 1.05 mM $[Mg^{2+}]_e$ group and the control group (Fig. 1). These results indicate that high $[Mg^{2+}]_e$ inhibits hBMSCs mineralization in vitro.

3.2. Effects of elevated $[Mg^{2+}]_e$ on frequency/amplitude of Ca^{2+} oscillations

Intracellular calcium is a key component in regulating extracellular mineralization [18]. As an antagonist for calcium, magnesium has been reported to modulate intracellular calcium signaling [19]. To test our hypothesis that high magnesium inhibit mineralizing capacity through interfering calcium signaling in hBMSCs, confocal imaging were used. Spontaneous and repetitive Ca^{2+} oscillations was successfully observed and was sustained in the HBSS containing 0.8 mM $[Mg^{2+}]_e$. About 56% of cells displayed robust and long lasting calcium oscillations. Fluorescence imaging revealed typical changes in $[Ca^{2+}]_i$ from hBMSCs (Fig. 2). Also, the quantitative data for the effects of high $[Mg^{2+}]_e$ on Ca^{2+} oscillatory frequency and amplitude are summarized. Increasing $[Mg^{2+}]_e$ from 0.8 to 1.3 mM did not evoke any statistically significant change in the average oscillatory amplitude or frequency. $[Mg^{2+}]_e$ at 1.8 mM and 3.8 mM significantly decreased calcium oscillation frequency by 37% ± 14% and 59% ± 18%, respectively. In addition, a substantial fraction of hBMSCs (32.5% and 64% respectively) no longer showed $[Ca^{2+}]_i$ oscillations. The average Ca^{2+} spike amplitude did not change significantly until hBMSCs were exposed to 3.8 mM $[Mg^{2+}]_e$ and only a slight reduction (10.5%) was observed (Fig. 3).