Magnesium ions facilitate integrin alpha-2 and alpha-3-mediated proliferation and partially promotes differentiation in hBMSCs

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Introduction: Recently, magnesium and its alloys have been proposed as a novel class of bone implant biomaterial due to its biodegradability and mechanical properties for medical applications [1]. Integrin signaling mediates cell proliferation, differentiation, and cell death in various cell types including osteoblasts [2]. The purpose of this current study is to identify whether magnesium ions, released abundantly from metals or alloys, affects proliferation and differentiation in hBMSCs.

Materials and Methods: hBMSCs were isolated from human bone marrow aspirated during hip joint arthroplasties after informed consent was obtained in accordance with the ASAN Medical Center Internal Review Board policy (Seoul, Korea). The cytotoxicity of hBMSCs in response to magnesium ions ranging from 1 to 10 mM was measured using flow cytometry with Annexin V and propidium iodide (PI). The proliferation of hBMSCs was measured using MTT assay in response to magnesium ions ranging from 1 to 10 mM. The expression of integrins subunits and osteogenic-related genes including alpha-2, alpha-3, beta-1, Runt-related transcription factor 2 (Runx2), alkaline phosphatase (ALP), and osteocalcin (OCN), in response to magnesium ions ranging from 1 to 10 mM was measured using RT-PCR. Knock-down experiment was conducted using gene-specific siRNA for integrin alpha-2 and/or alpha-3. The ALP activity and staining in response to magnesium ions ranging from 1 to 10 mM were conducted to evaluate osteogenic phenotype.

Results: Our findings demonstrated that high levels of magnesium ions (1-10 mM), more than normal levels in body fluid, did not induce cytotoxicity of hBMSCs. Next, proliferation of hBMSCs treated by magnesium was shown to have significantly increased at the 2.5 mM – 10 mM of ion content for 48-72 hours. To shed further light on the underlying mechanism associated, we further investigated the expression pattern of integrin subunits including alpha-2, alpha-3, and beta-1 in response to magnesium ions. The expression of integrin alpha-2 and alpha-3 were up regulated compared to the control and were shifted slightly from alpha-3 to alpha-2 in hBMSCs treated by magnesium ions, but not the beta-1 integrin subunits. Knock-down for integrin alpha-2 and/or alpha-3 significantly reduced magnesium-induced proliferation in hBMSCs. To identify whether magnesium ions promoted osteogenic differentiation, a battery of osteogenic-related genes including Runt-related transcription factor 2

(Runx2), alkaline phosphatase (ALP), and osteocalcin (OCN), and activity of ALP were measured. We found ALP gene expression and activities were profoundly enhanced in response to magnesium. In particular, ALP activities increased even when exposed to a relatively lower level (2.5 Mm) of magnesium ions.

Discussion and Conclusion: This findings indicate that the proliferation of hBMSCs is mediated by integrin alpha-2 and alpha-3 expression and the differentiation of hBMSCs is at least in part improved by enhanced ALP activity and expression exposed by magnesium ions. Collectively, exposure to magnesium facilitated proliferation rates via alpha-2 and alpha-3 expression, and partially promoted differentiation into osteoblast via the alteration of ALP expression and activity. Accordingly, magnesium can be utilized as a useful biomaterial for orthopedics.

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