SHORT COMMUNICATION

Simvastatin Protects Osteoblasts from the Deleterious Effects of the Liquid Milieu of Multiple Myeloma

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ABSTRACT

Lytic bone lesions are the main clinical manifestation of multiple myeloma. The intense variety in this cell microenvironment, composed mainly of fibroblasts, osteoblasts, osteoclasts, immune cells and mesenchymal cells, is influenced by the massive presence of neoplastic plasma cells. Studies with statins have reported their action in stimulating the formation and reducing bone resorption. The aim of this study was to verify the in vitro *response of human osteoblasts exposed to the supernatant (liquid milieu) of multiple myeloma. The data obtained indicate that simvastatin has positive effects on the growth of osteoblasts and protection against the anti-proliferative effects of multiple myeloma supernatant.*

Keywords*:* Multiple myeloma, osteoblast, simvastatin

La Simvastatina Protege los Osteoblastos de los Efectos Deletéreos del Medio Líquido del Mieloma Múltiple

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RESUMEN

Las lesiones líticas del hueso son la principal manifestación clínica del mieloma múltiple. La intensa variedad en este microambiente celular – compuesto principalmente por fibroblastos, osteoblastos, osteoclastos, células inmunes y células mesenquimales – es influenciada por la presencia masiva de células plasmáticas neoplásicas. Estudios realizados reportan que la acción de las estatinas estimula la formación y reducción de la resorción ósea. El objetivo de este estudio fue comprobar la respuesta in vitro *de osteoblastos humanos expuestos al sobrenadante (medio líquido) del mieloma múltiple. Los datos obtenidos indican que la simvastatina tiene efectos positivos sobre el crecimiento de los osteoblastos, y ofrece protección contra los efectos antiproliferativos del sobrenadante del mieloma múltiple.*

Palabras claves: Mieloma múltiple, osteoblastos, simvastatina

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INTRODUCTION

The accumulation of plasma cells more than 10% in bone marrow is one of the main features of multiple myeloma (1). The intense variety of cells in this microenvironment, composed mainly of fibroblasts, osteoblasts, osteoclasts, endothelial cells, immune cells and mesenchymal stem cells, is influenced by the massive presence of neoplastic plasma cells. In multiple myeloma, the liquid milieu, comprising cytokines, growth factors and chemokines, is able to induce autocrine and paracrine effects on proliferating status or of cells in the bone marrow microenvironment (2, 3).

Bone fractures are part of the pathology of multiple myeloma and are directly associated with deleterious effects on osteoblasts as Dickkopf-related protein 1 [DKK1] (4),

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which is a Wnt-signalling antagonist secreted by multiple myeloma cells. Interestingly, osteoblasts assist in multiple myeloma cell growth due to intense production of interleukin-6 [IL-6] (5).

Recently, our research showed the ability of simvastatin to differentiate amniotic fluid mesenchymal stem cells into osteoblasts (6). Here, we show the effect of supernatant of multiple myeloma and the protective effect of simvastatin on the osteoblast lineage.

MATERIALS AND METHODS

The multiple myeloma human cell line RPMI 8226 was cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS), 100 U/mL penicillin, streptomycin 100 µmL, 24 mM sodium bicarbonate ($NaHCO_3$) and maintained in moist carbon dioxide (CO_2) incubator (5%) to 37 ˚C.

The human fetal osteoblasts cell line (hFOB 1.19) was cultured in a mixture of F12 and Dulbecco's Modified Eagle's Medium with 10% FBS and penicillin 100 U/mL and kept in an oven at 34 ˚C.

Cytokines quantification

Quantification of cytokines and growth factors present in the supernatant of multiple myeloma were made through the method of enzyme-linked immunosorbent assay (ELISA) by ELISA reader (Lab Life MX PN 2001) in the range of 450/690 nm. Vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) were quantified using RayBio® kit as IL-6, and transforming growth factor-beta (TGF-β) were quantified using eBioscience® kit according to the methodology described by the manufacturer.

After a pool of ten confluent (2×10^6 cells) cell culture flasks, we obtained the following average results: VEGF = 247.3 pg/mL, IL-6 = 8033.3 pg/mL, TFG-β = 19.8118 pg/mL and basic FGF 9.8333 pg/mL (7).

The experiment was performed with different concentrations of the supernatant of multiple myeloma.

Cell viability

Cell viability was performed by MTT method; 4×10^4 cells were plated in triplicate. The groups were divided as control, then 5 µL, (S1), 15 µL (S2) or 30 µL (S3) of supernatant from multiple myeloma; 0.85 µM, 1.2 µM and 1.6 µM of simvastatin (Prati Donaduzzi, Toledo, Brazil). Simvastatin was activated by dissolving in 1.8 mL of ethanol, added to 19 mL of sodium hydroxide (NaOH; 0.1 M) and then incubated for 40 minutes at 40 ˚C to convert the lactone. To adjust the pH to 7.4, hydrochloric acid (HCl; 0.1 M) was added (8). Associations between the supernatant (S) and multiple myeloma simvastatin (Sim) were held in the proportions: 5 µL of S with 0.8 µM Sim (S1 + Sim1), 15 µL of S with 1.2 µM Sim (S2 + Sim2), 30 µL of S with 1.6 µM of Sim (S3 + Sim3), 0.8 µM of Sim with 30 µL of S (Sim1 + S3) and 5 µL of S with 1.6 µM of Sim (S1 + Sim3). The cells were incubated for 24, 48 and 72 consecutive hours. For quantification of viable cells, 200 µL of MTT reagent was added (0.5 mg/mL) and maintained in the greenhouse for four hours (37 ˚C) to form crystal formazam with dark blue colour. All content was removed from the wells and 200 µL of isopropyl alcohol was added with a reading of 595 nm and with a reference filter at 655 nm.

RESULTS AND DISCUSSION

Osteoblasts were exposed to multiple myeloma supernatant at different concentrations or treated with simvastatin, or with supernatant of multiple myeloma cells plus simvastatin.

The Figure shows the inhibitory effect of supernatant multiple myeloma, the stimulatory effect of simvastatin and the protective effect of the drug against the deleterious effects of the supernatant.

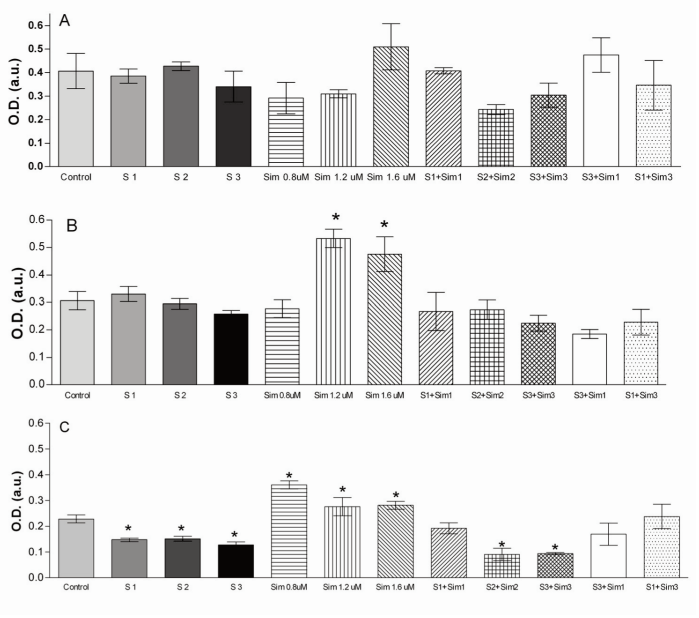

Figure: Simvastatin protects osteoblasts from the effects of liquid milieu of multiple myeloma (MM). Cells were cultured in the presence of supernatant of MM: 5 µL (S1), 15 µL (S2) or 30 µL (S3); or 0.85 µM, 1.2 µM and 1.6 µM of simvastatin (Sim); or 5 µL of S with 0.8 µM Sim (S1 + Sim1), 15 µL S with 1.2 µM Sim (S2 + Sim2), 30 µL of S with 1.6 µM of Sim (S3 + Sim3), 0.8 µM of Sim with 30 µL of S (Sim1 + S3), and 5 µL of S with 1.6 µM of (S1 + Sim3) for three days. Three wells of each condition were subjected to the MTT method. Data are expressed as mean and standard error of the mean. Statistical analysis was performed using analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison Test. *$p <$ 0.05 compared with the control group.

The lytic lesions in the bones are characteristic in more than 85% of patients suffering from multiple myeloma. The presence of plasma cells infiltrating the modified bone marrow varies between 10% and 60%, and bone lesions are proportional to the intensity of infiltration (9).

The adhesion of plasma cells with the bone marrow stroma induces the production of several factors, mainly IL-6, VEGF, TGF-β and IL-10. These factors have regulatory stimuli by autocrine and paracrine secretion (5).

The stimulation of osteoclast activity in this disease is well known and characterized by means of receptor activator of nuclear factor kappa-B ligand (RANKL) associated with macrophage inflammatory protein 1 alpha (MIP1α). At the same time, there is an inhibition of proliferation and differentiation of osteoblastic activity due primarily to the DKK1 protein (4).

Recently, our group demonstrated the induction of differentiation of stem cells into osteoblasts stimulated with simvastatin (6). Here, we have demonstrated the deleterious activity of the supernatant of multiple myeloma and protective activity of a strain of simvastatin on human osteoblasts. Together, these results point to the important effect of simvastatin on the therapeutic management of multiple myeloma.

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