

Cytotoxic effects of calcitriol on osteosarcoma cells

Osteosarkom hücrelerine kalsitriolün sitotoksik etkileri

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ABSTRACT

Introduction: It was aimed to examine the effect of calcitriol which has known anticancer activities, on the osteosarcoma cell line SAOS-2 cells [1,25(OH)2D₃].

Methods: SAOS-2 cells were grown in culture in conventional culture flasks in DMEM medium at 37°C and 5% CO₂. When the cells were 70-80% confluent, morphological changes were examined under an inverted microscope. The cells were passaged into 96 microplates, and after passage, different concentrations of calcitriol was applied to the cells (0.1; 0.5; 1; 5; 10; 25 nM/ml) was done. After administration, cytotoxic effect and proliferation rates/cell proliferation were analyzed by MTT method.

Results: The effect of calcitriol applied at different concentrations in cultured cells were 0.1, 1, 10, 25 nM/ml doses, in these groups proliferation was found to be statistically significantly reduced compared to the control group (p<0.05).

Conclusion: Our data imply that the antiproliferative effects of vitamin D applications can be benefited from in exosomal, combined and supportive treatments in osteosarcoma. Further trials are warranted to confirm and validate our findings.

Keywords: Cell culture techniques, cytotoxicity test, MTT formazan, calcitriol, osteosarcoma

ÖZ

Giriş: Çalışmada antikanser etkileri olduğu bilinen kalsitriolün osteosarkoma hücre hattı olan SAOS-2'ye etkilerinin değerlendirilmesi amaçlandı.

Yöntem ve Gereçler: SAOS-2 hücreleri klasik kültür flasklarında 37 C° ve %5 CO₂ varlığında DMEM besiyerinde kültüre alınarak büyütüldü. Hücreler %70-80 konfluent oldukları zaman inverted mikroskop altında morfolojik değişimler incelendi, hücreler 96'lık mikrolaklara pasajlandı ve pasaj sonrasında hücrelere farklı konsantrasyonlarda cinnamaldehyde ve methanandamid uygulaması (her iki ilaç için 0,675;1,25; 2,5; 5; 10; 20; 50; 100 nM/ml) yapıldı. Uygulama sonrası sitotoksik etki ve proliferasyon hızları/hücre proliferasyonu MTT yöntemi ile incelendi.

Bulgular: Farklı konsantrasyonlarda kültüre edilen kalsitriolün 0.1, 1, 10, 25 nM/ml dozlarının kontrol grubuna göre proliferasyonu istatistiksel olarak anlamlı ölçüde azalttığı tespit edildi.

Sonuç: Bulgularımıza göre osteosarkomun tedavisinde D vitamininin antiproliferatif etkisinden eksozomal, kombine ve destek tedavi olarak faydalanabileceğini düşünüyoruz. Bu konuda daha ileri çalışmaların yapılmasına ihtiyaç vardır.

Anahtar kelimeler: Hücre kültürü teknikleri, sitotoksikite testleri, MTT formazan, kalsitriol, osteosarkoma

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INTRODUCTION

Osteosarcoma, apart from leukemia and lymphoma, is the most common non-hematogenous primary malignant bone tumor in childhood and adolescence (1). Although the current therapies in high-grade tumors have improved prognosis, the long-term survival and outcomes of 30% of patients have not changed greatly over the past 20 years (2). The chemotherapy used in osteosarcoma protocols has remained unchanged for nearly 50 years with the use of high-dose methotrexate, doxorubicin, and cisplatin (3). The overall survival of patients with five-year metastatic osteosarcoma is less than 20% (4). Numerous new drug candidates to increase therapeutic efficacy have yet to identify more effective or less toxic regimens, although intensifying therapy or modulating the immune response (5). Therefore, there is an urgent need for new therapeutic approaches.

Calcitriol, the most biologically active hormonal form of vitamin D, [1,25(OH)₂D₃] is synthesized from 25(OH)D₃ in the kidneys by the cytochrome P450 enzyme CYP27B1 (6). Calcitriol is protective against bone metabolism diseases by regulating serum calcium and phosphate levels, which are essential for bone mineralization (7). In *in vitro* and *in vivo* animal models, it is known that calcitriol can limit or prevent cancer progression with anti-proliferative, pro-differentiating, and pro-apoptotic effects in cancer cells (6). Antiproliferative and anticancer effects have been demonstrated in prostate cancer (8), oral cancer (9), malignant pleural mesothelioma cells (10), breast cancer (11), and gastric cancer (12,13).

In our study, we aimed to evaluate the effects of calcitriol on the SAOS-2 osteosarcoma cell line to support surgical and medical treatments.

MATERIAL AND METHODS

Drugs, Reagents and Administration

Our study was performed under *in vitro* conditions cell culture laboratory in Bolu Abant İzzet Baysal

University Faculty of Medicine, Department of Physiology. Ethics committee approval is not required for cell culture studies.

In this study, a SAOS-2 human osteosarcoma cell line was used. The cell line was bought from ATCC (Manassas, VA, USA). Following, the cells proliferated from this colony in cell culture. The cell culture procedure was carried out as stated in earlier publications published (17-19). In a nutshell, cells were immediately defrosted in a water bath at 37°C and centrifuged for 4 minutes at 3000 rpm. All cells were then cultured in Dulbecco's Modified Eagle Medium (DMEM)/F-12 medium (Sigma-Aldrich, St. Louis, MO, USA) after centrifugation, which was supplemented with 10% fetal bovine serum (FBS) and a working dose of 100 IU/ml penicillin and 100 g/ml streptomycin (Sigma Aldrich, St. Louis, MO, USA). The growing media was replaced the next day to remove any lingering DMSO that might have been present in the freezing medium. At 37 degrees Celsius and 5% CO₂, cells were kept in monolayers on cell culture plates in a humid environment. When the cells reached 70-80% confluency, they were passaged via detaching with 0.25% Trypsin-EDTA (Invitrogen, Carlsbad, CA, USA).

After reaching the appropriate confluence, the cells were passaged. The concentrations of calcitriol (Monovit D₃, 50,000 IU/15 ml oral drops, Koçak Farma, Turkey) prepared to be 0.1; 0.5; 1; 5; 10; 25 nM/ml were given to the cells in equal volumes of µl and incubated for the defined times used. For each dose, 2 wells were inoculated and the cells were incubated at 37°C in a 5% CO₂ air mixture in a humid environment.

Cell viability assay

Trypan blue was used to determine cell viability and cell quantification. An automated cell counter (Bio-Rad TC20, Bio-Rad Laboratories, ABD) was used to measure cell viability and cell numbers. After cell counting and in 96-well plates, approximately 1.5x10⁴ cells/well were seeded in a total volume of 200 µL. Plates were then incubated at 37 °C for 24 hours for cell attachment.

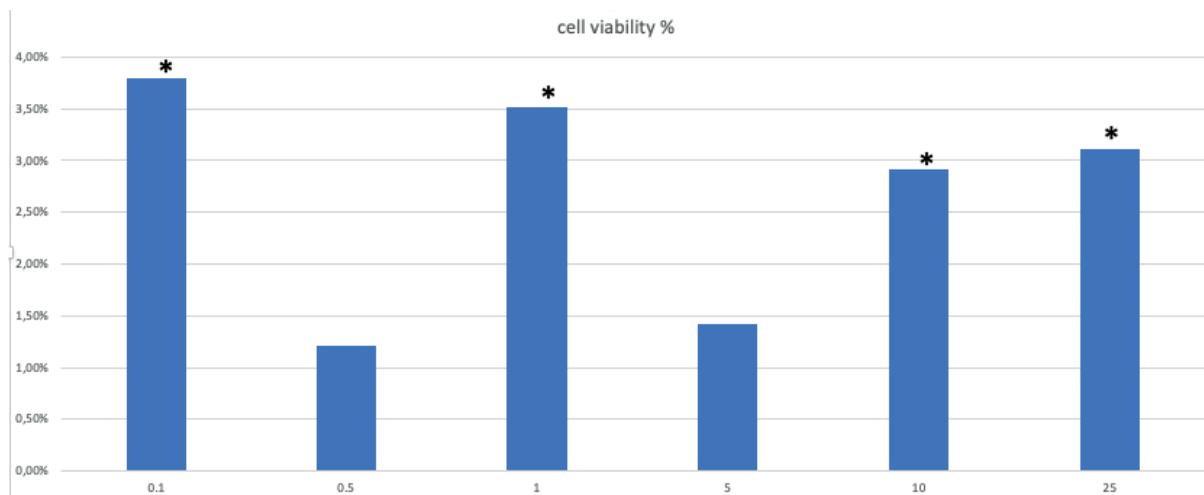


Figure 1. Effect of different doses of calcitriol on SAOS-2 cell line.

When the cell line was ready for 70-80% confluence in flasks, they were seeded into 96-well plates/plates and different dilutions of calcitriol (0.1; 0.5; 1; 5; 10; 25 nM/ml) for 48 hours. Cells incubated in 10% FBS were used as positive control and the viability of the cells was evaluated by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl- tetrazolium bromide (MTT) (Serva, Germany) method. The MTT test is a colorimetric method used to determine cell viability. It is based on the ability of NADPH-dependent cellular oxidoreductase enzymes to convert the tetrazolium dye MTT into insoluble formazan, which is purple. Briefly, 10 μ l of MTT reagent was added to each well and the 96-well plate was incubated at 37°C for 4 hours, then DMSO was added to the cells. Absorbance values/Change in Color were read at 570 nanometers with a colorimetric reader (spectrophotometer) (Epoch BioTek Instruments, Inc., Highland Park). The most appropriate proliferative and inhibitory doses of calcitriol were determined according to the cells. Thus, the effect of calcitriol on the viability of cultured cells and the duration of the effective dose were determined.

Statistical Analysis

One-way ANOVA with Proc GLM in SAS was used to evaluate the variations in cell counts (version 9.2, SAS Institute, Cary, NC, USA). The Shapiro-Wilk test of normality and box-plot graphs were used to assess the normal distribution of the

numerical data. Dunnett's multiple comparison tests were used when there was a substantial difference. Statistics were judged significant at $p < 0.05$. The mean and standard error of the mean were used to present all data (SEM).

RESULTS

The ID50 (Inhibition dose 50%) dose was determined for calcitriol by recording the total cell counts. Six different doses, 0.1; 0.5; 1; 5; 10; 25 nM/ml, were administered to see the effects of calcitriol on SAOS-2. According to the results, calcitriol showed antiproliferative effects on the SAOS-2 cell line at a dose of 0.1, 1, 10, and 25 nM (Figure 1).

DISCUSSION

Known as a fat-soluble vitamin, vitamin D is a potent precursor of steroid hormones that regulates a wide range of physiological processes (14). In recent years, in addition to its known roles in bone metabolism, preclinical, cellular, and clinical research, it has been demonstrated that vitamin D plays a key role in the prevention and treatment of many skeletal/non-skeletal diseases such as cancer (15). This study illuminates the role of calcitriol, the active form of vitamin D₃, on a well-known pediatric malignancy, osteosarcoma for its antiproliferative properties. Several studies documented antiproliferative role along with

some other anti-cancer properties of calcitriol in some adulthood and pediatric cancers in the past: lung (16), breast (17), prostate (18), and acute lymphoblastic leukemia (19). Various anticancer features of vitamin D₃ have been demonstrated with various effects on cancer development and progression (20). Antiproliferation (21), induction of apoptosis (22), metastasis, invasion (23), and inhibition of angiogenesis in cancer cells are some of the anticancer properties of vitamin D₃ (24). This study has focused on the antiproliferative effect of this substance on human osteosarcoma cell lines SAOS-2. According to the results of our study, calcitriol at a dose of 0.1, 1, 10 and 25 nM showed cytotoxic and antiproliferative effects on SAOS-2 osteosarcoma cells.

In summary, this study has suggested that calcitriol can be used to inhibit proliferative the feature of osteosarcoma cells, SAOS-2. Because of the constancy of historically low survival rates of osteosarcoma (25), working in experimental and clinical research evaluated by molecular techniques may make it easier to identify new treatments. Understanding vitamin D metabolism and its function in cancer, successful vitamin D-based therapies may allow the development of promising new strategies for cancer.

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