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Arsenic trioxide exerts a double effect on osteoblast growth in vitro

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ABSTRACT

Arsenic trioxide (ATO) is a promising antitumor agent used to treat acute promyelocytic leukemia (APL) and, recently solid tumor. The present study was designed to evaluate the effect of ATO proliferation of osteoblast that plays very important roles in maintaining the structure integrity and function of bone.

Cell survives, apoptosis, collagen, and molecular targets were identified by multiple detecting techniques, including MTT assay, electron microscopy, collagen detecting kit, TUNEL kit, and western blot in hFOB1.19 human osteoblasts cell line. The results showed that low dose of ATO (0.25, 0.5, and 1 μM) remarkably enhanced the viability of cultured osteoblasts in a concentration- and time-dependent manner. Intriguingly, a dual effect of high dose of ATO (5, 10, and 20 μM) was also observed showing significant reduction in viability of culture osteoblasts at concentration- and time-dependent fashion. Moreover, low dose of ATO promoted secretion and synthesis of collagen, whereas high dose of ATO induced typical morphological characteristics of apoptosis in osteoblasts. Mechanically, western blot results demonstrated that low dose of ATO dramatically up-regulated TGF-β1 protein and activated p-AKT proliferative signaling. And, high dose of ATO increased Bax/Bcl-2 ratio in a time-dependent fashion and activated caspase-3 apoptotic signaling. These results demonstrate at the first time that ATO exerts a double effect on osteoblast function depending upon the concentration and provide a clue to rationally use ATO for clinicians to pay more attention to protect bone from the adverse effects of therapeutic dose of ATO during tumor therapy.

1. Introduction

Arsenic trioxide (As_2O_3; ATO) is very effective in clinical management of patients with acute promyelocytic leukemia (APL) and some solid tumors (Sanz and Lo-Coco, 2011; Zhou, 2012). Recently, a multicenter, open-label, phase I/II dose escalation study also revealed the safety and efficacy of ATO/bortezomib/ascorbic acid combination therapy for the treatment of relapsed or refractory multiple myeloma

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(Berenson et al., 2007; Held et al., 2013). However, excessive exposure to arsenic has also been associated with increased incidence of cancers, bone necrosis, and cardiovascular toxicity in humans (Chu et al., 2012; Ferreccio et al., 2013; Li et al., 2013; Shi, 2008; Yavuz et al., 2008).

Bone is a complex tissue composed of several cell types including osteoblasts, osteoclasts, osteocytes, and lining cells. Bone is continuously undergoing a process of renewal and repair termed "bone remodeling" (Brunner et al., 2013). The osteoblasts are cells with single nuclei in the bone tissue, which synthesize bone collagen and contribute to form new bone. Hu et al. found that, in primary osteoblasts, ATO produces oxidative stress and causes DNA tailing and consequently affects bone remodeling by effects on osteoblast differentiation and function. Moreover, but does not induce apoptosis (Hu et al., 2012, 2013). In contrast, Tang et al. revealed that ATO induces cell apoptosis in cultured osteoblasts through endoplasmic reticulum stress, suggesting that excessive arsenic exposure may alter bone formation by inducing apoptosis of osteoblasts, resulting in bone-related diseases, such as osteoporosis (Tang et al., 2009). Obviously, previous studies regarding the effects of ATO on osteoblasts have conflicted and its molecular mechanism has not been fully elucidated. Therefore, in present study we re-evaluate the effect of ATO on growth and apoptosis in hFOB1.19 osteoblast cell line in vitro and provide an evidence and theory of protecting bone from the adverse effects while treating cancer with ATO.

2. Materials and methods

2.1. Reagents

ATO was a gift from Harbin YI-DA Pharmaceutical Company (Harbin, China). Caspase-3 assay kit was purchased from Promega (Madison, WI, USA). Other reagents were purchased from Sigma (St. Louis, MO, USA).

2.2. Cell culture

Human osteoblast cell line (hFOB1.19) was purchased from a cell collection of the Chinese Academy of Science (Shanghai, China). The hFOB1.19 cells were cultured in complete culture medium containing 0.3 mg/mL of G418, 90% F12 medium and 10% fetal bovine serum at 34 °C with 5% CO_2. The non-adherent cells were removed with medium changes and the adherent cells were kept in the culture dish for further growth. The medium was changed every 3 days.

2.3. Cell viability assay

Cells were seeded in 96-well plate with the same starting cell number per well (2.5×10^4 cells/well) and allowed attaching for 24 h. After treatment, the media in each well were replaced with PBS solution containing 5 mg/mL MTT (Sigma–Aldrich, St. Louis, MO) and then the plate was further incubated at 37 °C for 3 h. All the remaining supernatant was then removed and 100 mL of DMSO was added to each well and mixed thoroughly to dissolve the formed crystal formazan. After 10 min of incubation to ensure all crystal formazan were dissolved, the cell viability was detected by measuring the absorbance of each well at 570 nm. Relative cell viability was calculated by the absorbance percentage of the ATO-treated group to the control group.

2.4. TUNEL assay and DAPI staining

Apoptotic cells were detected in situ by a Cell Death Detection Kit and POD analysis, which was performed with a commercially available kit for immunohistochemical detection and quantification of apoptosis (programmed cell death) at single cell level, based on labeling of DNA strand breaks according to the manufacturer's instructions (Roche Molecular Biochemicals). Cultured mice cardiac fibroblasts were prepared on cover slips in six-well plates. Briefly, cells were fixed in 4% paraformaldehyde and pretreatment of ethanol, proteinkinase K, Triton X-100, and pepsin. After washing with PBS for three times, the samples were then treated with the TUNEL reaction mixture terminal deoxynucleotidyl transferase (TdT) and TUNEL dilution buffer at the ratio of 1:9, and incubated in a humidified chamber at 37 °C for 1 h. After washing with PBS, anti-digoxigenin peroxidase conjugate was added, and incubation continued in a humidified chamber for another 30 min at room temperature (22–23 °C). The samples were washed with PBS, stained nuclei with DAPI (Roche Molecular Biochemicals) at room temperature for 5 min, and observed by confocal microscope (Olympus, FV-100). Further, the ratio of apoptotic (TUNEL-positive) cells to total (DAPI-stained nuclei) was calculated ($n = 3$). Measurements were performed using Scion Image software (Beta 4.03; Scion Corporation, MD, Frederick). All the measurements were performed in a double-blind manner by two independent researchers.

2.5. Electron microscopy

The samples were prepared by routine methods for transmission electron microscopy (TEM) analysis as previously described in detail in our previous studies (Li et al., 2013). Briefly, samples were fixed in 2.5% glutaraldehyde (pH 7.4) at 4 °C overnight. The fixed samples were washed with distilled water, dehydrated through graded acetone, and embedded in Epon medium. Ultrathin sections (60–70 nm) were stained with uranyl acetate and lead citrate, and then analyzed under JEOL 1200 electron microscope (JEOL Co., Japan).

2.6. Measurement of collagen content

Total collagen content was determined by using a colorimetric reaction with picrosirius red, as described previously (Chu et al., 2011, 2012). Briefly, cells (2×10^5) after various treatments (at 85% confluence) were lysed, and the lysate (100 mL) was dehydrated and stained with picrosirius red in saturated picric acid (0.1% w/v) in a 96-well plate. The dye was solubilized, and absorbance read at 540 nm. Readings were converted to protein units using a linear calibration curve generated from standards (Vitrogen 100; Angiotech Biomaterials, Palo Alto, CA, USA) and normalized to the wet weight of each cell sample (1.2–1.5 mg).