Effect of arsenic trioxide on proliferation and apoptosis of U266 cells and its relationship with the expression variation of VEGF

Rong ZHAN (✉), BM, Qinghong YU, MM, Haobo HUANG, MM
Fujian Institute of Hematology, Department of Hematology, The Affiliated Union Hospital, Fujian Medical University, Fuzhou 350001, China

Abstract The aim of this article is to explore the effect of arsenic trioxide (As₂O₃) on the proliferation and apoptosis of myeloma cell line U266 and its relationship with the expression variation of vascular endothelial growth factor (VEGF). The viability and apoptosis of U266 cells were observed by methylthiazolyl-tetrazolium (MTT) assay and terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL). The effect of As₂O₃ on the VEGF expression of U266 cells were tested by enzyme linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) analysis. We found that As₂O₃ could significantly inhibit the growth of U266 cells, and the concentration for 50% growth inhibition (IC₅₀) was 2 µmol/L. After treatment with 2, 5, 10 µmol/L As₂O₃ for 36 hours, dose-dependent apoptosis of U266 cells was observed. After treatment with 2, 5, 10 µmol/L As₂O₃ for 72 hours, a dose-dependent reduction of VEGF in the supernatant of U266 cells culture was found. As far as single cells are concerned, nevertheless, the expression of VEGF mRNA did not vary. So we draw the conclusion that As₂O₃ could induce the apoptosis of U266 cells and inhibit their proliferation, decrease the tumor load, and lead to the reduction of VEGF in the culture supernatant, but not change the expression of VEGF in single U266 cells.

Keywords arsenic trioxide; myeloma; proliferation; apoptosis; vascular endothelial growth factor

1 Introduction

Multiple myeloma (MM) is a plasma cell tumor characterized by the malignant proliferation of monoclonal plasma cells and the secretion of monoclonal immunoglobulin. It mainly involves the bone marrow and also invades extramedullary tissues, leading to lytic bone disease, frequent infections, anemia, hypercalcemia, hyperviscosity syndrome and renal insufficiency. To date, MM is still an incurable disease. In recent years, clinical researches have demonstrated that arsenic trioxide (As₂O₃) has an effect in the therapy of relapsing and refractory MM [1]. Basic researches have also demonstrated that vascular endothelial growth factor (VEGF) plays an important role in the pathogenesis of MM and the generation of its clinical characteristics through various pathways and various mechanisms [2]. In the present study, we use myeloma cell line U266 as the in vitro model to explore the effect of As₂O₃ on the proliferation and apoptosis of myeloma cell line U266 and its relationship with the expression variation of VEGF.

2 Materials and methods

2.1 Materials

The cell line and cell culture Human multiple myeloma cell line U266 was a kind gift from professor Hou Jian (Department of Hematology, Shanghai Changzheng Hospital). It was maintained in RPMI1640 supplemented with 10% heat-inactivated fetal bovine serum in a CO₂ incubator (95%O₂ and 5%CO₂). Cells in logarithmic growth phase were collected for experiments.

2.2 Drugs and reagents

As₂O₃ was purchased from Yida Company (Heilongjiang, China). Terminal-deoxynucleotidyl transferase mediated nick end labeling (TUNEL) and reverse transcription-polymerase chain reaction (RT-PCR) kits were purchased from Promega Company (USA). Trizol was purchased from Invitrogen Company (USA). Human VEGF enzyme-linked immunosorbent assay (ELISA) KIT was purchased...
from Pierce Company (USA). RPMI 1640 was purchased from Gibco Company (USA). Fetal bovine serum was purchased from Sijiqing Company (Hangzhou, China).

2.3 MTT assay

The inhibitory effect of As$_2$O$_3$ on the growth of U266 cells was determined by the MTT dye uptake method. Briefly, U266 cells were treated with various concentrations (0, 2, 5, 10 μmol/L) of As$_2$O$_3$ for 48 hours in 96-well plates. U266 cells were treated with As$_2$O$_3$ at the concentration which caused 50% inhibition of cell proliferation for 0, 24, 48, 72 hours in 96-well plates. Each group contained six wells. The optical density (OD) at 492 nm was read by using a 96-well multiscanner autoreader. The following formula was used for calculations: cell proliferation rate (%) = (OD of the experimental samples/OD of the control) × 100% (n = 6; $\bar{x} \pm s$).

2.4 TUNEL analysis

U266 cells treated with various concentrations (0, 2, 5, 10 μmol/L) of As$_2$O$_3$ for 36 hours were collected and smeared on slides. The slides were affixed with paraformaldehyde (4 g/L), then processed and stained according to the manufacturer’s instruction. The slides were observed under a microscope with high power lens. Apoptotic cells were dark brown after DAB coloration while the background was light brown.

2.5 RT-PCR analysis

Cells treated with 2 μmol/L As$_2$O$_3$ at various time points were collected. Total RNA was extracted using TRIZOL (Invitrogen, USA). First strand cDNA was synthesized according to the manufacturer’s instruction. VEGF and β-actin were amplified using the standard protocol with two pairs of primers: VEGF (Forward: 5’-gaagtggtgaagttcatggatgtc-3’, reverse5’-cgatcgttctgtatcagtctttcc-3’) for the VEGF gene, β-actin (Forward: 5’-cgetgcgttcctgctgaca-3’, reverse5’-gtaacgcaagttccgct-3’) for the β-actin gene. All reactions were carried out on the sequences of VEGF and β-actin with the following PCR conditions: initial denaturation at 99°C for 5 min, 32 cycles of 94°C for 30 s, 64°C for 60 s and 72°C for 1 min for amplification, followed by a final extension for 10 min at 72°C. Finally, equal volumes of RT-PCR products were electrophoresed on 2% agarose gel, imaged and analyzed with GEL system.

2.6 ELISA

The VEGF level in the culture supernatant of the U266 cells was measured by ELISA according to the manufacturer’s instruction. These standard and unknown samples were duplicated. The optical density at 450 nm was read using a 96-well multiscanner autoreader.

2.7 Statistical analysis

SPSS software package (version 11.5) was used for statistical analysis. For univariate analysis, $t$-test was employed in two-group data, then one-factor analysis of variance was employed in multiple group data. Differences were considered significant at $P < 0.05$.

3 Results

3.1 Effect of As$_2$O$_3$ on the proliferation of U266 cells

We investigated the effects of As$_2$O$_3$ at various concentrations (0, 1, 2, 5, 10 μmol/L) and time points (24, 48, 72 hours) on the proliferation of U266 cells. We found that fifty percent growth inhibition (IC50) in U266 cells at the 48th hour was at 2 μmol/L (Fig. 1). We found that As$_2$O$_3$ inhibited the growth of U266 cells in a dose-dependent (Fig. 1) as well as time-dependent (Fig. 2) manner.

![Fig. 1 Effects of various concentrations of As$_2$O$_3$ on the viability of U266 cells.](image1)

![Fig. 2 Effects of 2 μmol/L As$_2$O$_3$ at various time points on the viability of U266 cells.](image2)

3.2 Effect of As$_2$O$_3$ on the apoptosis of U266 cells

After treatment with various concentrations (0, 2, 5, 10 μmol/L) of As$_2$O$_3$ for 36 hours, apoptotic cells were seen in the treatment groups but not in the control group.