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The optimal dose of arsenic trioxide induced opposite efficacy in autophagy between K562 cells and their initiating cells to eradicate human myelogenous leukemia

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ABSTRACT

Ethnopharmacological relevance: Arsenic trioxide (As2O3), a main component of arsenolite which is a common traditional Chinese medicine (TCM) wildly used as a therapeutic agent for more than 2400 years in china, has been accepted as a standard treatment for the patients with acute promyelocytic leukemia (APL) based on the principle in TCM of "using a poison to fight against other poisons or malignancy illnesses". However, it remains unknown that which mechanism is actually responsible for the therapeutic effects against these blood malignancies.

Aim of the study: The purpose of this study was to explore the actual mechanism that ATO exerts its effects in K562 cells and their initiating cells (K562s).

Materials and methods: K562s cells were separated and enriched for CD34+/CD38- cells using magnetic microbeads. Cell proliferation was determined by incorporation of BrdU. Cell apoptosis was evaluated by Annexin-V binding and PI uptake. Autophagy was estimated by acridine orange and immunofluorescence staining of LC3-B and p62. MC colonic formation was used to examine cell self-renew. ROS generation inside living cells was measured by DCFH-DA. Cell differentiation was assessed by the benzidine staining. The SA-β-gal assay was used to detect cell senescence. Protein expression was examined by western blotting and immunohistochemical staining.

Results: K562s cells were stronger in self-renew and resistance to ATO cytotoxicity and starvation-induced apoptosis than K562 cells. Unexpectedly, we found that ATO at a dose of 0.5 μM which had no effect on cell proliferation resulted in maximum suppression on self-renew in both cells and maximum starvation-induced apoptosis in K562s cells but minimum starvation-induced apoptosis in K562 cells. Next, we found that ATO no more than 0.5 μM selectively induced K562s cell differentiation indicated by benzidine staining, γ-globin and CD235a expression. More importantly, we found that ATO no more than 0.5 μM led to opposite efficacy in autophagy between K562 and K562s cells, and the opposite autophagy could induced late-phase senescence in both cells. Finally, we used the optimal dose of ATO to eradicate leukemia cells and obtained a satisfied therapeutic outcomes in vivo.

Conclusions: Our results suggest that the used dose of ATO may determine the fate of cell differentiation senescence or malignant transformation, and the optimal dose of ATO induced opposite efficacy in autophagy between K562 cells and their initiating cells and ultimately leads both cells to late-phase senescence.

1. Introduction

Arsenic has long been known to act as a carcinogen, paradoxically, arsenic has also long been demonstrated to have anticancer activity in some cases. In traditional Chinese medicine, arsenic trioxide (As2O3) named Pishuang as a main component of arsenolite which is a common traditional Chinese medicine (TCM) and has played a significant therapeutic role in various diseases for over 2400 years in china

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(Liang et al., 2012), was recorded in the Compendium of Materia Medica by Mr Li Shi-Zhen (1518–1593). In Western medicine, arsenous oxide (Fowler's solution) was used as a treatment of choice for chronic myeloid leukemia (CML) in the 19th century (Zhang et al., 2001). Arsenic trioxide (ATO) has been accepted as a standard treatment for the patients with acute promyelocytic leukemia (APL) based on the therapeutic principle of traditional Chinese medicine (TCM) of "using a poison to fight against other poisons or malignancy illnesses" in China (Wu et al., 2011), United States multicenter study of arsenic trioxide in relapsed acute promyelocytic leukemia subsequently established ATO as a highly effective therapy for patients with relapsed APL (Soignet et al.,2001), and the therapeutic range has been extended to chronic myelocytic leukemia (CML), malignant lymphoma (mL), myelocytic dysfunction syndrome (MDS) and multiple myloma (MM) (Hua et al., 2011). The pharmacological effects of ATO are mainly through modulation of the posttranslational modification and proteasomal degradation of key proteins involved in the pathogenesis of these diseases (Wang et al., 2015). However, it remains unknown that which mechanism is actually responsible for the therapeutic effects against these blood malignancies because ATO is also a major environmental toxin and exposure to ATO in humans may have proinflammatory and carcinogenic effects in multiple organs including the hematopoietic system (Huff et al., 2000). Understanding the detail mechanism that ATO exert its effects may provide a more efficient therapeutic option.

K562 cells is a human chronic myelogenous leukemia (CML) cell line that is established from a patient in a blast crisis of chronic myeloid leukemia and possesses variable capacities of differentiation toward erythroid and megakaryocytic cell lineages (Randrianarison-Huetz et al., 2010). CML is clinically characterized by three phases: an initial chronic phase displaying almost normal myeloid differentiation, followed by an accelerated phase and then the final blast crisis, in which myeloid and lymphoid blasts failed to differentiate and led to abnormal accumulation of immature leukemic blast cells in blood and bone marrow (Roche-Lestienne et al., 2002). CML is also a clonal hematopoietic stem cell disorder that the malignant clone progressively loses the capacity for terminal differentiation. Some valuable progress has been achieved in biological or chemical agents that could induce terminal differentiation for CML therapy (Shiotsu et al., 2000). Autophagy is a conserved protein degradation pathway with important roles in mammalian cell differentiation, particularly within the hematopoietic system (Watson et al., 2011). Previous studies have shown that all-trans retinoic acid (ATRA)-induced autophagy enhanced the differentiation of myeloid cells (Yang et al., 2015). Recently, Isakson et al. reported that autophagy is essential for differentiation therapy of CML patients and that autophagy could be activated by chemotherapeutic drugs in leukemia cell lines (Isakson et al., 2010). These data support the development of strategies to stimulate autophagy as a novel approach to improve clinical outcomes for CML treatment. However, the autophagy has recently been explored for providing putative survival signals to Leukemia initiating cells (LICs), implying that stimulating autophagy is in potential danger for LIC formation. Since LICs may also require niche cells for their maintenance just as normal stem cells require niche cells for survival (Chiarini et al., 2012), we hypothesized that the autophagic ability between differentiated cancer cells and its initiating cells may determine the fate of cell differentiation senescence or malignant transformation. Herein, we examined whether the ATO efficacy on differentiation, proliferation, apoptosis, autophagy, self-renew and senescence was associated with used dose in K562 cells and their initiating cells (K562s). Our studies demonstrated that 1) K562s cells were stronger in self-renew and resistance to ATO cytotoxicity and starvation-induced apoptosis than K562 cells, and 2) that the optimal dose of ATO induced opposite efficacy in autophagy between K562 cells and their initiating cells and ultimately leads both cells to late-phase senescence.

2. Materials and methods

2.1. Reagents

ATO, Yi-Tai-Da injection (Arsenious acid and sodium chloride injection), a commercialized product and supplied by Yida Pharmaceutical Co., Ltd., Harbin, China; Spec: 10 mL containing 10 mg As2O3 and 90 mg sodium chloride; Authorized document number: Country medicine accurate character H19990191; Batch number: 20141102; Executive standard: WS1-(X-039)–2003Z. Basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), 5-bromo-2V-deoxyuridine (BrdU), methylcellulose (MC), benzidine, 2-[N-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]–2-deoxyglucose (2-NBDG), insulin and H2O2 were purchased from Sigma Chemical Co. RPMI1640 was obtained from Cellgro (Mediatech, USA). Fetal bovine serum (FBS) was obtained from Hangzhou sijiqing biological engineering materials co. (China). The antibodies used include: Nanog, Oct4, BCR-ABL, p16INK4a, LC3-B, p62, γ-globin and CD235a were from BD Pharmingen. Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG polyclonal antibody and 3-amino-9-ethylcarbazole (AEC) were from Nichirei Bioscience (Tokyo, Japan). Rapamycin (an autophagic enhancer), chloroquine (an autophagic inhibitor), sodium azide , TritonX-100 , acridine orange (AO), ethidium bromide (EB), Annexin-V and prodium iodide (PI) were obtained from Solarbio (China). 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA) was obtained from Wako Ltd (Osaka, Japan). Senescence-associated-β-galactosidase (SA-β-gal) staining solution was obtained from Chemicon (USA). Nucleoprotein extracted kit and Strept avidin-biotin complex (SABC) were purchased from Boster (China).

2.2. Cell culture and isolation

K562 cells were grown in RPMI1640 medium supplemented with 10% (v/v) fetal bovine serum and 1% penicillin/streptomycin at 37 °C with a humidified atmosphere of 5% CO_2 and 95% air. K562s cellswere separated and enriched for CD34+/CD38- cells using magnetic microbeads (Miltenyi Biotec, Auburn, CA, USA). Cells were analyzed on a Magnetic Sorter unit, and the purity and viability of isolated cells were routinely greater than 95%.

2.3. Cell proliferation assay

The cell proliferation was determined by incorporation of BrdU according to previously described method. Briefly, K562 or K562s cells were seeded at a concentration of 1×10^4/well in a 96-well plate and were incubated at 37 °C for 48 h in medium or in medium supplemented with various concentration of ATO (ATO injection was diluted to working concentrations with complete medium before use) or an equal volume of normal saline control; each condition was tested in six replicates. Then, BrdU labeling solution (10 mL/well) was added to the culture, the cells were further incubated for 18 h. The plate was centrifuged and labeling solution was removed. The cells were fixed for 30 min and dried for 1 h, followed by addition of peroxidase-conjugated anti-BrdU monoclonal antibody and incubation for 90 min at room temperature. The bound peroxidase was detected by subsequent substrate reaction and the absorbance was measured on a microplate reader (Elx-800; Bio-Tek, USA) at 450 nm. The cell proliferation are presented as BrdU incorporation.

2.4. Cell apoptosis and autophagy detection

Cell apoptosis was evaluated by Annexin-V binding and PI uptake using an Annexin V-FITC/PI kit. Briefly, ATO treated or untreated K562/K562s cells for 72 h was centrifuged and resuspended in Annexin V binding buffer. The cells were stained with Annexin V-FITC for 15 min, washed, and then stained with PI. The samples were