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Research paper

Effects of arsenic disulfide on apoptosis, histone acetylation, toll like receptor 2 activation, and erythropoiesis in bone marrow mononuclear cells of myelodysplastic syndromes patients in vitro

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ABSTRACT

Objective: As the main component of traditional Chinese medicine realgar, arsenic disulfide (As_2S_2) is widely used in treating myelodysplastic syndromes (MDS). The goal of the current study is to assess the effects of As_2S_2 on bone marrow mononuclear cells (BMMNC) of MDS.

Methods: BMMNCs were obtained from 10 lower risk MDS patients, 5 higher risk MDS patients, and 3 healthy controls. Then, the cells were treated with As_2S_2 for 48 h, using vorinostat (also known as SAHA) as control. Cell proliferation and apoptosis were detected. mRNA and protein levels of histone deacetylase-1 (HDAC1), Toll-like receptor 2 (TLR2), and erythroid transcription factor (GATA-1) were detected by quantitative real-time PCR and western blot analysis.

Results: After As_2S_2 treatment in concentrations ranging from 3.125 to 100 μmol/L, cell proliferation was inhibited in both lower risk and higher risk MDS. Fifty percent inhibitory concentrations were 24.4 μmol/L and 23.6 μmol/L, respectively, for lower and higher risk MDS. Apoptotic cells significantly increased in both types of MDS. mRNA and protein levels of HDAC1 and TLR2 were reduced, whereas GATA-1 was increased in both types of MDS.

Conclusions: As_2S_2 could inhibit cell proliferation and induce apoptosis through histone acetylation modulation in MDS. Similar to SAHA, As_2S_2 could reduce TLR2 activation and increase GATA-1 expression. Current data suggest epigenetic and immunological alternations are involved in therapeutic mechanisms of realgar in the treatment of MDS.

1. Introduction

Myelodysplastic syndromes (MDS) are a group of hematologic malignancies characterized by clonal bone marrow stem cell disorder such as ineffective hematopoiesis, which customarily might lead to pancytopenia and infection in many cases [1]. Unfortunately, the pathogenesis of MDS remains unclear. Studies focusing on the pathogenesis of MDS have transformed into epigenetic modification and microenvironment from molecular genetics and immune-phenotype. Hopefully, some achievements have been made in its clinical treatments in recent years, including hematopoietic stem cells and epigenetic drugs such as decitabine [2].

It was reported that increased Toll-like receptor 2 (TLR2) in the MDS microenvironment might be connected to the genesis of aberrant hematopoietic stem cells [3]. Furthermore, an MDS-associated mutation of TLR2 (TLR2-F217S) has been observed, and short hairpin RNA (shRNA) knockdown of TLR2 in MDS $CD34^+$ cells led to a dramatic increase in erythroid progenitors in colony formation assays [4]. The

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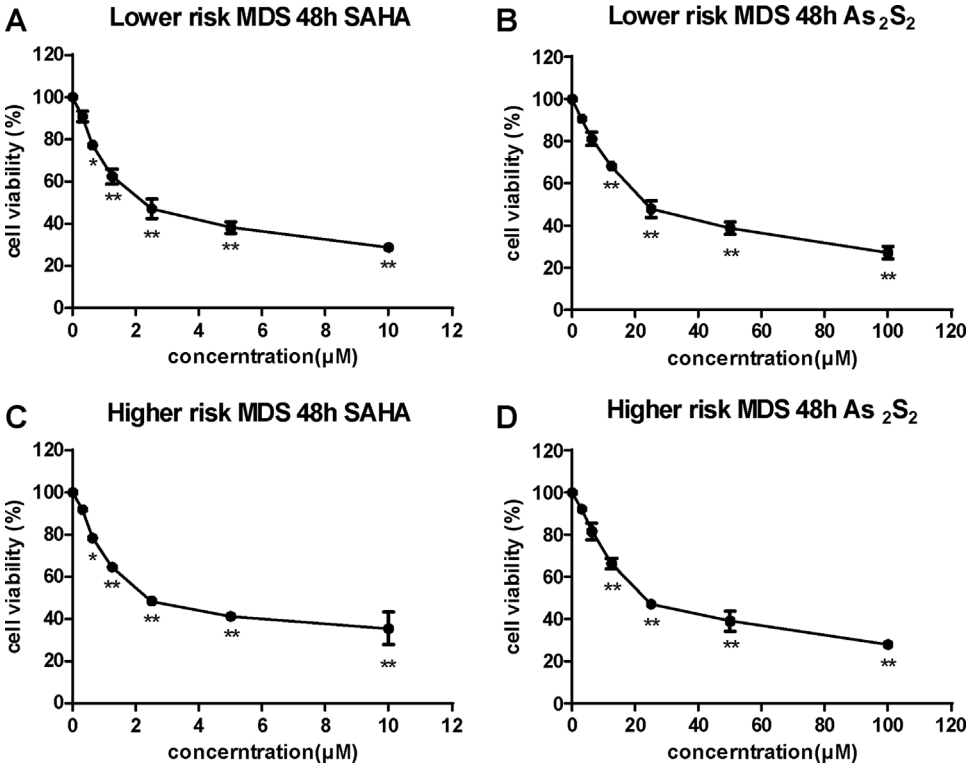

Fig. 1. Effects of As_2S_2 on viability of lower risk and higher risk MDS BMMNCs (n = 3). Notes: Lower risk MDS BMMNCs were treated with SAHA at different concentrations ranged from 0.3125 μmol/L to 10 μmol/L for 48 h (A), lower risk MDS BMMNCs were treated with As_2S_2 at concentrations ranged from 3.125 μmol/L to 100 μmol/L for 48 h (B), higher risk MDS BMMNCs were treated with SAHA at concentrations ranged from 0.3125 μmol/L to 10 μmol/L for 48 h (C), higher risk MDS BMMNCs were treated with As_2S_2 at concentrations ranged from 3.125 μmol/L to 100 μmol/L for 48 h (D). Cell proliferation was determined by an MTT assay ($^{*}p < 0.05$, $^{**}p < 0.01$).

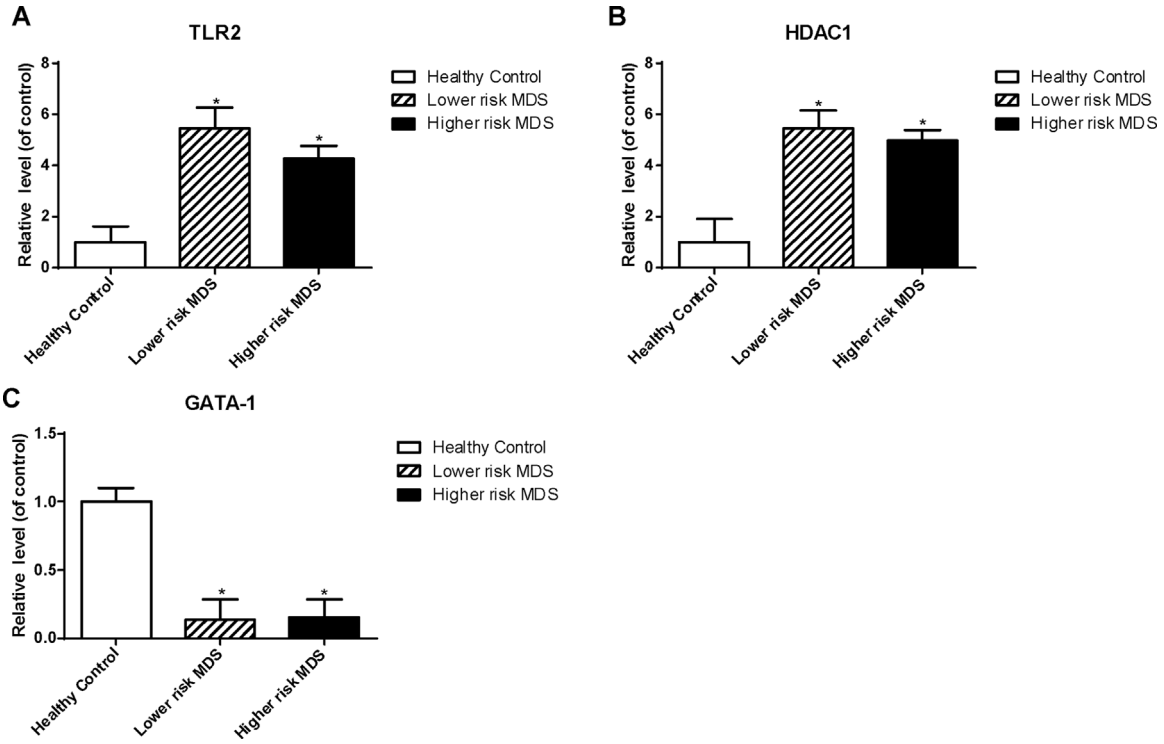

Fig. 2. TLR2, HDAC1, and GATA-1 mRNA expression of healthy control, lower risk and higher risk MDS BMMNCs (n = 3) Notes: Expression of TLR2 (A), HDAC1 (B) and GATA-1 (C) mRNA in BMMNCs from healthy control, lower risk MDS and higher risk MDS cases as determined by quantitative real-time PCR. The bars indicate median values. Student's *t*-test was used for statistical analysis ($^{*}p < 0.05$ versus healthy control).

disturbed erythroid differentiation in low-risk MDS patients could appear as high expression of GATA binding protein 1 (GATA-1) in the more differentiated cells ($CD235A^{+}/CD34^{-}$) [5]. Recent studies have shown that epigenetic modulation is becoming a promising approach.

Outcomes of MDS varied widely. The International Prognostic Scoring System was developed to classify patients to low/intermediate-1 (lower risk group), and intermediate-2/high (higher risk group) risk MDS according to the percentage of marrow blasts, cytogenetics, and number of peripheral cytopenias. Current treatments for lower-risk non-del (5q) MDS include red blood cell transfusion, recombinant human EPO, iron chelation therapy, immunosuppressive therapy, and hypomethylating agents [6]. Vorinostat (also known as SAHA) is a