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In vitro dual effect of arsenic trioxide on hemopoiesis: Inhibition of erythropoiesis and stimulation of megakaryocytic maturation

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Abstract

Although the arsenic compounds are now widely utilized in clinics in the treatment of various tumors, their effects on normal hematopoiesis do not seem to have been explored. In the present study, we provide evidence that arsenic trioxide (As_2O_3) exerts in vitro a potent inhibitory effect on normal erythropoiesis and a stimulatory action on megakaryocytic differentiation.

The effect of As_2O_3 on erythroid and megakaryocytic differentiation was evaluated on both erythroleukemic cell lines K562 and HEL and on normal hemopoietic progenitor cells (HPCs) induced to selective erythroid or megakaryocytic differentiation.

The inhibitory effect of As_2O_3 on erythropoiesis is related to: (a) the inhibition of Stat5 activation with consequent reduced expression of the target genes Bcl-X_L and glycophorin-A; (b) the activation of an apoptotic mechanism that leads to the cleavage of the erythroid transcription factors Tal-1 and GATA-1, whose integrity is required for erythroid cell survival and differentiation; (c) the reduced expression of heat shock protein 70, required for GATA-1 integrity. The stimulatory effect of As_2O_3 on normal megakaryocytopoiesis is seemingly related to upmodulation of GATA-2 expression and to stimulation of MAPK activity. These observations may have implications for the patients undergoing anti-leukemic treatment with this compound.

Keywords: Arsenic; Erythropoiesis; Megakaryocytes; Hemopoiesis; Transcription factors

Introduction

Arsenic is a semimetal commonly found in soil, water and air. Arsenic compounds have been used therapeutically from the time of the ancient Greek medicine. Recently, a considerable interest has developed around arsenic trioxide for its anticancer properties.

Thus, hematologists in China have introduced arsenic trioxide, alone or in combination with chemotherapeutic agents, as a therapy for patients with acute promyelocytic leukemia (APL), achieving a high rate of complete remissions [1–4]. The length of the remissions were long, and the treatment was relatively well tolerated, with transient gastrointestinal side effects [1–4]. Additional studies carried out in the United States have confirmed that low doses of arsenic trioxide can induce durable complete remissions in relapsed APL patients [5,6]. All-*trans* retinoic acid and As_2O_3 combination yielded durable remissions and survival in newly diagnosed APL patients [7]. At the moment, the anticancer properties of arsenic trioxide are also explored in other clinical settings, such as multiple myeloma and some solid tumors (reviewed in [8]).

These observations have prompted a series of studies focused to elucidate the molecular mechanisms through which arsenic trioxide exerts its anticancer properties.

These studies, mainly focused on the APL model, have provided evidence that arsenic trioxide exerts remarkable biological effects on several cellular functions, including induction of apoptosis, inhibition of cell differentiation and inhibition of angiogenesis (reviewed in [9]). In APL cells, the block in myeloid differentiation is caused by the fusion protein PML/RAR-α generated by the t(15;17) translocation. This block is released by treatment with pharmacologic doses of retinoic

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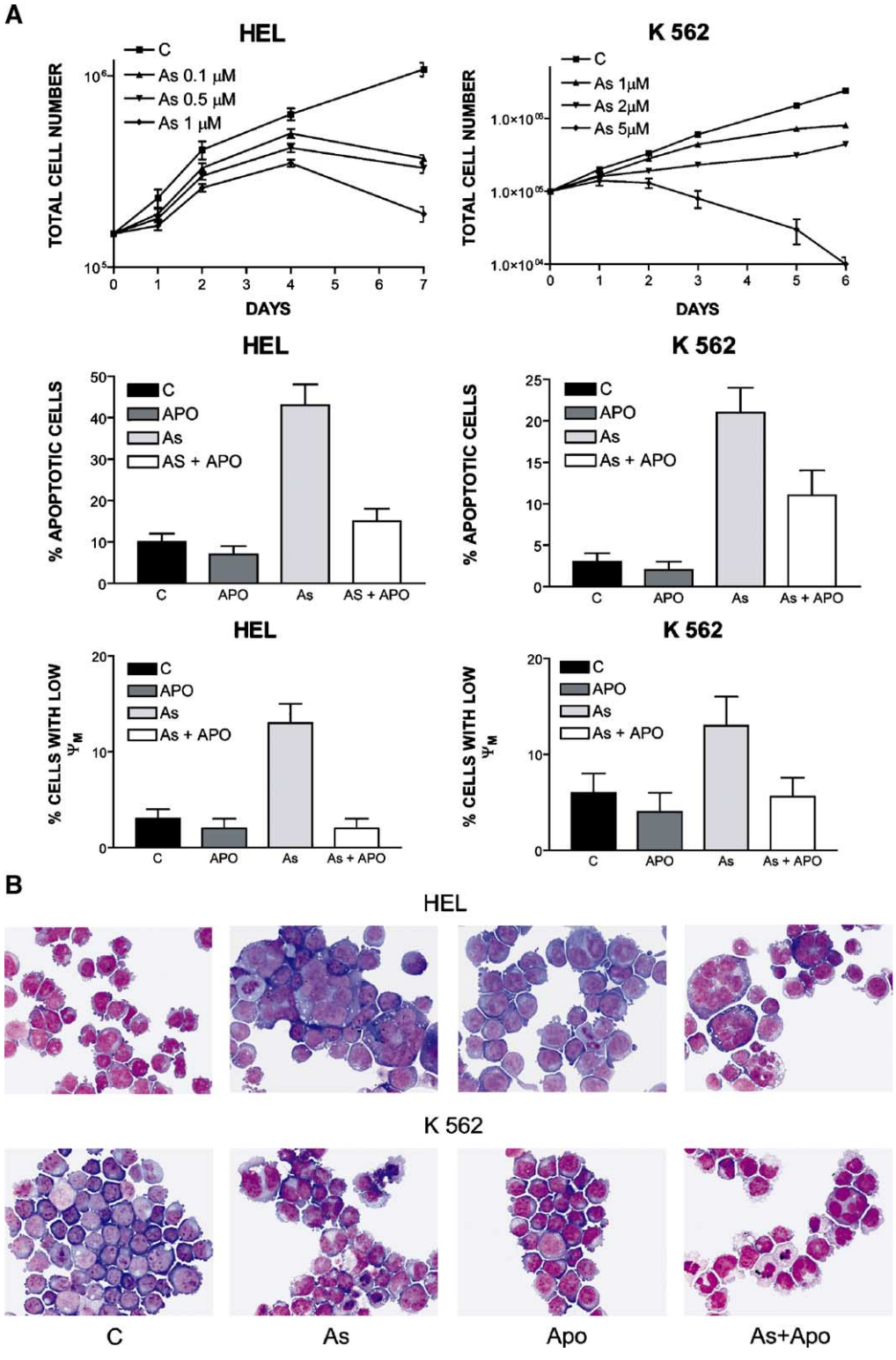

Fig. 1. (A) Top panel: effect of different As_2O_3 concentrations on the growth of HEL and K562 cells. Mean values ± SEM observed in three separate experiments. Middle panel: effect of As_2O_3 on the induction of apoptosis, as evaluated by the annexin V binding assay, In HEL and K562 cells. The cells have been grown for 4 days either in the absence (C) or in the presence of either Apoptame Q (APO) or As_2O_3 (As) or As_2O_3 + Apoptame (As + APO), and the percentage of apoptotic cells was then evaluated by flow cytometry using the Annexin V/PI staining. Bottom panel: effect of As_2O_3 on the mitochondrial membrane potential of K562 and HEL cells grown as reported in the middle panel. (B) Morphology of K562 and HEL cells grown for 4 days either in the absence of additives (C) or in the presence of As_2O_3 (As) or in the presence of Apoptame Q (APO) or in the presence of both As_2O_3 and Apoptame Q (As + APO). (C) Effect of As_2O_3 on the expression of membrane erythroid (top panels) and megakaryocytic (bottom panels) markers on HEL cells. In one set of experiments, HEL cells have been grown for different periods of time (0, 2, 4 and 7 days) in the presence of As_2O_3, and the expression of c-kit and glycophorin-A was determined by flow cytometry. One representative experiment out of three is reported. In the other set of experiments, HEL cells have been grown for 4 days either in the absence (C) or in the presence of As_2O_3 (As), and the reactivity with anti-CD61–CD41a and Thrombin-R was determined by flow cytometry. One representative experiment out of three is reported.