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# Lycorine induces apoptosis and down-regulation of Mcl-1 in human leukemia cells

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ABSTRACT

Lycorine is an alkaloid isolated from the bulb of the Amaryllidaceae Lycoris. Here, we report that treatment with lycorine resulted in survival inhibition and apoptosis induction in human leukemia cell lines. Lycorine induced apoptosis in human leukemia cells via intrinsic mitochondria pathway and caused a rapid-turnover of protein level of Mcl-1 which occurred before caspases activation. Furthermore, pronounced apoptosis accompanied by the down-regulation of Mcl-1 was also observed in blasts from patients with acute myeloid leukemia. Our findings suggest that lycorine may be a good candidate therapeutic agent against leukemia in worth of further evaluation.



## 1. Introduction

Acute myeloid leukemia (AML) represents a group of aggressive hematological malignancies. Currently, AML patients are treated mainly by conventional chemotherapy combined with cytarabine or daunorubicin. While this therapy may induce complete remissions in 60–80% of young and 40–55% of elderly adult patients, long-term survivors among patients with complete remissions are few [1]. Thus, the development of novel chemical agents that are more effective in the selective killing of AML cells retains high priorities in leukemia research.

A key mechanism by which antileukemia agents kill leukemia cells is to activate the apoptosis pathways of the cells [2]. Apoptosis can be initiated through either the death receptor or the mitochondrial pathway. Both pathways are executed and regulated by Bcl-2 family of proteins [3]. One member of this family is myeloid cell leukemia-1 protein or Mcl-1, which is a pro-survival member. Mcl-1 was originally discovered as an early induced gene during the differentiation of the myeloid cell line ML-1 [4]. More recent studies have suggested that Mcl-1 may play an important survival role in a variety of tumor cells. First of all, Mcl-1 has been shown to be highly expressed in a variety of cancers including certain forms of leukemia [5]. Secondly, the targeted down-regulation of Mcl-1 by SiRNA triggers apoptosis in leukemia cells, while enhanced Mcl-1 expression contributes to a malignant phenotype in certain tumor cells [6,7]. More relevantly, Mcl-1 level has been shown to be elevated at the time of leukemia recurrence after chemotherapy in AML patients [8]. It has also been found that the down-regulation of Mcl-1 potentiates histone deacetylase inhibitor (HDACi)-induced apoptosis [9]. These together suggest that Mcl-1 may serve as a molecular target in antitumor therapy. One focus of intense interest is to develop treatments that can diminish the cellular level of the Mcl-1 protein.

In this work, we investigate the cytotoxic effects of the drug compound lycorine (Fig. 1) on human leukemia cell lines and the possible involvements of Mcl-1 in such effects. Lycorine is an active alkaloid isolated from Lycoris. It possesses various biological effects including antitumor [10], antiviral [11], antimalarial [12], antiinflammation [13]. It may inhibit the enzyme acetylcholinesterase [14] and the ascorbic acid biosynthesis [15]. The drug is

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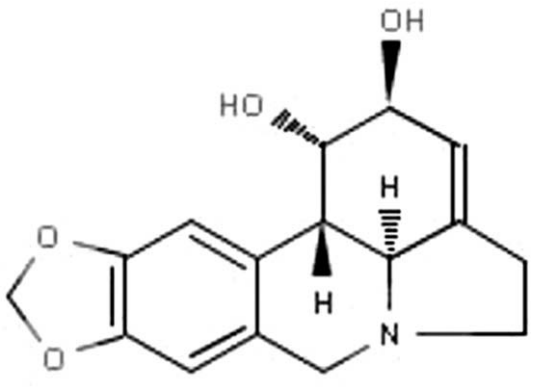


**Fig. 1.** Chemical structure of lycorine.

clinically used in Russian as an expectorant to treat chronic and acute inflammatory processes in lungs and bronchial diseases [16,17]. Several studies have shown that lycorine has selective cell type-dependent cell-killing effects on tumor cells. It inhibits the growth of leukemia Molt 4 and cervical HeLa cells heavily but has weak effects on hepatoma HepaG2 cells [10,18]. More recently, it has been shown to induce apoptosis through regulation of the cell cycle in leukemia HL-60 cells and multiplemeyloma cell line KM3 [19,20]. Another recent study reported that lycorine exhibits anti-tumor activity against HL-60 cells in SCID mice [21]. So far, there has been no reported studies demonstrating and elucidating the mechanism of cytotoxicity of lycorine against leukemia cells. Our present studies demonstrate that lycorine is potent in inhibiting the growth and inducing the apoptosis in human leukemia cells. At the molecular level, lycorine causes a rapid-turnover of protein levels of Mcl-1. These results suggest that lycorine may be a good candidate therapeutic agent against leukemia.

## 2. Materials and methods

### 2.1. Reagents

Lycorine ($C_{16}H_{17}NO_4HCl$, $M_W = 323.77$) (Fig. 1) and 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl tetrazolium (MTT) were purchased from Sigma Chemical Co., St. Louis, MO, USA. Boc-D-fmk and MG-132 were purchased from Calbiochem, San Diego, CA, USA. All reagents were prepared and used as recommended by their suppliers.

### 2.2. Cell Lines and cell culture

K562, U937, and HL-60 cell lines were kindly provided by Dr. Jun Yin (Shantou University, Shantou, China). 6T-CEM cell line was purchased from Cell Bank in Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China. The imatinib-resistant K562/G01 cell line was purchased from Institute of Hematology, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China [22]. Fresh peripheral blood mononuclear cells (PBMNCs) from five healthy subjects and bone marrow specimens from three AML patients were collected after their informed consent had been obtained. Mononuclear cells were separated by Ficoll–Hipaque density sedimentation. The percentage of blasts for patients was >80%. Cells were cultured separately in RPMI 1640 supplemented with penicillin, streptomycin, and 10% FBS. K562/G01 cells were maintained in RPMI1640 containing or lacking 4 μM of imatinib. Cells were collected at a concentration of $1 \times 10^5$ cells/ml, to which were added the designated drugs and maintained in a 37 °C, 5% $CO_2$, fully humidified incubator for the indicated time.

### 2.3. MTT assay

Cells were treated with different concentrations of lycorine. At certain times after treatment initiation, cell viability was estimated by the modified MTT-assay described previously [23]. Briefly, 10 μl of MTT solution (5 mg/ml in $ddH_2O$) was added to each well. Plates were then incubated for 4 h at 37 °C. Intracellular formazan crystals were dissolved by addition of 100 μl of isopropanol with 0.04 N HCl to each well, until the solution turned purple and absorbance analyzed in an enzyme-linked immunosorbent assay (ELISA) plate reader at 490 nm. Rate of inhibition was calculated by using the equation: Rate of inhibition = $(Ac - At)/Ac \times 100$, where At and Ac represent the absorbance in treated and control cultures, respectively. $IC_{50}$, the drug concentration causing a 50% decrease in cell survival, was determined by interpolation from dose–response curves.

### 2.4. Annexin V-FITC/propidium iodine FACS

Apoptosis of cells exposed to lycorine for 24 h was determined by flow cytometry using a commercially available Annexin V-FITC/propidium iodine apoptosis detection kit (KeyGen Biotech Co., Ltd., NanJing, China). After drug treatment, cells were collected and washed twice in ice cold PBS and resuspended in 500 μl of binding buffer at $1 \times 10^5$ cells/ml and incubated with 1 μl of AnnexinV/FITC and 5 μl of propidium iodine in the dark for 15 min at room temperature. Finally, samples were analyzed by flow cytometry and evaluated based on the percentage of early apoptotic cells for AnnexinV positive and PI negative.

### 2.5. DNA fragmentation assay

DNA fragmentation was analyzed after the extraction of DNA from cells exposed to 10 μM of lycorine for 24 h using Apoptotic DNA ladder kit (Applygen Technologies Inc., Beijing, China). The DNA was separated on a 1.5% agarose gel and visualized under UV light by ethidium bromide staining.

### 2.6. Cytochrome C release assay

Assay kits for cytochrome *C* release apoptosis (Calbiochem, San Diego, CA, USA) was used to assess the release of cytochrome *C* from mitochondria to cytosol. Briefly, cell samples were harvested and washed once with ice-cold phosphate-buffered saline (PBS) by centrifugation at 600g for 5 min at 4 °C. Cell pellets were resuspended in cytosol extraction buffer and Incubated on ice for 10 min