

CYTOTOXICITY AND INDUCTION OF APOPTOSIS BY 4-AMINO-3-ACETYLQUINOLINE IN MURINE LEUKEMIA CELL LINE L1210

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Nitrogen heterocyclic compounds are used in the pharmaceutical industry, in medicine and in agriculture for their biological activity. 4-Amino-3-acetylquinoline, a new synthetically prepared quinoline derivative, was the most effective compound in our primary cytotoxic screening. In this study, we evaluated cytotoxic/antiproliferative activity of quinoline using murine leukemia cell line L1210. Its ability to induce apoptosis was studied, too. Quinoline derivative acted cytotoxically on tumor cell line L1210, the IC₁₀₀ value were 50 µg/ml (for 24 h), 25 µg/ml (for 48 h) and 10 µg/ml (for 72 h). The IC₅₀ values was found to be less than 4 µg/ml, a limit put forward by the National Cancer Institute (NCI) for classification of the compound as a potential anticancer drug. The cytotoxic concentrations of 4-amino-3-acetylquinoline induced morphological changes of L1210 cells and the apoptotic DNA fragmentation.

INTRODUCTION

Nitrogen heterocyclic compounds are used in the pharmaceutical industry, in medicine and in agriculture for their biological activity. They have been the subject of chemical and biological studies due to their antimicrobial, antipyretic, analgesic, anti-inflammatory, herbicidal, fungicidal, antiviral and leishmanicidal properties. As documented in the literature, many derivatives act as anticancer active compounds¹⁻⁴. They are photosynthetic electron transport inhibitors, cyclooxygenase 2 (COX-2) inhibitors, inhibitors of tumor necrosis factor- α production, selective inhibitors of monoamine oxidase (MAO-A, MAO-B), alcohol dehydrogenase inhibitors, phosphodiesterase 4 (PDE4) inhibitors, adenosine kinase inhibitors, neutral proteases inhibitors, dihydrofolate reductase inhibitors, tyrosine kinase inhibitors and cyclin-dependent kinase inhibitors. Some of nitrogen heterocyclic compounds induce apoptosis, disruption of mitochondrial membrane potential, DNA single-strand breaks and activation of caspase 9, are single or dual inhibitors of DNA topoisomerase I and DNA topoisomerase II and interact with DNA⁵⁻⁷. There are now a number of DNA topoisomerase inhibitors in development. These have broad spectrum of activity against different kinds of human cancers. Some of these (celecoxib) is now in preclinical testing.

With the aim of obtaining new antitumor agents, a series of substituted nitrogen heterocyclic compounds were prepared. These compounds were firstly tested for cytotoxic properties *in vitro* on selected microorganisms and human

tumor cell line HeLa. The widest activity has been manifested by the derivatives 4-methyl-2-(3-nitrophenyl)-pyrimidine-5-carbonitrile and 4-amino-3-acetylquinazoline⁸.

The main aim of this study was to investigate the cytotoxic effect of 4-amino-3-acetylquinoline on further cell line murine leukemia L1210 cells. Its ability to induce of apoptosis was studied, too.

MATERIAL AND METHODS

We used murine leukemia cell line L1210 (obtained from American Type Culture Collection, Rockville, MD, USA) which was grown in supplemented RPMI 1640 medium (Biocom Bratislava) at 37 °C in a humidified atmosphere containing 5% CO₂. Cell viability were assessed by 0.4% trypan blue staining. 4-Amino-3-acetylquinoline (AAQ) prepared by Černuchová et al.⁹, was dissolved in dimethyl sulfoxide (DMSO). All other chemicals were obtained from Sigma Chemicals (St Louis, MD).

Antiproliferative activity *in vitro* was measured by the cell growth inhibition assay. After 24 h of incubation, the L1210 cells (the starting inoculum of 8 × 10⁴ cells/ml) were treated with AAQ (concentrations of 50, 25, 10, 1, 0.1 and 0.01 µg/ml). Control cells were treated with DMSO, its final concentration never exceeded 1%. After 24, 48 and 72 h of AAQ treatment, the number of cells per culture dishes was counted in Bürker chamber. Cell growth and viability were assessed by direct counting of 0.4% trypan blue dye-excluding cells.

For detection of apoptotic cells (internucleosomal DNA fragmentation), the electrophoretic determination was used. The untreated L1210 cells (control) and the cells (1×10^6) treated with AAQ concentrations of 50, 25, 10 and 1 $\mu\text{g}/\text{ml}$ for 24 h, 48 h and 72 h were harvested, washed in PBS and then lysed in 100 μl of solution (10 mM Tris, 10 mM EDTA, 0.5% Triton X-100) supplemented with proteinase K (1 mg/ml). Samples were then incubated at 37 °C for 1 h and heated at 70 °C for 10 min. Following lysis, RNA-ase (200 $\mu\text{g}/\text{ml}$) was added and repeated incubation at 37°C for 1 h followed. The samples were subjected to electrophoresis at 40 V for 3 h in 2% (w/v) agarose gels complemented with ethidium bromide (1 $\mu\text{g}/\text{ml}$). Separated DNA fragments were visualized using UV transilluminator (254 nm, Ultra-Lum Electronic UV Transilluminator, USA).

RESULTS AND DISCUSSION

The growth of L1210 cells exposed to 4-amino-3-acetylquinoline concentrations ranging from 0.01 to 50 $\mu\text{g}/\text{ml}$ was monitored within 72 h of culturing (Fig. 1). As shown in figure, the addition of AAQ to the medium reduced L1210 viable cell number. After 24 h, the highest concentration tested (50 $\mu\text{g}/\text{ml}$) had an acute cytotoxic effect manifested by degeneration (lysis-necrosis) of cell populations. Two concentrations of AAQ (25 and 10 $\mu\text{g}/\text{ml}$) induced delayed cytotoxic effect. While in the first 24 h, 31.8 – 45.4 % of the cell population proliferated, in the next 48 and 72 h 12.6 – 51.9 % of the population degenerated. The antiproliferative effect of AAQ at the next concentrations tested (1, 0.1 and 0.01 $\mu\text{g}/\text{ml}$) was directly proportional to the concentration and the time of influence. The inhibition of cell proliferation was 9.7–64.2 %. These changes in viable cell number were also observed when aliquots of the cultures were examined by light microscopy.

The sensitivity of the cells to AAQ assessed by appearance of apoptotic internucleosomal DNA fragmentation in L1210 cells detected after 24 h, 48 h and 72 h of incubation with AAQ (Fig. 2). The apoptotic DNA fragmentation was observed in L1210 cells treated with 50, 25 and 10 $\mu\text{g}/\text{ml}$ of quinoline derivative after 48 and 72 h of influence. 4-Amino-3-acetylquinoline after 24 h of treatment did not induce the apoptotic DNA fragmentation in L1210 cells.

On the basis of the obtained results we can conclude that 4-amino-3-acetylquinoline acted cytotoxically on the cell line L1210. The apoptotic DNA fragmentation after 48 and 72 h treatment by concentrations of 50, 25 and 10 $\mu\text{g}/\text{ml}$ was found.

Apoptosis has been described as multiple pathways converging from numerous different initiating events and insults. Numerous studies have demonstrated that apoptosis may be involved in cell death induced by chemotherapeutic agents including cisplatin, gemcitabine, etoposide, taxol. Apoptosis can be executed through two basic signalling pathways: one is mediated by death receptors on the cell surface – sometime referred to as

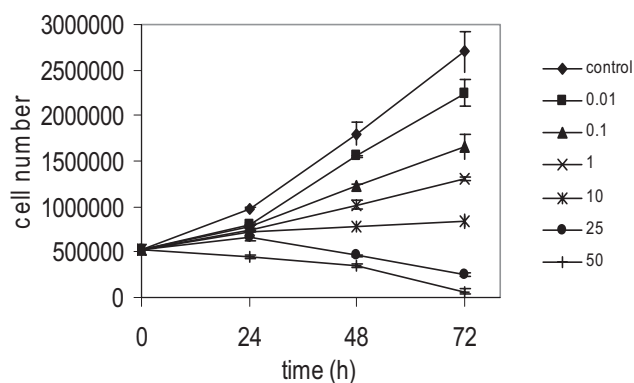


Fig. 1. Effect of 4-amino-3-acetylquinoline on L1210 cell proliferation.

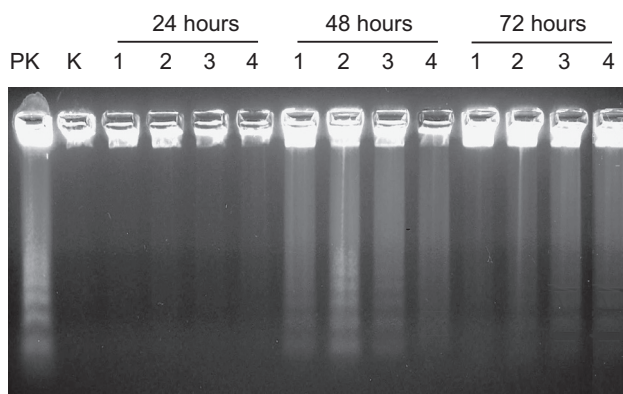


Fig. 2. Electrophoretic detection of internucleosomal fragmentation of cellular DNA after 24 h, 48 h and 72 h treating of L1210 cells with 4-amino-3-acetylquinoline. PC = positive control (cis-platine). AAQ concentration ($\mu\text{g}/\text{ml}$): C = control, 1 = 1.0, 2 = 10.0, 3 = 25.0, 4 = 50.0.

the „extrinsic pathway“, the other is mediated by mitochondria – referred to as the „mitochondrial or intrinsic pathway“. Accumulating evidence showed that efficacy of antitumor agents is related to the intrinsic propensity of the target tumor cells to respond to these agents by apoptosis. Morphological changes of apoptosis are considered the results of complex cellular biochemical pathways. In mammals, apoptosis is result of the proteolysis of various cellular components initiated by activated caspases (a family of cysteine protease). Progression of the caspase cascade ends with the activation of caspase 3 that occurs in early apoptosis, long before DNA-fragmentation appears. Once caspase 3 has been activated, there is no way back to normal viability, the program for cell death is irreversibly activated¹⁰. Therefore, in the next, time-dependend analysis of caspase 3 activation in murine leukemia cells treated with 4-amino-3-acetylquinoline will be examined.

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