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Research paper

Apoptotic events induced by synthetic naphthylchalcones in human acute leukemia cell lines

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ABSTRACT

Acute leukemia is a disorder of the hematopoietic system characterized by the expansion of a clonal population of cells blocked from differentiating into mature cells. Recent studies have shown that chalcones and their derivatives induce apoptosis in different cell lines. Since new compounds with biological activity are needed, the aim of this study was to evaluate the cytotoxic effect of three synthetic chalcones, derived from 1-naphthaldehyde and 2-naphthaldehyde, on human acute myeloid leukemia K562 cells and on human acute lymphoblastic leukemia Jurkat cells. Based on the results, the most cytotoxic compound (**A1**) was chosen for further analysis in six human acute leukemia cells and in a human colon adenocarcinoma cell line (HT-29). Chalcone **A1** significantly reduced the cell viability of K562, Jurkat, Kasumi, U937, CEM and NB4 cells in a concentration and time-dependent manner when compared with the control group (IC_{50} values between ~1.5 μM and 40 μM). It was also cytotoxic to HL-29 cells. To further examine its effect on normal cells, peripheral blood lymphocytes collected from healthy volunteers were incubated with the compound. It has also been incubated with human fibroblasts cultured from bone marrow (JMA). Chalcone **A1** is non-cytotoxic to PBL cells and to JMA cells. **A1** caused significant cell cycle arrest in all phases according to the cell line, and increased the proportion of cells in the sub G0/G1 phase. To evaluate whether this chalcone induced cell death via an apoptotic or necrotic pathway, cell morphology was examined using fluorescence microscopy. Cells treated with **A1** at IC_{50} demonstrated the morphological characteristic of apoptosis, such as chromatin condensation and formation of apoptotic bodies. Apoptosis was confirmed by externalization of phosphatidylserine, which was detected by the Annexin V-FITC method, and by DNA fragmentation. The results suggest that chalcone **A1** has potential as a new lead compound for cancer therapy.

1. Introduction

Acute leukemia is a disorder of the hematopoietic system characterized by the expansion of a clonal population of cells that is blocked from differentiating into mature cells, which may occur in bone marrow or in lymphoid tissues [1,2]. The leukemic clone may arise at different stages of differentiation of the lymphoid and myeloid precursors, which characterizes this type of cancer as a heterogeneous disease in biological and morphological terms [3–5].

Despite being the most effective treatment for leukemia, standard chemotherapy is still associated with patient relapse, high morbidity and high mortality rates [6]. The killing of tumor cells by these therapies is mediated primarily by induction of apoptosis, which suggests that resistance of tumor cells to therapy can be caused by a failure in the ability to initiate apoptosis [7]. Apoptosis is a term introduced in 1972 to distinguish one type of programmed cell death with characteristic morphology and highly regulated endogenous mechanisms [8]. The homeostasis of normal cells is the result of a balance between proliferation, differentiation and apoptosis whereas malignant processes are characterized by the excessive expansion of tumor cells due to failures in one or more of these processes [9]. Therefore, treatment strategies with new antineoplastic drugs that have greater specificity in inducing apoptosis of

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tumor cells are needed, not only to improve the cure rate but also the patients' quality of life [10]. In order to achieve this, understanding the mechanisms of cell cycle regulation and apoptosis is critical in relation to identifying new targets for antileukemic therapy.

Chalcones are naturally-occurring open-chain flavonoids isolated from some plants and include plant allelochemicals and insect hormones and pheromones [11]. They are prepared by condensing aryl ketones with aromatic aldehydes in the presence of suitable condensing agents [12]. They undergo a variety of chemical reactions and have been used in the synthesis of a variety of synthetic heterocyclic compounds [13–15]. Several biological effects suggest a potential pharmaceutical use of chalcones, such as antimicrobial [16], anti-inflammatory [17,18], analgesic [19], antiviral [20] and anticancer [13]. Studies have also shown that chalcones have antiproliferative and cytotoxic effects [13,14,21], and may also arrest the cell cycle in different tumor cell lines [22–25]. Several studies have reported that chalcones can block the cell cycle in the G2/M phase [25–28], while others show blocking in the G0/G1 phase [29,30]. However, the effect of chalcones and their derivatives in the transduction of signals which control the cell cycle and induce the apoptosis of cells are still controversial.

We have previously reported the cytotoxic effects of a series of chalcones derived from 2-naphthaldehyde. We found that chalcone (2*E*)-3-(2-naphthalenyl)-1-(3′-methoxy-4′-hydroxy-phenyl)-2-propene-1-one showed a strong cytotoxic effect, with an IC_{50} value of 54 μM being obtained in tests on mouse acute lymphoblastic leukemia cells (L-1210). The mechanisms of apoptosis were investigated and it was found that this compound causes blocking of the cell cycle in the G2/M phase and apoptosis via the mitochondrial pathway. This finding was explained by the decreased expression of Bcl-2 and increased expression of Bax, which results in caspase-3 activation and cell death [25]. However, despite possessing the pharmacological properties required for the development of an antitumor drug, the IC_{50} value of 54 μM is prohibitively high.

In this context, three structural analogs of (2*E*)-3-(2-naphthalenyl)-1-(3′-methoxy-4′-hydroxy-phenyl)-2-propene-1-one were prepared: (2*E*)-1-(3,4,5-trimethoxy-phenyl)-3-(2-naphthyl)-2-propene-1-one (**C24**), (2*E*)-1-(3,4,5-trimethoxy-phenyl)-3-(1-naphthyl)-2-propene-1-one (**A23**) and (2*E*)-1-(2,5-dimethoxy-phenyl)-3-(1-naphthyl)-2-propene-1-one (**A1**) (Fig. 1). These compounds had been previously synthesized by our group [31], but no tests had been carried out to identify possible cytotoxic effects on leukemia cells.

The main purpose of this study was to assess the cytotoxic effect of these three synthetic chalcones (**C24**, **A23** and **A1**) on a human acute myeloid leukemia cell line (K562) and a human acute lymphoblastic leukemia cell line (Jurkat). Based on the results obtained, we selected the most cytotoxic compound for further analysis. Considering the different biological characteristics and therapeutic responses of acute leukemia, we investigated the effects of the most active chalcone in six different types and subtypes of human leukemia cells (K562, Jurkat, U937, Kasumi, NB4 and CEM). In addition, we investigated the effect of this compound on the cell cycle and on apoptosis induction, in order to clarify its mechanism of action, seeking a potential lead compound in the search for new antitumor agents.

2. Materials and methods

2.1. Synthesis

The reagents used were obtained commercially from Sigma–Aldrich® and solvents from Vetec. The chalcones were prepared by aldolic condensation using methanol as the solvent under basic conditions (KOH 50% w/v) at room temperature for 24 h. Distilled water and 10% hydrochloric acid were added to the reaction for total precipitation of the compounds, which were then obtained by vacuum filtration and later recrystallized in dichloromethane and hexane. The structures were identified by melting point, infrared spectroscopy (IR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy and elementary analysis, as previously described by our group [31].

2.2. Cell culture

Six human leukemia cell lines were used in this study: chronic myeloid leukemia in blast crisis (K562), acute lymphoblastic leukemia (Jurkat), monocyte-like histiocytic lymphoma (U937), (8:21)-kit mutant model for acute myeloid leukemia (Kasumi), acute promyelocytic leukemia (NB4) and T-cell leukemia (CEM). It also has been used human colon adenocarcinoma cells (HT-29) and human fibroblasts cultured from bone marrow (JMA) as a solid tumor model and as a control for actively proliferating stable cells, respectively. Cells were cultured in Roswell Park Memorial Institute Medium (RPMI) or Dulbecco's Modified Eagle's Medium (DEMEM) (GIBCO, São Paulo, SP, Brazil) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES under 5% CO_2 humidified atmosphere in75 cm^2 flasks. JMA cells were cultured in Iscove's Modified Dulbecco Medium (IMDM) (GIBCO, São Paulo, SP, Brazil) supplemented with 20% FBS and 100 U/ml penicillin and streptomycin. All these cell lines were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) or Rio de Janeiro Cell Bank (BCRJ, Rio de Janeiro, RJ, Brazil). Cell viability counts were obtained using the Trypan blue exclusion assay.

2.3. Samples from healthy volunteers

This study was approved by the Medical Ethics Committee 238/03. Five non-smoking healthy volunteers who had never been treated with any cancer drugs entered this study between August 2011 and September 2011 at the *Polydoro Ernani de São Thiago* University Hospital (Florianópolis, Brazil). Blood samples were collected and human peripheral blood lymphocytes were isolated by the Ficoll–Hypaque method [32]. Briefly, after diluting blood with PBS, lymphocytes were isolated by centrifugation over a density gradient of Ficoll–Hypaque (density = 1.070 g/ml) for 30 min at 2000 rpm. Cells were washed with PBS twice and then suspended in RPMI with 10% fetal bovine serum. The viability of the isolated lymphocytes was measured by the Trypan blue exclusion assay and found to be around 95%.

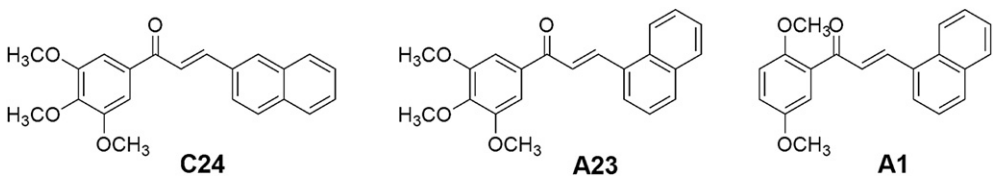

Fig. 1. Structures of chalcones **C24**, **A23** and **A1**.

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