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# Apoptosis of Human T-Cell Acute Lymphoblastic Leukemia Cells by Diphenhydramine, an H1 Histamine Receptor Antagonist

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Recently, it has been demonstrated that histamine plays an important role in the proliferation of normal and malignant cells. We have examined the effects of histamine, diphenhydramine, and cimetidine (H1 and H2 histamine receptor antagonists, respectively) on the in vitro proliferation of two human T-cell acute lymphoblastic leukemia cell lines, namely CCRF-CEM and Jurkat. Exogenous histamine did not alter the proliferation or viability of these cells. In contrast, diphenhydramine induced apoptosis in a dose- and time-dependent manner in both cell lines, whereas cimetidine failed to induce significant effects at similar concentrations. Diphenhydramine-induced apoptosis was evaluated in terms of morphology, flow cytometry, and the release of cytochrome *c* to the cytosol. The latter was partially mitigated by Bcl-2 overexpression. In human peripheral blood mononuclear cells, diphenhydramine inhibited cell proliferation without inducing apoptosis. Our findings indicate that endogenous histamine may be an important factor for the survival of CCRF-CEM, Jurkat, and peripheral blood mononuclear cells, and point to the potential application of H1 receptor antagonists as cytotoxic agents for the specific treatment of certain types of leukemia.

Key words: Histamine antagonist; Diphenhydramine; Cimetidine; Apoptosis; Mitochondrion

Apoptosis is a normal physiological form of cell death, characterized by certain morphological and biochemical alterations that distinguish it from necrosis. Recent studies have demonstrated that most anticancer drugs act by inducing apoptosis in target cells through two major pathways: the mitochondrial and cell death receptor pathways (1). Mitochondria were first discovered to be involved in the cell death machinery with the discovery that the Bcl-2 antiapoptotic protein is present in the mitochondrial outer membrane (2,3). Cytochrome *c* is one of the most potent activators of mitochondrial-dependent apoptosis; its release to the cytosol activates a cascade of proteolytic enzymes called caspases, which play a pivotal role in the initiation and termination of apoptotic process (4).

Histamine is a biogenic amine that exerts its effects on target cells through a family of G-protein-coupled receptors known as H1–H4 histamine receptors, which differ in their location, signal transduction, and histamine-binding characteristics. In addition, different histamine receptor agonists and antagonists can bind to different portions of the receptor complex (5). H1 and H2 receptors are expressed on the cell surface of several types of normal and malignant cells, including normal lymphocytes and leukemia cells (6,7). The effect of histamine on tumor growth was observed many years ago (8). Since then, multiple in vitro and in vivo studies have been performed to examine the effect of histamine on cell proliferation and tumor growth, but with many contradictory findings (6,9).

Diphenhydramine (DPH[2]) is a well-known classical H1 histamine receptor antagonist that is employed for the treatment of allergic diseases. In contrast, cimetidine, an H2 receptor antagonist, has been used for many years as a treatment and prophylaxis for human gastric ulcer. It has also been shown to have beneficial effects in colorectal cancer (10).

Acute lymphoblastic leukemia (ALL) is a cancer of immature lymphocyte cells known as lymphoblasts. It is classified into the B- and T-cell lymphoblastic leukemias based on the type of lymphocyte lineage affected. The ALL represents about 75–80% of leukemia in chil-

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[2]Abbreviations used: DPH, diphenhydramine; ALL, acute lymphoblastic leukemia; PBMC, peripheral blood mononuclear cell; PHA-M, phytohemaglutinin-M; LDH, lactate dehydrogenase; ROS, reactive oxygen species.

dren. Although the survival rate of ALL has improved in the last 20 years (11), new therapeutic modalities are needed to be investigated for this disease.

Interestingly, histamine has been described as an autocrine regulator of cell proliferation in B-cell ALL, and H1 receptor antagonists have been reported to inhibit leukemia cell proliferation (12). However, the role of histamine in T-cell leukemia is still not clear.

To better characterize the effect of histamine and its antagonists on the survival of human T-cell ALL, we have studied the effect of histamine, DPH, and cimetidine on the viability of CCRF-CEM and Jurkat cell lines and of normal human peripheral blood mononuclear cells (PBMCs). Our results indicate that endogenous histamine is a survival factor for these cells. Moreover, DPH was found to induce cell death by apoptosis via the mitochondrial pathway in CCRF-CEM and Jurkat cells, but not in human PBMCs.

## MATERIALS AND METHODS

### *Cells and Culture Conditions*

CCRF-CEM, Jurkat, and CEM-C7H2-10E1 [CEM cells stably transfected with a Bcl-2 cDNA-containing vector overexpressing Bcl-2 (13); kindly provided by Dr. Kofler] cell lines were routinely maintained in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, and 100 μg $ml^{-1}$ gentamicin. The density of cultured cells was maintained between 2 and $3 \times 10^5$ cells $ml^{-1}$. PBMCs were isolated from healthy volunteers and were activated with phytohemaglutinin-M (PHA-M) for 72 h and cultured as described previously (14).

Cells were incubated for 12, 24, and 48 h with different concentrations of histamine, DPH, and cimetidine. All products were purchased from Sigma Chemical Co. (St. Louis, MO). In all the experiments cell cultures had viability of around 95%.

### *Proliferation and Cytotoxicity Assays*

Cells were seeded into flat-bottomed 96-well microtiter plates, at a density of $2 \times 10^4$ cells per well in 100 μl of culture medium. Cells were incubated with various concentrations (1 μM–1 mM) of histamine, DPH, and cimetidine for different time periods and cell proliferation and survival were subsequently determined by a colorimetric assay in accordance with the indications of the supplier (Cell Proliferation kit II.XTT, Roche). Absorbance at 490 nm was measured using a microtiter (ELISA) plate reader and cell survival percentages were calculate in each experiment in relation to control, nontreated samples. Proliferation assays were also performed in the same way with inactive and PHA-M activated PBMCs.

The potential specific cytotoxicity of agents was determined by measuring the release of lactate dehydrogenase (LDH) from the cytosol of damaged cells into the culture medium by means of an LDH cytotoxicity detection kit (Roche). To assess the maximum available LDH, cells were homogenized with 1% Triton X-100 and considered as the highest cytotoxicy control and untreated cells were considered as the low cytotoxicity control. The extinction of supernatants at 490 nm was measured using a microtiter ELISA plate reader. To determine the cytotoxicity percentage, we calculated the average absorbance values and subtracted from each of these the absorbance value obtained in the background control. The resulting values were substituted in the following equation: % cytotoxicity = (experimental value − low control/high control − low control) × 100.

The cellular viability before experiments and in the untreated control cells always was >95%. All tests were carried out with six replicates and experiments were repeated at least three times.

### *Apoptosis Assays*

*Transmission Electron Microscopy (TEM).* CCRF-CEM, Jurkat, and PBM cells from untreated and DPH-treated cultures (0.5 and 1 mM) were incubated for 24 h. They were then harvested and centrifuged at $625 \times g$ for 10 min and processed as described previously (15). Ultrathin sections were stained with uranyl acetate and examined with a Philips EM 208 electron microscope (Philips Electronic Instruments, Eindhoven, Netherlands).

*Flow Cytometry.* An Annexin V-FITC apoptosis detection kit (Oncogene, San Diego, CA) was used to quantify apoptosis and necrosis. Treated and untreated CCRF-CEM and Jurkat cells were harvested and processed according to the manufacturer's protocol.

Analysis of hypodiploid cells was performed with both exponentially growing leukemia cells and PHA-M-activated human PBMCs, which were treated with various concentrations of DPH for 12, 24, and 48 h. Untreated and treated cells were fixed and stained as described previously (16).

Flow cytometric analysis was performed on at least 10,000 cells using a Coulter EPICS ELITE ESP (EPICS Division Coulter Corp.). During analysis of hypodiploid cells, the intensity of fluorescence emitted by bound propidium iodide is directly proportional to the amount of DNA in each sample. Results were analyzed using the WinMDI 2.8 program and expressed as a percentage of hypodiploid cells locating in the sub-$G_0/G_1$ phase of the cell cycle.

*Measurement of Intracellular Generation of Reactive Oxygen Species (ROS).* To measure the production of reactive oxygen species (principally hydrogen peroxide

and hydroxyl radicals), we used the oxidation-sensitive fluorescent 2′,7′-dichlorodihydrofluoresceindiacetate reagent (DCFH-DA, Molecular probes, Eugene, OR) as described by Asumendi et al. (14). CCRF-CEM and Jurkat cells from untreated and DPH-treated cultures (0.5 and 1 mM) were analyzed and fluorescent intensity was measured at 350 nm after excitation at 485 nm in a FL500fluorimeter (Bio-Tek Instruments). Six wells were used for each treatment and the experiment was repeated three times.

### *Morphological Evaluation by DAPI Staining*

Cells were harvested 24 h after treatment with different concentrations of test compounds. The cells were pelleted, washed with PBS, fixed with methanol at 4°C for 15 min, and then stained with 2 mg $ml^{-1}$ of 4′,6-diamidino-2-phenylindole (DAPI). Digital images were obtained with a fluorescent microscope (Axioplan, Zeiss, Germany) connected to a digital camera (AxioScan, Zeiss).

### *Detection of Cytochrome* c *Release From the Mitochondria to the Cytosol*

CCRF-CEM and CEM-C7H2-10E1 cells treated with or without 0.5 mM DPH for 12 and 24 h were harvested and cytosol and mitochondrial extracts were obtained as previously described (14). Samples of the cytosolic and mitochondrial extracts containing 2 μg of protein were used to measure the level of cytochrome *c* using a Cytochrome *c* ELISA kit (R&D Systems Europe, Ltd., UK) following the manufacturer's instructions. Four wells were used for each treatment and the experiment was repeated three times.

To further verify cytochrome *c* release into the cytosol during DPH-induced apoptosis, Western blotting was carried out by subjecting 40 μg of protein from cytosolic extracts to electrophoresis in 15% polyacrylamide gels as previously reported (14). Anti-cytochrome *c* monoclonal antibody (BD Pharmingen, San Diego, CA) was used at a dilution of 1:500. An anti-actin antibody (Chemicon International Inc., Temecula, CA) at a dilution of 1:200 was used as a control.

### *Statistical Analysis*

The level of statistical significance between sample means was determined using the Student *t*-test; $p < 0.05$ was considered statistically significant. Nonlinear regression to calculate the $IC_{50}$ for DPH effects was performed using GraphPad Prism, version 3 for Windows (GraphPad Software, San Diego, CA).

## RESULTS

### *Effect of Histamine, DPH, and Cimetidine on Cell Proliferation and Viability*

We studied the effects of several doses of histamine, ranging from 1 μM to 1 mM, on the cell survival and proliferation of T-ALL cell lines. Exogenous histamine was not found to alter the proliferation of either cell line (Fig. 1A), either 24 or 48 h after incubation, or in the presence or absence of 10% FCS. To evaluate the effect of endogenous histamine on cell proliferation, cells were incubated with various concentrations (1 μM–1 mM) of DPH and cimetidine for 24 h in medium containing 10% FCS. DPH was found to inhibit cell survival in a dose-dependent manner, while no effect was observed when cells were cultured with cimetidine at the same range of concentrations. The maximum inhibitory effect of DPH was observed at 1 mM (Fig. 1A) and the half-maximal inhibitory concentration ($IC_{50}$) values for DPH in CEM and Jurkat cell lines were $550 \pm 70$ and $420 \pm 60$ μM, respectively.

Cytotoxicity was evaluated by detecting LDH in the supernatants of treated cell cultures. Neither histamine nor cimetidine had any significant effect, after 48 h of incubation. However, DPH was found to induce cell death in a dose-dependent manner (Fig. 1B).

### *DPH-Induced Apoptosis in Human CCRF-CEM and Jurkat ALL Cells*

Ultrastructural characteristics of cell death induced by DPH were evaluated by means of TEM. Morphological changes induced by 24-h incubation with 0.5 mM DPH were observed to be characteristic of apoptosis in both cell lines (Fig. 2). These alterations included chromatin condensation and margination (Fig. 2C, F), impaction of organelles at one cell pole (Fig. 2C), cisternal hyperdilatation of the nuclear membrane around prefragmented nuclei (Fig. 2D), dispersal of nuclear fragments into the cytoplasm (Fig. 2E), and clustering of nuclear pore complexes in between the nuclear fragments at the site opposite the condensed chromatin (Fig. 2D, F).

### *Apoptosis Induced by DPH in CCRF-CEM and Jurkat Cells Is Dose and Time Dependent*

Quantitative studies to evaluate the extent of apoptosis induced by DPH in both cell lines were carried out by flow cytometric analysis, using Annexin V-FITC and propidium iodide. Viable cells do not fluoresce with these reagents. In contrast, early apoptotic cells bind Annexin V-FITC but exclude propidium iodide, while cells at late apoptotic stages or undergoing secondary necrosis are both Annexin V-FITC and propidium iodide positive. These assays revealed that apoptosis induced by DPH is

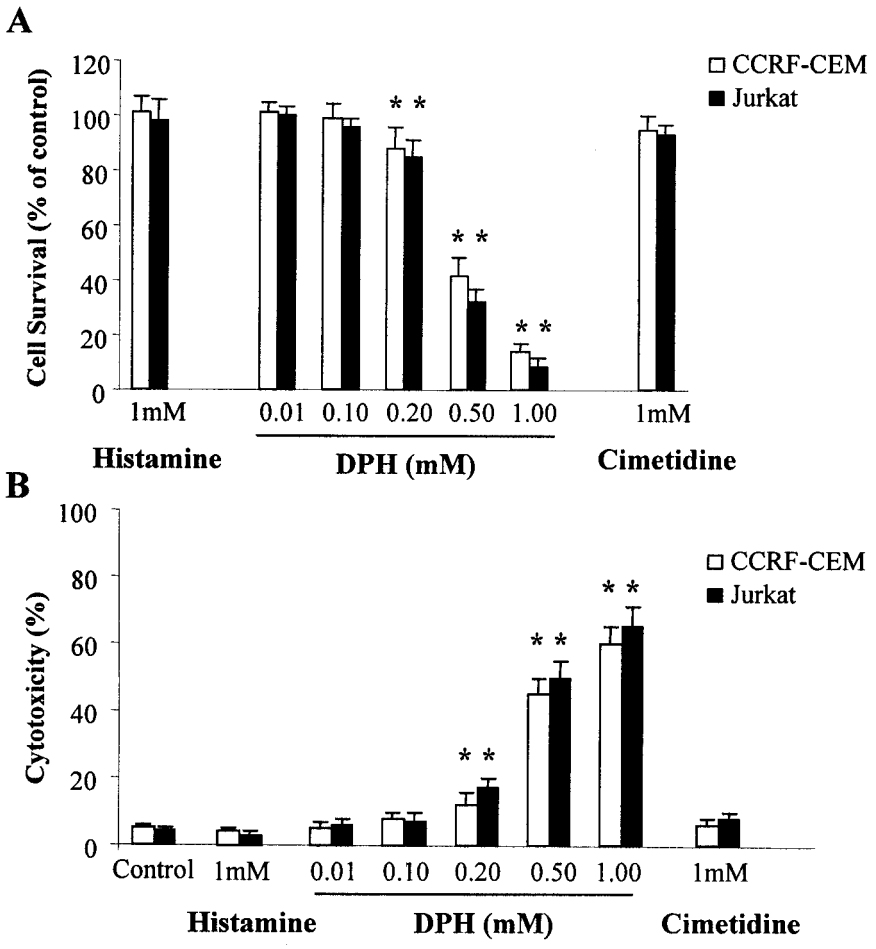


**Figure 1.** Effects of histamine, DPH, and cimetidine on CCRF-CEM and Jurkat cells. (A) Cell proliferation. (B) LDH cytotoxicity. Cell cultures were treated with various concentrations of histamine, DPH, and cimetidine for 24 h in RPMI supplemented with 10% FCS. For cytotoxicity assays, supernatants were collected after 48 h of treatments. Results are expressed as cytotoxicity percentage obtained from the following equation: % cytotoxicity = (experimental value – low control/high control – low control) × 100. Data represent mean ± SD of six determinations of three separate experiments for each cell line. Only DPH inhibited cell proliferation in a dose-dependent manner. The $IC_{50}$ values for DPH in CCRF-CEM and Jurkat cell lines were 550 ± 70 and 420 ± 60 mM, respectively. $*p < 0.05$ (Student *t*-test).

dose and time dependent in both cell lines (Fig. 3A, B). The percentage of cells at early and late apoptosis was found to be proportional to the concentration and duration of incubation of DPH. In contrast, no significant effects were observed when cells were treated with 1 mM histamine or cimetidine for 24 h.

These results were further confirmed by detection of hypodiploid cells, characteristic of apoptosis, as cells with low DNA content located in the sub-$G_0/G_1$ phase of the cell cycle. DPH induced an increase in the percentage of hypodiploid cells in this phase of the cell cycle in a dose-dependent manner, in both CCRF-CEM and Jurkat cells. Thus, the percentage of cells in the sub-$G_0/G_1$ phase of the cell cycle in untreated CCRF-CEM and Jurkat cells was 5.8% and 5.7%, respectively. After 48 h of incubation with 1 mM DPH, the corresponding sub-$G_0/G_1$ peaks were 44.5% and 75.5% for treated CCRF-CEM and Jurkat cells, respectively.

### *DPH Does Not Induce Apoptosis in Human PBMCs*

Nonactivated and PHA-M-activated human PBMCs were cultured with various concentrations of DPH for 24 h. DPH was found to inhibit cell proliferation in a dose-dependent manner in activated human PBMCs, whereas no significant effects were observed in inactive lymphocytes (Fig. 4A).

To evaluate if DPH induced apoptosis in human

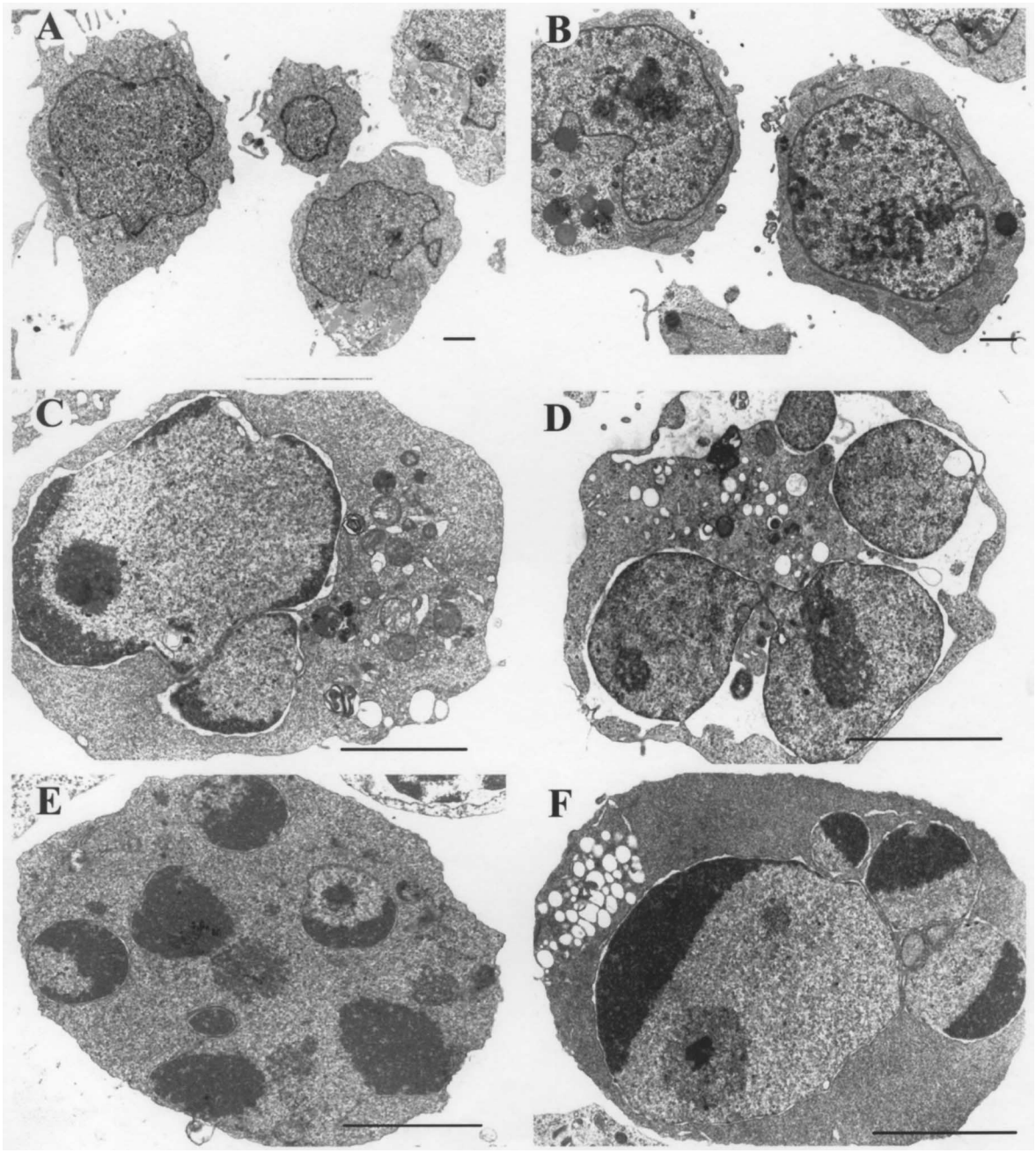


**Figure 2.** DPH-induced apoptotic ultrastructural cellular changes in CCRF-CEM and Jurkat cells. Transmission electron micrographs show apoptosis-like morphological changes induced by 0.5 mM DPH after 24-h treatment in CCRF-CEM cells (A, C, E) and in Jurkat cells (B, D, F). (A, B) Control CCRF-CEM and Jurkat cells, respectively. (C) Chromatin condensation and margination of an apoptotic CCRF-CEM cell with impaction of cellular organelles at one cellular pole. (D) Cisternal hyperdilatation around the prefragmented nucleus of an apoptotic Jurkat cell. (E) An apoptotic CCRF-CEM cell with nuclear fragments spreading into the cellular cytoplasm. (F) Chromatin condensation and margination with clustering of nuclear pores in a dilated nuclear membrane, distal to the compacted chromatin in an apoptotic Jurkat cell. Scale bars: (A, B) 2.5 μm; (C–F) 6 μm.

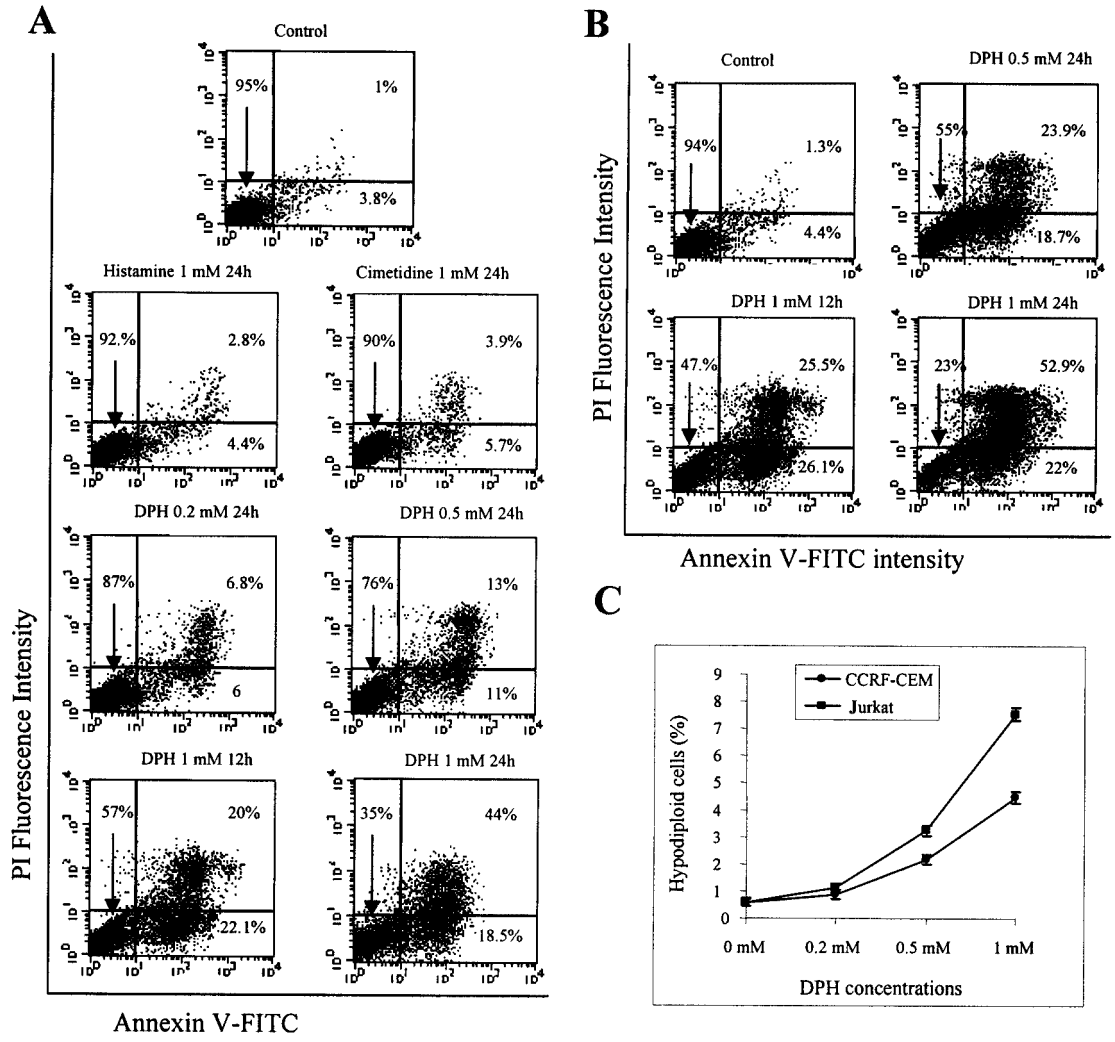


**Figure 3.** Flow cytometric analysis showing that DPH induces time- and dose-dependent apoptosis. (A) CCRF-CEM cells. (B) Jurkat cells. Untreated and treated CCRF-CEM and Jurkat leukemia cells were analyzed by flow cytometry. After 24-h incubation, cells were harvested and stained with Annexin V-FITC and propidium iodide. The percentage of early apoptotic cells in the lower right quadrant (Annexin V-FITC +ve/propidium iodide −ve cells), as well as late apoptotic cells located in the upper right quadrant (Annexin V-FITC +ve/propidium iodide +ve cells) increased in a time- and dose-dependent manner in DPH-treated cultures. DPH induced more apoptosis in Jurkat (74.9%) than in CCRF-CEM (62.5%) cells. These results are representative of three independent experiments. (C) Histogram of the DNA content of untreated and DPH-treated CCRF-CEM and Jurkat cells measured by flow cytometric analysis of the cell cycle. The percentage of hypodiploid cells at sub-$G_0/G_1$, which represent apoptotic cells with low DNA content, is presented. Cultures treated with DPH showed a dose-dependent increase in their percentage of hypodiploid cells. This percentage rose to around 75% in the case of Jurkat cells treated with 1 mM DPH for 24 h and to around 44% in the case of identically treated CEM cells.

PBMCs, analysis of hypodiploid cells was performed. The percentage of hypodiploid cells in the sub-$G_0/G_1$ peak was found to be unaltered in response to DPH treatment (Fig. 4B). This result was also verified by morphological evaluation of DPH-treated PBMCs at the electron microscopic level (data not shown). Thus, although DPH inhibited the proliferation of activated human PBMCs, it did not induce cytotoxicity in these cells.

### *DPH-Induced Apoptosis in T-ALL Cells Involves Bcl-2 and Cytochrome* c

To study the apoptotic mechanism involved, we analyzed the mitochondrial events in DPH-induced apoptosis by treating Bcl-2-overexpressing CEM-C7H2-10E1 and parental CCRF-CEM cells with various concentrations of DPH and measuring cell viability using the XTT assay. We found increased cell viability in CEM-C7H2-

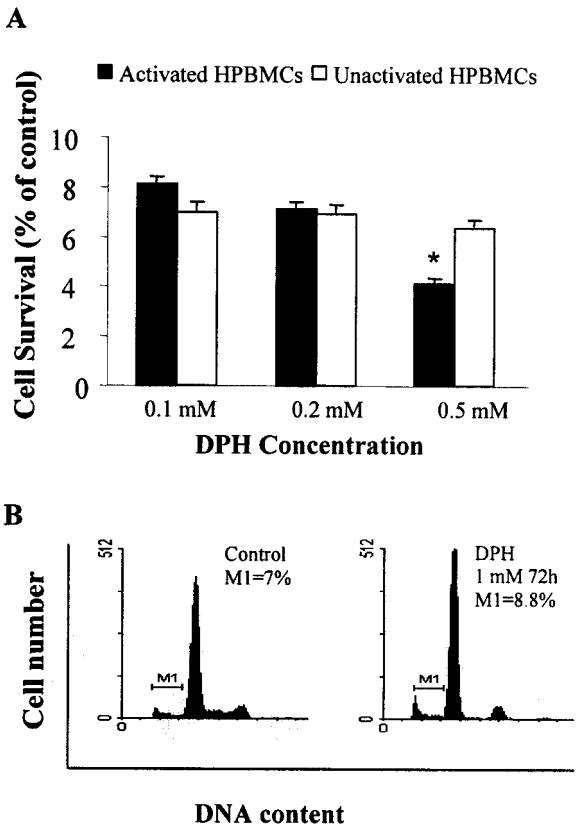


**Figure 4.** Effect of DPH on human PBMCs. (A) Cell proliferation assay. Nonactivated and 72-h PHA-M-activated human PBMCs were treated for 24 h with various doses of DPH. Cell proliferation and survival were evaluated using an XTT proliferation kit. The inhibitory effect on activated PBMC proliferation was mainly observed with 0.5 mM of DPH. $*p < 0.05$. (B) Graphs illustrating the DNA content of untreated and treated human PBMCs. DPH did not increase the sub-$G_0/G_1$ population in activated human PBMCs.

10E1 cells in comparison to parental CCRF-CEM cells. Thus, the $IC_{50}$ of DPH shifted from $550 \pm 70$ μM in parental CCRF-CEM cells to $820 \pm 50$ μM in CEM-C7H2-10E1 cells (Fig. 5A). Furthermore, DAPI staining revealed more cells with nuclear fragmentation and condensed chromatin in parental CCRF-CEM cells in comparison to DPH-treated CEM-C7H2-10E1 cells (Fig. 5B, C). The fact that overexpression of Bcl-2 significantly attenuated DPH-induced apoptosis points to the involvement of mitochondria in the apoptotic process. However, unexpectedly, we did not find elevated ROS levels in any of the DPH-treated cell lines (data not shown).

We next measured mitochondrial release of cytochrome *c* to the cytosol in response to DPH treatment (Fig. 6A). This effect was clearly reduced in Bcl-2-overexpressing CEM-C7H2-10E1 cells (Fig. 6B). Furthermore, Western blotting analysis revealed an increase in cytochrome *c* in the cytosolic protein fraction 24 h after treatment with DPH. This increase was attenuated in Bcl-2-overexpressing cells (Fig. 6C). Overall, these findings suggest that Bcl-2 may be exerting its antiapoptotic effects by attenuating the release of cytochrome *c* from the mitochondrion to the cytosol.

## DISCUSSION

In the present study, we have demonstrated that the H1 histamine receptor antagonist DPH induces apoptosis in two human T-cell ALL cell lines. The apoptosis was dose and time dependent and involves mitochondrial pathway. DPH, which is a proven, clinically safe agent without major side effects, does not induce cytotoxicity in PBMCs, raising the possibility that this histamine antagonist may have beneficial therapeutic effects in treatments aimed to selectively kill ALL-type cancer cells.

Various in vitro and in vivo studies have been carried out to examine the effects of exogenously applied histamine on different cell lines. Most of these studies revealed that exogenous histamine can act as a mediator of cell growth with either an inhibitory or stimulatory effect on cell growth (6). In contrast, in our study with two T-cell ALL cell lines and PBMCs, histamine applied exogenously at a wide range of concentrations up to 1 mM, in the presence or absence of FCS, was not found to modulate the viability of these cells. It is thus possible that these cells synthesize large quantities of endogenous histamine that are sufficient to occupy all the histamine receptors and that the addition of exogenous histamine represents saturating concentrations of the agonist.

On the basis of the present results, it appears that endogenous histamine is acting as a stimulatory factor for cell survival through H1 histamine receptors in the studied leukemia cell lines. In normal PBMCs, the absence of apoptosis indicates that histamine may act on the proliferation rather than survival signaling. Curiously, histamine has been found as an inhibitory factor for cell proliferation in different cell lines such as the pancreatic carcinoma cell line PANC-1 (9), experimental mammary carcinoma (6), and Leydig cell sarcoma-LTW (17), suggesting that the response of cells to histamine or its antagonists is cell-type specific. Similarly, we observed that histamine acts through H1 receptors in the studied cell lines to inhibit cell survival, in keeping with other reports (18,19). Nevertheless, in other cell lines, the actions of histamine are mediated by H2 receptors (20). It is thus likely that different types of tumor cell lines are characterized by a different receptor phenotype and that CCRF-CEM and Jurkat cells may express the H1 receptor predominantly.

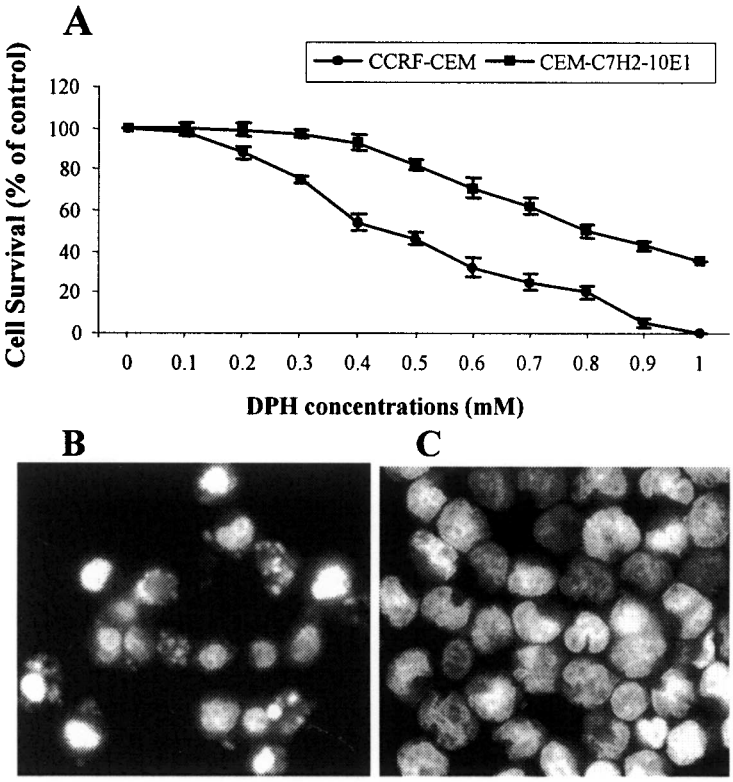


**Figure 5.** Overexpression of the Bcl-2 antiapoptotic protein partially protects CCRF-CEM cells from the effects of DPH. (A) The effect of DPH on the viability of parental CCRF-CEM cells and of CEM-C7H2-10E1 cells stably transfected cDNA containing a vector that overexpresses Bcl-2. Bcl-2-overexpressing cells are more resistant to DPH-induced apoptosis. (B) DAPI staining of parental CCRF-CEM cells treated with 0.5 mM DPH for 24 h. (C) DAPI staining of CEM-C7H2-10E1 cells. Extensive nuclear condensation and fragmentation in response to DPH were observed in parental CCRF-CEM cells in comparison to CEM-C7H2-10E1 cells in which much fewer condensed or fragmented nuclei were seen.

The cytotoxic effects evoked by DPH were observed by fluorescence and electron microscopy and consisted of morphological changes characteristic of apoptosis. Furthermore, the apoptotic process induced by DPH progressed rapidly, and secondary necrosis could be observed frequently by electron microscopic analysis. This fact may explain the high levels of LDH that were detected in the supernatants of DPH-treated cell cultures.

The in vitro effects of histamine and its receptor antagonists on lymphocytic leukemia cell proliferation have not been extensively studied to date. However, it has been demonstrated that in different B-cell ALL cell lines, histamine can act as an autocrine regulator of cell proliferation and DPH can inhibit in vitro clonogenic growth (12). On the other hand, Radvany et al. (19) demonstrated that endogenously produced histamine stimulates a T-cell ALL cell line and antigen-specific mouse lymphocytes through histamine H1 receptors. In the present work, we have shown that DPH produces cell death by apoptosis in T-cell ALL cell lines. Recently it has been demonstrated that another H1 histamine receptor antagonist (terfenadine) can also induce apoptosis in human hepatoma, colorectal cancer, and fibroblast cell lines (21). Histamine is thought to play an important role in the rapid proliferation of certain tissues and tumors (6,22) and it is thus not surprising that the interruption of histamine-mediated vital events can lead to the induction of an apoptotic program. Particularly, it is well known that histamine through the H1 receptor can modulate the intracellular $Ca^{2+}$ levels and activation of protein kinase C (23).

Mitochondria play a central role in apoptotic processes. In response to an apoptotic signal, cytochrome *c* is released from mitochondria to the cytosol to initiate the activation of caspases and finally the characteristic fragmentation of DNA. Bcl-2 directly or indirectly prevents the release of cytochrome *c* from mitochondria, so overexpression of Bcl-2 prevents cells from undergoing

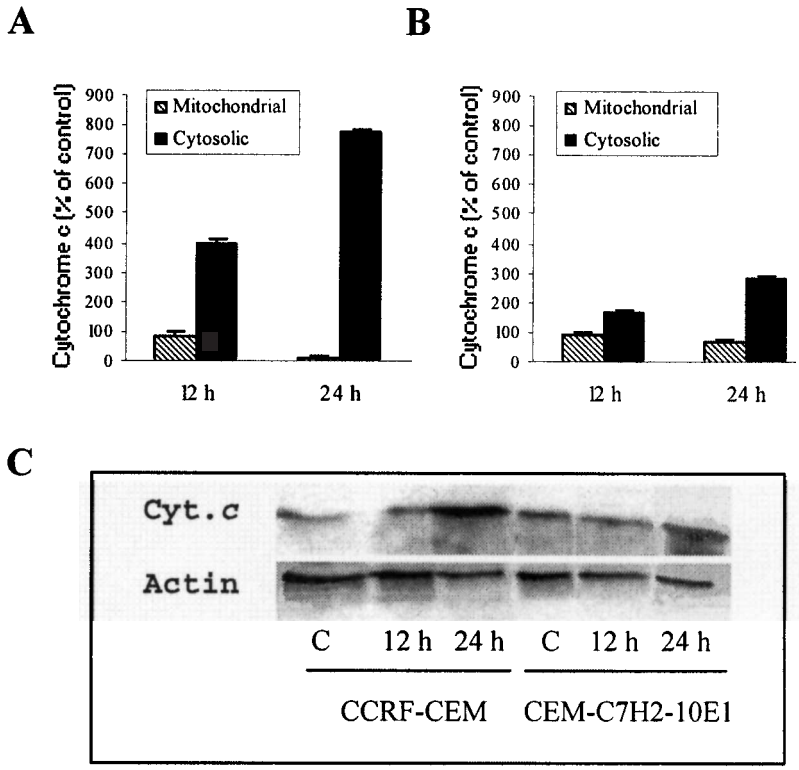


**Figure 6.** Cytochrome *c* release from mitochondria into the cytosol in CCRF-CEM and CEM-C7H2-10E1 cells induced by DPH. (A) Parental CCRF-CEM cells treated with 0.5 mM DPH for 12 and 24 h. The level of cytochrome *c* in mitochondria decreased progressively while it increased very briskly in the cytosol. (B) CEM-C7H2-10E1 cells that overexpress Bcl-2 show a less pronounced shift in the distribution of cytochrome *c* from mitochondria to the cytosol in response to DPH. The results are presented as the mean ± SD of three independent experiments (triplicate wells). Percentages are referred to untreated control cells. (C) Western blot using cytosolic protein fractions of parental CCRF-CEM cells and CEM-C7H2-10E1 cells following treatment with 0.5 mM DPH for 12 and 24 h. The levels of cytosolic cytochrome *c* increase in parental CCRF-CEM cells in response to DPH; this increase is much less apparent in Bcl-2 transfected CEM cells.

apoptosis in response to a variety of stimuli (24,25). Accordingly, we found that DPH-induced apoptosis was substantially mitigated in CEM-C7H2-10E1 cells, which stably overexpress the Bcl-2 protein, in comparison to parental CCRF-CEM cells. The mechanism by means of which Bcl-2 inhibits the release of cytochrome *c* from mitochondria is still not clear.

Although accumulation of ROS has been proposed to be an early essential event in apoptotic responses induced by a variety of proapoptotic factors (26,27), we found that DPH induced apoptosis in T-ALL cells without generating significant levels of ROS. This finding corroborates that of other studies, which concluded that ROS generation is not essential for the induction of apoptosis, particularly when the death signals for cytochrome *c* release and mitochondrial membrane potential collapse are abundant (28). Together, these findings indicate that DPH induces Bcl-2-dependent apoptosis in T-cell ALL cell lines mainly through the mitochondrial pathway.

Future studies will be aimed at elucidating the precise components of the signal transduction cascade associated with DPH proapoptotic effects and evaluating the specificity of these effects for ALL-type cancers. The effectiveness of other antihistaminic agents to induce apoptotic effects in ALL and other types of cancers should also be examined.

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