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Biochemical Pharmacology 62 (2001) 1439–1447

Biochemical Pharmacology

Induction of G_2/M phase arrest and apoptosis by a new synthetic anti-cancer agent, DW2282, in promyelocytic leukemia (HL-60) cells

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Received 1 February 2001; accepted 2 May 2001

Abstract

We studied the effect of DW2282-,{(*S*)-(+)-4-phenyl-1-[*N*-(4-aminobenzoyl)-indoline-5-sulfonyl-4,5-dihydro-2-imidazolone]·hydrochloride}, a newly developed anti-cancer agent, on cell proliferation, cell cycle progression, and induction of apoptosis in human promyelocytic leukemia (HL-60) cells. DW2282, a diarylsulfonylurea compound, was cytotoxic to HL-60 cells, with an IC_{50} of 1.0 μg/mL. Treatment with DW2282 fragmented DNA in a concentration- and time-dependent manner, suggesting that these cells underwent apoptosis. Flow cytometric analysis further confirmed that DW2282-treated HL-60 cells were hypodiploid, in terms of DNA content, and were arrested at the G_2/M phase. The cell cycle arrest was reversible upon the removal of DW2282. HL-60 cells also underwent distinct morphological changes in response to DW2282 treatment, including the appearance of elongated cells with conical tails and other apoptotic characteristics. G_2/M phase cell cycle arrest was accompanied by a decrease in the levels of cdc2, a protein that plays a critical role for progression through the G_2/M phase. Treatment of HL-60 cells with DW2282 was also associated with decreased levels of the anti-apoptotic protein Bcl-2, activation of caspase-3, and proteolytic cleavage of poly(ADP-ribose) polymerase. Taken together, these results demonstrate that DW2282 dramatically suppressed HL-60 cell growth by inducing apoptosis after G_2/M phase arrest. These findings are consistent with the possibility that G_2/M phase arrest was mediated by the down-regulation of cdc2 levels in HL-60 cells. The data also suggest that DW2282 triggered apoptosis by decreasing Bcl-2 levels and activating caspase-3 protease. These results provide important new information towards understanding the mechanisms by which DW2282 and other diarylsulfonylureas mediate their therapeutic effects.

Keywords: DW2282; G_2/M phase arrest; Apoptosis; cdc-2; Caspase-3; HL-60 cells

1. Introduction

A modified form of diarylsulfonylurea, DW2282 {(*S*)-(+)-4-phenyl-1-[*N*-(4-aminobenzoyl)-indoline-5-sulfonyl-4,5-dihydro-2-imidazolone]·hydrochloride}, has been developed as a new anti-cancer agent. Diarylsulfonylureas are a class of anti-cancer agents with significant therapeutic efficacy against certain rodent and human models of cancer [1–3]. One compound from this series, Sulofenur, has progressed to phase I and phase II clinical trials [4–6]. However, serious side-effects such as methemoglobinemia and anemia limit the doses of the drug that can be used in humans [4,5,7]. The formation of its toxic metabolite, *p*-chloraniline, causes Sulofenur toxicity in humans [4–6]. A related compound, DW2143 {4-phenyl-1-[1-(4-aminobenzoyl)-indoline-5-sulfonyl]-4,5-dihydro-2-imidazolone hydrochloride}, was designed to reduce the toxicities observed with other diarylsulfonylurea anti-cancer agents. The *S*-enantiomer of DW2143, termed DW2282, was selectively cytotoxic to murine and cancer cells [8,9]. However, the mechanism of action of DW2282 remains unknown.

Some anti-cancer agents cause cell death by apoptosis [10–14]. Apoptosis is a selective physiological process that plays an important role in the balance between cell replication and cell death. A wide range of stimuli can be integrated to trigger the irreversible decision to die. Perturbation of progression of the cell cycle and inappropriate activity of

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Abbreviations: FBS, fetal bovine serum; PARP, poly(ADP-ribose) polymerase; and MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

0006-2952/01/$ – see front matter
PII: S0006-2952(01)00796-1

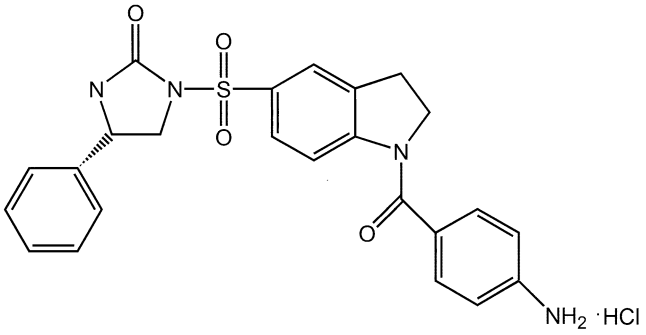

Fig. 1. Structure of DW2282.

the cyclin-dependent protein kinases are signals that can trigger apoptotic cell death [11,15]. G_2/M phase arrest is exhibited in cells exposed to a variety of DNA-damaging agents, including x-irradiation [16], microtubule-stabilizing agents [17], and topoisomerase II inhibitors [18,19]. The process by which cell cycle progression is controlled has been investigated intensively. A protein kinase (cdc2) that interacts with cyclin B has been found to play an important role in the regulation of the cell cycle [20]. Activation of the cdc2/cyclin B complex is required for transition from the G_2 to the M phase of the cell cycle [21]. Several studies have reported that various cytotoxic drugs can induce G_2/M phase arrest [12,19,22,23].

The mechanism by which diarylsulfonylureas exert cytotoxicity remains largely unknown. Several reports have suggested that the actions of diarylsulfonylureas are unrelated to the cell cycle and that these drugs do not inhibit DNA or RNA synthesis [5,24]. Diarylsulfonylureas are membrane-active and weak uncouplers of mitochondrial oxidative phosphorylation. Thus, the mitochondria have been suggested as a possible site of action [24–26]. However, it has also been reported that the anti-cancer activity of diarylsulfonylureas is not the result of their uncoupling action [26]. The purpose of this study was to characterize the mechanism of action of a newly developed diarylsulfonylurea, DW2282, in the human promyelocytic leukemia HL-60 cell line. Here we report that DW2282 induces cytotoxicity, cell cycle arrest, and apoptosis in HL-60 cells.

2. Materials and methods

2.1. Cell culture and drug treatment

Human promyelocytic leukemia cells (HL-60, ATCC CCL240) were maintained in the logarithmic phase of growth in RPMI 1640 (Gibco BRL) supplemented with 10% FBS, 2 mM L-glutamine, and 1 mM sodium pyruvate. Logarithmically growing HL-60 cells were used for all experiments. DW2282 (Fig. 1) was synthesized by the Dong Wha Pharmaceutical Ind. Co. Ltd. DW2282 was dissolved in DMSO at a concentration of 1 mg/mL and diluted in tissue culture medium before use.

2.2. Cytotoxicity analysis

Exponentially growing HL-60 cells were seeded at 5×10^4 cells/well in a 96-well plate and treated with DW2282 or vehicle, as indicated. After incubation for various times, the general viability of cultured cells was determined by assaying for the reduction of MTT to formazan [27]. Cell viability was also measured by trypan blue exclusion. The beginning number of HL-60 cells was 5×10^5 cells/mL. Cells that were (a) morphologically similar to the control viable cells and (b) were greater than 50% of the average size of these controls were designated as viable. Cells that did not meet these criteria were counted as dead cells. All experiments were performed in triplicate.

2.3. Morphology observation

Cells used in this study were observed constantly under an inverted phase-contrast microscope (Olympus). Logarithmically growing HL-60 cells were placed in a 24-well plate at a concentration of 2×10^6 cells/mL. Photographs were taken after cells were incubated with various concentrations of DW2282 for various time periods, as described in the figure legends.

2.4. DNA fragmentation analysis

DNA was purified as previously described [28]. The HL-60 cells were grown to a density of 2×10^6 cells/mL and exposed to DW2282 for different time periods at concentrations as described in the figure legends. Cells were rinsed with ice-cold PBS, centrifuged, and resuspended in TE buffer (10 mM Tris–Cl, 1 mM EDTA, pH 8.0) to a concentration of 5×10^7 cells/mL. The resulting purified DNA was subjected to electrophoresis on 1.5% agarose gels and visualized with ethidium bromide staining. Results shown are an example of three different experiments.

2.5. Flow cytometry analysis

The effects of DW2282 on cell proliferation were evaluated by measuring the distribution of the cells in the different phases of the cell cycle by flow cytometry. Cells were treated with DW2282 for the indicated times and harvested by centrifugation at $750 \times g$ for 5 min at room temperature. Cell pellets were rinsed with PBS, suspended in a 1:1 (v/v) solution of PBS and 0.2 M Na_2HPO_4-0.1 M citric acid (pH 7.5), and fixed with cold ethanol at 4° for 1 hr. Fixed cells were washed with PBS and resuspended in a staining solution containing propidium iodide (10 μg/mL) and DNase-free RNase (100 μg/mL). The cell suspensions were incubated at 37° for 1 hr in the dark and analyzed on a fluorescence-activated cell sorter flow cytometer (FACS-

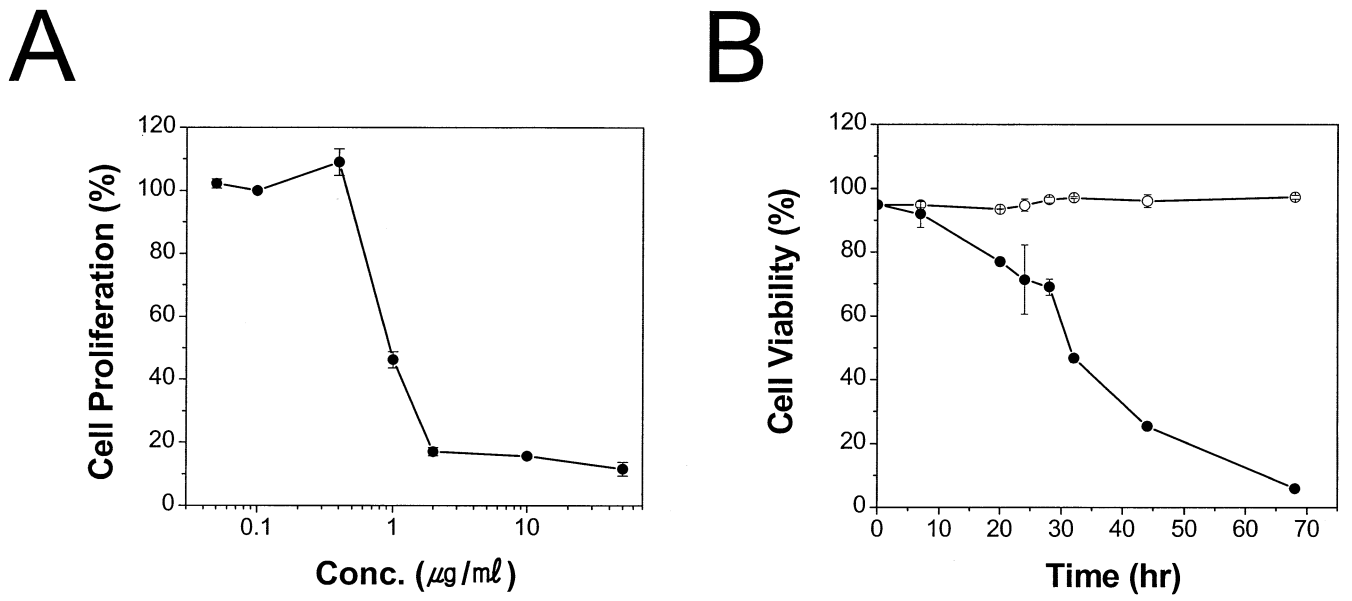

Fig. 2. Antiproliferative effect of DW2282 on HL-60 cells. (A) Cells were incubated with 0, 0.01, 0.1, 1.0, or 10 μg/mL of DW2282 for 48 hr, and cell survival was measured by the MTT assay. (B) The trypan blue dye exclusion assay was used to measure the survival of cells after treatment with (●) or without (○) 1.0 μg/mL of DW2282. Values are means ± SD, N = 3.

caliber, Becton Dickinson). Results shown are an example of three different experiments.

2.6. Western blot analysis

After DW2282 treatment, cells were washed with PBS and lysed in a buffer containing 20 mM Tris–Cl, 150 mM NaCl, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM $NaVO_3$, 100 mM NaF, 10% glycerol, 1 mM EGTA, 10 mM sodium pyrophosphate, and 1 mM phenylmethylsulfonyl fluoride, pH 7.5. Cell lysates were centrifuged at 12,000 *g* for 125 min at 4°, and the protein content was determined by the method of Lowry *et al.* [29]. After SDS–PAGE, proteins were transferred to nitrocellulose membranes (S&S) for 2 hr at 80 mA. Blots were probed with mouse monoclonal anti-human anti-Bcl-2 (Oncogene), anti-caspase-3 (Transduction Laboratory), and rabbit monoclonal anti-human anti-PARP (Santa Cruz) antibodies. Immunoreactivity was detected using either an anti-mouse (Santa Cruz) or anti-rabbit (Amersham) peroxidase-conjugated secondary immunoglobulin G antibody followed by enhanced chemiluminescence (ECL, Amersham). Experiments were repeated at least three times.

3. Results

3.1. Inhibition of the proliferation, and alteration of the morphology, of HL-60 cells by DW2282

The effect of DW2282 on cellular proliferation was evaluated using the MTT assay. A 48-hr exposure to DW2282 dramatically decreased the proliferation of HL-60 cells in a concentration-dependent manner (Fig. 2A). The concentration required to inhibit growth by 50% (IC_{50}) was approximately 1.0 μg/mL. A shorter period of treatment (24 hr) with 1.0 μg/mL of DW2282 reduced growth inhibition only slightly (45.1% inhibition, data not shown). Relative cell survival was also assessed at various times after exposure to 1.0 μg/mL of DW2282, as shown in Fig. 2B. Prolonged exposure to DW2282 markedly decreased the viability of these cells, such that after 68 hr, virtually no viable cells remained. Control cells treated with vehicle alone showed no changes in cell proliferation or viability.

Morphological analysis of HL-60 cells treated with DW2282 revealed striking changes. Cellular morphology progressively changed with increasing duration of exposure to DW2282. At the start of treatment, cells were rounded in shape but became elongated (with conical tails) with time. These morphological changes first appeared after 3 hr of treatment with the drug, and the number of cells displaying such changes increased with increasing time of incubation (Fig. 3A). These morphological changes also occurred in a concentration-dependent manner (Fig. 3B). As little as 0.1 μg/mL of DW2282 induced distinct morphological changes in HL-60 cells. Along with the appearance of elongated cells, disintegrated cells, as evidenced by apoptotic bodies, and cells with condensed nuclear chromatin appeared in time- and concentration-dependent fashions in response to DW2282 treatment (Fig. 3, A and B). The elongated morphology preceded the appearance of apoptotic cells.

3.2. Induction of apoptosis after G_2/M phase arrest

To investigate the mechanism by which DW2282 induces cell death, we analyzed chromosomal DNA from

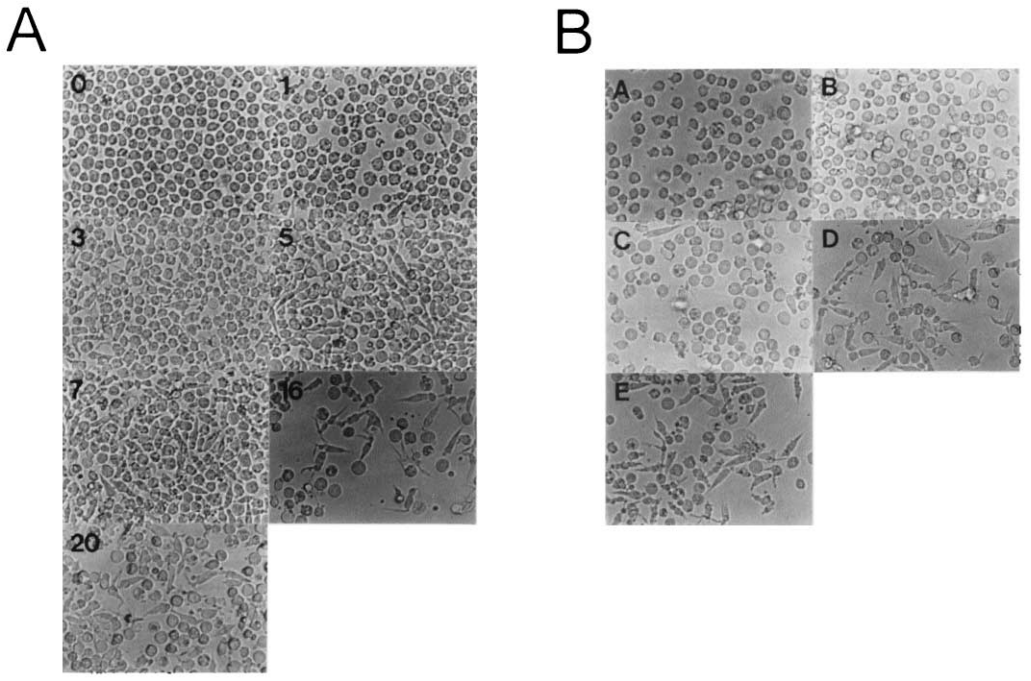

Fig. 3. Morphological changes in HL-60 cells in response to DW2282. (A) Cells were treated with 1.0 μg/mL of DW2282 for 1, 3, 5, 7, 16, or 20 hr, as indicated. (B) Cells were treated with 0 (a), 0.01 (b), 0.1 (c), 1.0 (d), or 10 μg/mL (e) of DW2282 for 16 hr. Cells were observed under a phase-contrast microscope after drug treatment. Morphologically distinct and apoptotic cells were observed among the DW2282-treated HL-60 cells.

control and DW2282-treated cells. Compared with DNA from control cells, treatment with DW2282 induced apoptosis, as shown by the formation of distinct internucleosomal DNA fragments (Fig. 4). The intensity of the DNA banding ladder progressively increased in a time- and concentration-dependent manner. The DNA fragmentation was first observed after a 7-hr incubation with DW2282 (Fig. 4A), and occurred at concentrations of DW2282 of 1.0 μg/mL and higher (Fig. 4B).

We further investigated the effects of DW2282 on the progression of HL-60 cells through the cell cycle and on the induction of apoptotic bodies. HL-60 cells were cultured for various times in the presence or absence of DW2282 and analyzed by flow cytometry. As shown in Fig. 5, DW2282 induced a time- and concentration-dependent accumulation of HL-60 cells in the G_2/M phase of the cell cycle. Cell cycle arrest at the G_2/M phase first appeared after 7 hr in response to treatment with DW2282 (1.0 μg/mL). Maximal G_2/M phase arrest was observed after a 24-hr exposure to DW2282. When cells were treated with various concentrations of DW2282 for 24 hr, a similar level of G_2/M phase arrest was observed in response to 0.1, 1.0, and 10 μg/mL.

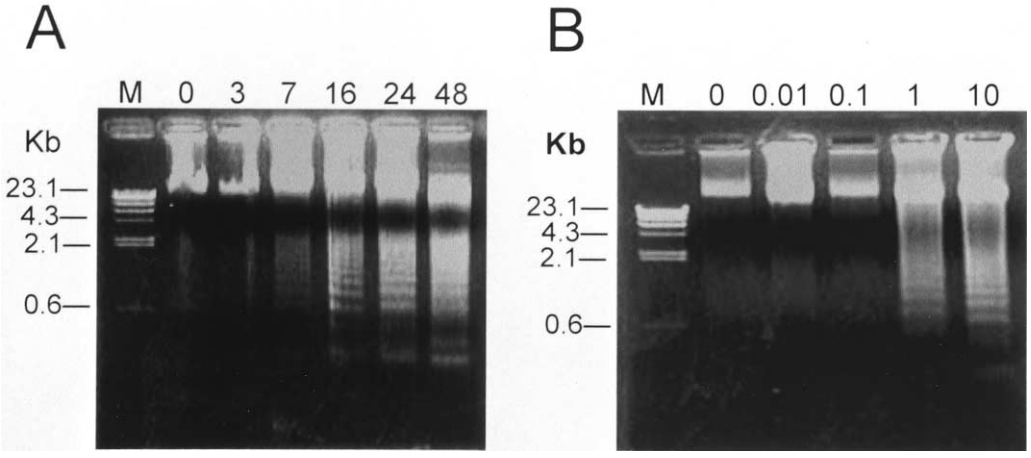

Fig. 4. Induction of time- and concentration-dependent fragmentation of nuclear DNA by DW2282 treatment. (A) HL-60 cells were incubated with 1.0 μg/mL of DW2282 for 0, 3, 7, 16, 24, or 48 hr, as indicated. (B) HL-60 cells were incubated with 0, 0.01, 0.1, 1.0, or 10 μg/mL of DW2282 for 24 hr, as indicated. DNA was isolated from the cells and analyzed on 1.5% agarose gels by ethidium bromide staining, as described in "Materials and methods."

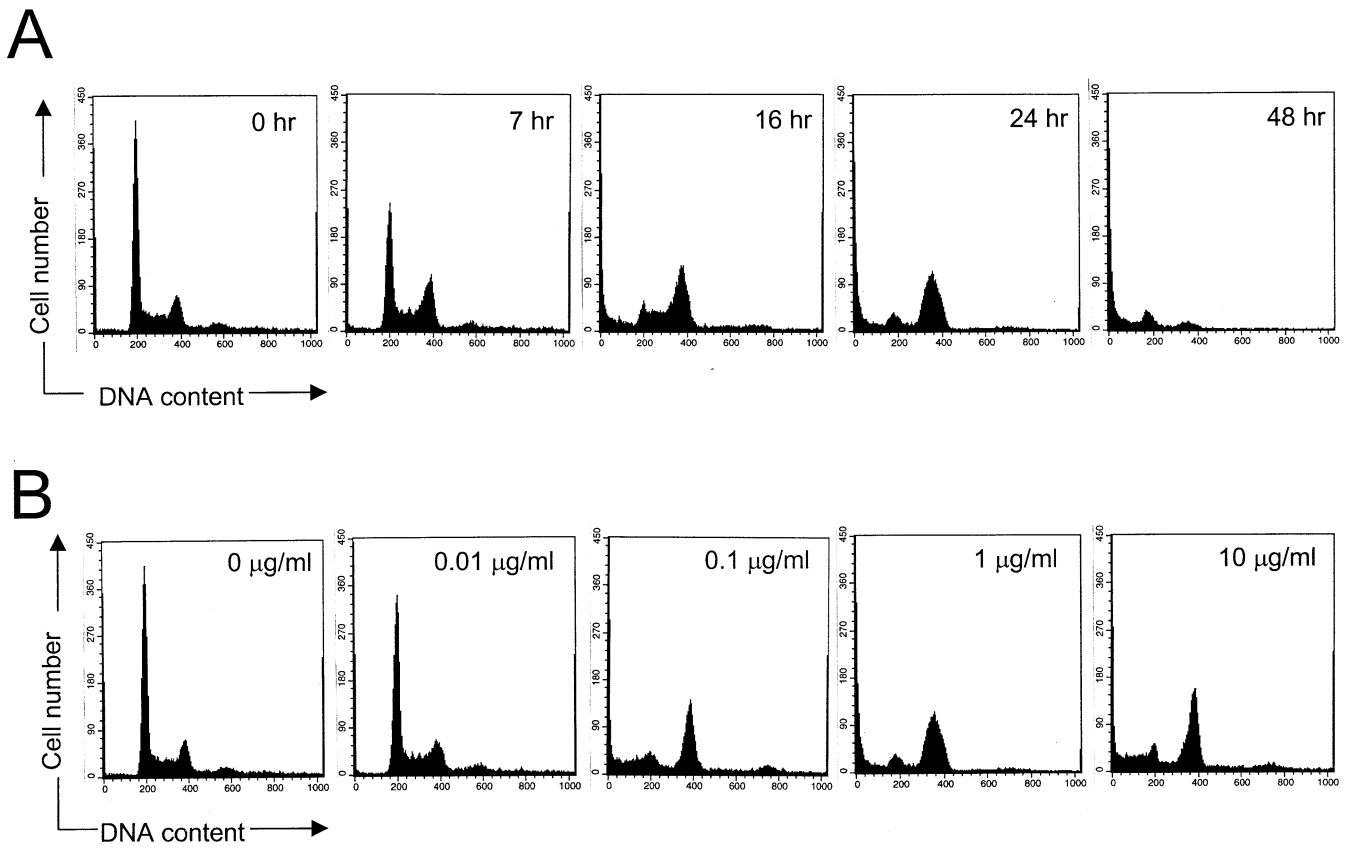

Fig. 5. Effect of DW2282 on progression through the cell cycle. HL-60 cells were cultured either (A) in the presence of 1.0 μg/mL of DW2282 for the indicated incubation times, or (B) for 24 hr with various concentrations of DW2282, as indicated. Cells were stained with propidium iodide and analyzed by flow cytometry, as described in "Materials and methods."

Cell cycle progression was not arrested following treatment with medium containing DMSO alone (0 hr and 0 μg/mL in Fig. 5). After cell cycle arrest at the G_2/M phase, DW2282-treated cells underwent apoptosis. Cells with sub-G_1 levels of DNA were scored as apoptotic, as described in a previous study [30]. After a 24-hr treatment with 1.0 μg/mL of DW2282, 45% of the cells displayed an apoptotic/hypodiploid peak. Of the total number of cells, 3.3 and 29% were contained in the apoptotic region after 7- and 16-hr exposures to DW2282, respectively. After 48 hr of exposure to DW2282, 86% of the cells underwent apoptosis. In addition, the relative ratio of cells in the apoptotic/hypodiploid peak corresponded proportionately to the concentration of DW2282 used. No apoptotic cells were observed in samples treated with levels lower than 0.01 μg/mL for 24 hr. At concentrations of 0.1, 1.0, and 10 μg/mL of DW2282 for 24 hr, about 40% of the cells were localized within the apoptotic/hypodiploid peak.

3.3. Reversibility of DW2282-induced G_2/M phase arrest

We next determined whether the G_2/M phase arrest that was induced by DW2282 was reversible. HL-60 cells were cultured in medium containing 1.0 μg/mL of DW2282 for 16 hr. Then the cultures were washed three times with PBS, replenished with fresh medium in the absence of DW2282, and returned to the incubator at 37° for an additional 1, 3, 6, or 24 hr. Flow cytometric analyses of HL-60 cells harvested at time points post release are depicted in Table 1. After 16 hr of treatment with 1.0 μg/mL of DW2282 (0 hr in Table

Table 1
Time course analysis of the cell cycle in DW2282-treated cells after release from DW2282 treatment

Time (hr)	Hypodiploid cells (%)	G_1 (%)	S (%)	G_2/M (%)
Control	0	61.9	20.07	17.4
0	23.0	18.3 (23.8)[a]	12.0 (15.6)	46.7 (60.6)
1	19.1	23.1 (28.5)	12.1 (15.0)	45.7 (56.5)
3	29.7	39.0 (55.5)	9.9 (14.1)	21.4 (30.4)
6	24.3	53.7 (70.9)	6.7 (8.9)	15.3 (20.2)
24	26.5	47.2 (64.2)	13.4 (18.2)	12.9 (17.6)

HL-60 cells were treated with DW2282 (1.0 μg/mL) for 16 hr, at which time the cells were washed and incubated in medium lacking DW2282, as described. Then cells were harvested at the indicated time points after DW2282 washout and analyzed by flow cytometry. Data shown are the means of duplicate experiments.

[a]Percentage of cells in cell cycle except for hypodiploid cells is shown in parentheses.

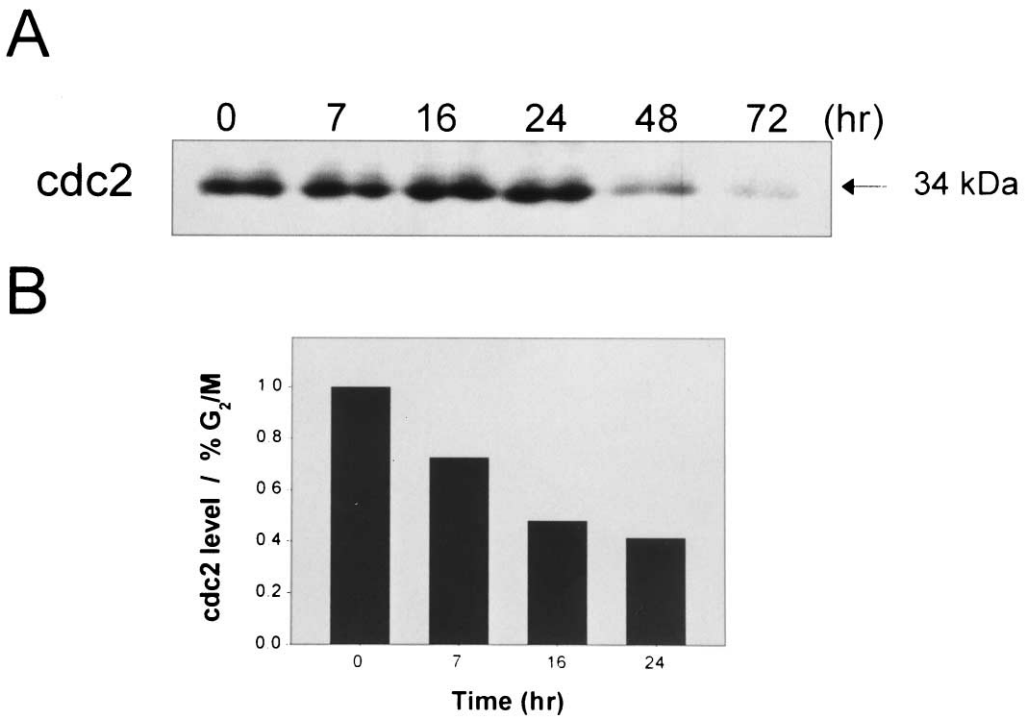

Fig. 6. Down-regulation of cdc2 levels by DW2282 treatment in HL-60 cells. (A) Cells were treated with DW2282 (1.0 μg/mL) for various times between 0 and 72 hr, as indicated. The resulting cell lysates were subjected to immunoblotting with an anti-cdc2 antibody, as described in "Materials and methods." (B) Level of cdc2 in DW2282-treated cells normalized to the percentage of cells in G_2/M.

1), 60.6% of the cells were blocked in the G_2/M phase. After DW2282 was washed out, the percentage of cells in the G_1 phase increased in a time-dependent manner. These data indicated that between 6 and 24 hr after removal of DW2282 from the cells, the majority of G_2/M arrested cells had progressed through mitosis and exhibited a normal DNA cell cycle profile. About 20% of the total number of cells were hypodiploid after 16 hr of treatment with DW2282. These cells did not resume cycling, but rather appeared to undergo apoptotic cell death.

3.4. Down-regulation of cdc2 levels in response to DW2282

Because DW2282 arrested HL-60 cells at the G_2/M phase, it was of interest to test the effects of this drug on cdc2 levels. No major effect on steady-state levels of cdc2 immunoreactivity was observed for up to 24 hr of DW2282 treatment (Fig. 6A). However, cdc2 immunoreactivity levels were reduced significantly in cells treated with DW2282 for 48 or 72 hr. It is important to note that the percentage of cells in the G_2/M phase was increased in DW2282-treated cells, as shown in Fig. 5. When the band intensity of cdc2 was normalized to the higher percentage of cells in the G_2/M phase after 0, 7, 16, and 24 hr of exposure to DW2282, a time-dependent reduction of cdc2 immunoreactivity was clearly evident (Fig. 6B). Thus, we conclude that DW2282 treatment reduced the steady-state levels of cdc2 during the HL-60 cell cycle.

3.5. Induction of the down-regulation of Bcl-2, activation of caspase-3, and the cleavage of PARP by DW2282 treatment

To investigate the mechanism by which DW2282 causes apoptosis, we also tested the effects of this compound on levels of Bcl-2, an important regulator of apoptotic signaling pathways [31]. As shown in Fig. 7, western blot analysis revealed that DW2282 treatment decreased Bcl-2 protein levels. We also found that DW2282 induced the proteolytic processing of caspase-3 in a time-dependent manner. Activation of caspase-3 leads to the cleavage of a number of proteins, one of which is PARP. Although PARP is not essential for cell death, the cleavage of PARP is another hallmark of apoptosis. DW2282 treatment also induced a time-dependent proteolytic cleavage of PARP, with concomitant accumulation of the 85 kDa form and the disappearance of the full-size 116 kDa molecule (Fig. 7). Taken together, these findings suggest that DW2282 induced apoptosis through down-regulation of Bcl-2 and activation of caspase-3.

4. Discussion

DW2282, a member of the family of diarylsulfonylureas, is a novel anti-cancer agent that inhibits cell proliferation in several cancer cell lines [9]. This compound was developed in hopes of reducing the negative side-effects associated

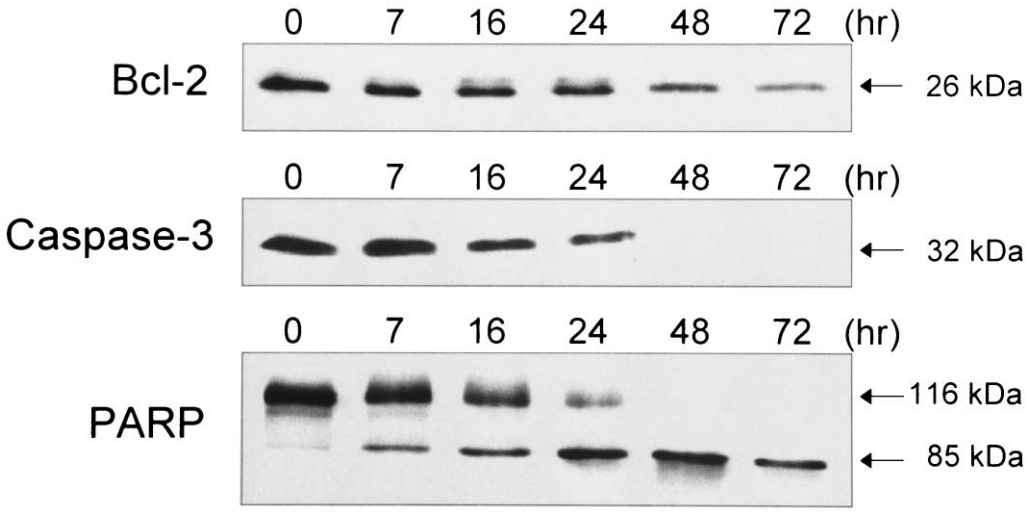

Fig. 7. Down-regulation of Bcl-2, activation of caspase-3, and induction of PARP cleavage by DW2282 treatment. Exponentially growing HL-60 cells were treated with 1.0 μg/mL of DW2282 for the indicated periods of time. Cells were washed with PBS and lysed, and the resulting cell lysates were subjected to immunoblotting with anti-Bcl-2, anti-caspase-3, or anti-PARP antibodies, as described in "Materials and methods."

with other diarylsulfonylureas, as discussed above. Indeed, DW2282 appears to be less toxic, in that it does not induce methemoglobinemia or hypoglycemia [32]. Thus, this compound represents a promising candidate as an anti-cancer agent. The mechanism of anti-cancer activities of diarylsulfonylureas is not yet clear. Previous studies have found no evidence that these compounds target the cell cycle or inhibit DNA, RNA, or protein synthesis [7,24].

Our study demonstrated that DW2282, a novel anti-cancer agent, inhibits cell proliferation and induces apoptosis by arresting human promyelocytic leukemia HL-60 cells in the G_2/M phase. Alterations in cell morphology, fragmentation of DNA, and the appearance of hypodiploid cells all indicate that DW2282 induced apoptosis in these cells. Flow cytometric analysis revealed that DW2282-treated HL-60 cells were arrested in the G_2/M phase. In addition, we showed that this G_2/M phase arrest is reversible by removal of DW2282 from the culture medium.

Many anti-cancer agents and DNA-damaging agents arrest the cell cycle at the G_1, S, or G_2/M phase and then induce apoptotic cell death [33–38]. The cell cycle checkpoints may function to ensure that cells have time for DNA repair [39,40], whereas apoptotic cell death may function to eliminate irreparable or unrepaired damaged cells [41]. DW2282 arrested HL-60 cells in the G_2/M phase. To further analyze the molecular mechanism by which DW2282 causes cell cycle arrest, we evaluated cdc2 protein levels. The cdc2/cyclin B complex is one of the major regulatory elements governing the G_2 to M progression [42]. Cdc2 is activated by phosphorylation and by binding to cyclin B, which is synthesized during the S and G_2 phases of the cell cycle. We found that DW2282 treatment reduced cdc2 immunoreactivity levels when the data were normalized to the percent of cells in G_2/M as a function of time exposed to DW2282, as shown in Fig. 6. Thus, our data suggest that cell cycle arrest is mediated by limitation of the supply of cdc2 to cdc2/cyclin B complex formation, which is an essential step in regulating passage into mitosis. G_2/M phase arrest was largely reversible when DW2282 was removed from the culture medium. The DW2282-damaged cells entered mitosis, and then they digested their DNA in a manner characteristic of apoptosis. When DW2282 was removed from the medium, a normal distribution of cells throughout the cell cycle was restored. However, about 20% of the cells remained hypodiploid even after removal of DW2282 from the medium (Table 1). These cells most likely represent damaged cells that could not be repaired and restored to progress normally through the cell cycle, and hence die by apoptosis.

Pronounced morphological changes correlated with the cell cycle arrest induced by DW2282. HL-60 cells became elongated with conical tails. This morphology has also been associated with G_2/M phase arrest in rat neuronal cells and human prostate carcinoma cells [43,44]. Apigenin, a flavone, has been reported to induce G_2/M phase arrest and morphological elongation and arborization of neutrites in rat neuronal cells [44]. Genistein, a natural isoflavonoid phytoestrogen, also blocked the cell cycle at the G_2/M transition and induced morphological changes. Genistein-treated human prostate carcinoma cells developed a dendrite-like structure that became progressively elongated over time [44]. In addition to the appearance of these elongated cells, HL-60 cells with apoptotic characteristics, namely chromatin condensation, DNA fragmentation, and cell shrinkage, appeared after longer incubation times in DW2282-treated cells.

Apoptosis is an active process that ultimately leads to the activation of endonucleases and cleavage of DNA into fragments of about 180–200 base pairs [45]. Exposure to DW2282 fragmented cellular DNA in a pattern character-

istic of apoptotic cell death. Cells with sub-G_1 DNA content were also detected after G_2/M phase arrest by FACS analysis, which is also consistent with apoptosis. The onset of these cytofluorimetric alterations closely correlated with the time- and concentration-dependence of the biochemical indicators of apoptosis.

One of the best-characterized regulators of apoptosis is the Bcl-2 family of proteins. Bcl-2 is an intracellular suppressor of apoptosis and thus serves a cyto-protective function in cells [46]. We found that DW2282 treatment decreased Bcl-2 immunoreactivity levels in HL-60 cells. Bcl-2 functions by heterodimerizing with its pro-apoptotic relative, Bax [47]. Therefore, the ratio of Bcl-2:Bax is proportional to the relative sensitivity or resistance of cells to a wide variety of apoptotic stimuli [48]. The induction of apoptosis by DW2282 may be caused by the reduction in Bcl-2 levels. In addition, the cleavage of caspase-3 appears to correlate with DW2282-induced apoptosis in HL-60 cells. Caspase-3, a cysteine protease that exists as an inactive zymogen in cells, is activated by sequential proteolytic events that cleave the 32-kDa precursor at an aspartic acid residue to generate active heterodimers comprised of 20- and 12-kDa subunits [49]. The activity of caspase-3 was confirmed by the cleavage of PARP.

In conclusion, DW2282, a novel anti-cancer agent that was developed to reduce the negative side-effects observed in other diarylsulfonylurea drugs, arrested the cell cycle at the G_2/M phase and induced apoptosis of HL-60 cells. DW2282 exposure also reduced cdc2 levels in cells in the G_2/M phase. Our studies suggest that HL-60 cells with irreparable damage from DW2282 treatment underwent apoptosis. We further hypothesize that apoptosis was induced by a decrease in the level of Bcl-2 and a concomitant activation of caspase-3 in HL-60 cells treated with DW2282. Taken together, these findings provide important new insights into the possible molecular mechanisms of the anti-cancer activity of DW2282.

Acknowledgments

This work was supported by a grant from the Good Health R&D project (HMP-98-DA-7-0015), the Ministry of Health and Welfare, Korea. W. Piao is a graduate fellow of the Brain Korea 21 program of the Ministry of Education, Korea.

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