Geldanamycin and Its Analogue 17-Allylamino-17-demethoxygeldanamycin Lowers Bcr-Abl Levels and Induces Apoptosis and Differentiation of Bcr-Abl-positive Human Leukemic Blasts

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Abstract

HL-60/Bcr-Abl cells, with ectopic expression of p185 Bcr-Abl tyrosine kinase (TK), and K562 cells, with endogenous expression of p210 Bcr-Abl TK, display a high degree of resistance against antileukemic drug-induced apoptosis (G. Fang et al., Blood, 96: 2246–2256, 2000). Present studies demonstrate that treatment with ansamycin antibiotic geldanamycin (GA), or its less toxic analogue 17-allylamino-17-demethoxygeldanamycin (17-AAG), induces cytosolic accumulation of cytochrome c and cleavage and activities of caspase-9 and caspase-3, triggering apoptosis of HL-60/Bcr-Abl and K562 cells. GA or 17-AAG down-regulated intracellular Bcr-Abl and c-Raf protein levels, as well as reduced Akt kinase activity. Similar to Raf-1, v-Src, and Her-2-neu, Bcr-Abl TK has caspase association with heat shock protein 90 (Hsp90). By binding and inhibiting Hsp90, GA or 17-AAG treatment shifted the binding of Bcr-Abl from Hsp90 to Hsp70 and induced the proteosomal degradation of Bcr-Abl, because cotreatment with proteasome inhibitor PSC341 reduced both GA (or 17-AAG)-mediated down-regulation of Bcr-Abl levels and inhibited apoptosis of HL-60/Bcr-Abl and K562 cells. These data establish the in vitro activity of GA and 17-AAG against Bcr-Abl-positive leukemic cells and support the in vivo investigation of 17-AAG against Bcr-Abl-positive leukemias.

Introduction

The Bcr-Abl fusion gene encodes for the p210 or p185 TK2 implicated in the pathogenesis of CML or ALL, respectively (1, 2). Ectopic expression of p185 Bcr-Abl in human myeloid leukemia HL-60 (HL-60/Bcr-Abl) or the endogenous expression of p210 Bcr-Abl in the CML blast crisis K562 cells confers resistance against apoptosis even when exposed to high doses of antileukemic drugs (3, 4). Bcr-Abl expression was demonstrated to block the mitochondrial permeability transition (Δψm) and release of cyt c, thereby inhibiting the activation of the executioner caspases and apoptosis (4). Ectopic or endogenous Bcr-Abl expression up-regulates several antiapoptotic mechanisms, including the levels of Bcl-xL, as well as the activities of NF-κB and Akt kinase (3–6). Recent studies have shown that inhibition of Bcr-Abl TK activity by a relatively specific inhibitor, STI571, induces differentiation and apoptosis as well as causes in vitro and in vivo eradication of Bcr-Abl-positive human leukemia cells (7, 8). Exposure to STI571 lowered Bcl-xL levels and Akt kinase and NFκB activities as well as induced intracellular Hb and differentiation in Bcr-Abl-positive acute leukemia cells (7). Preclinical studies have indicated that agents that lower Bcr-Abl expression could also be highly effective against Bcr-Abl-positive leukemias (9–12). Although arsenic trioxide (As2O3) is an active agent against acute promyelocytic leukemia cells (13), it was also demonstrated to lower Bcr-Abl levels and induce apoptosis of HL-60/Bcr-Abl and K562 cells (14). Taken together, these reports indicate that multiple strategies that lower the levels or activity of Bcr-Abl TK may have efficacy against Bcr-Abl-positive leukemias.

The benzoquinone ansamycin antibiotic GA and its analogue 17-AAG bind strongly to the Hsp90 and specifically disrupt its chaperone function for several transcription factors (e.g., steroid, hormone, and retinoid receptors and hypoxia-inducible factor) and protein kinases (e.g., v-Src, Raf-1, Ick, Wee 1, and cyclin-dependent kinase 4; Refs. 15–21). This results in their reduced stability and promotes degradation by the proteasome-based mechanism (15, 16, 22). This may be biologically significant, because another ansamycin, herbimycin A, a known tyrosine kinase inhibitor, can revert the morphological phenotype of v-Src-transformed cells (20). Previous studies have also demonstrated that herbimycin A can induce differentiation (erythroid) of K562 cells (23). Therefore, we investigated whether GA or 17-AAG would also affect Bcr-Abl levels and its antiapoptotic effects in HL-60/Bcr-Abl and K562 cells. In the present report, we demonstrate that GA and 17-AAG decreased the association of Bcr-Abl with HSP90, resulting in its degradation through the proteasomes. Consequently, GA or 17-AAG treatment lowers Bcr-Abl levels, thereby inducing apoptosis and Hb in HL-60/Bcr-Abl and K562 cells.

Materials and Methods

Reagents. GA and 17-AAG were kindly provided by the developmental therapeutics branch of Cancer Therapy Evaluation Program/National Cancer Institute/NIH (Bethesda, MD). Proteasome inhibitor PSC341 was kindly provided by Dr. Peter Elliott (Proscript, Inc., Cambridge, MA). The Akt kinase assay kit was purchased from New England Biolabs (Beverly, MA). Anti-Bid and anti-caspase-9 antibodies were kindly provided by Dr. Xiaodong Wang (University of Texas, Southwestern School of Medicine, Dallas, TX; Ref. 24). Monoclonal anti-XIP, anti-Hsp90, anti-Hsp70, or anti-caspase-9 antibodies were purchased from StressGen Biotechnologies Corp. (Victoria, British Columbia, Canada). Polyclonal anti-poly(ADP-ribose) polymerase was purchased from Pharmingen, Inc. (San Diego, CA). Anti-c-Raf antibody was purchased from Transduction Labs (Cincinnati, OH; Ref. 24).

Cell Culture and Cell Growth Inhibition. Human leukemia cells HL-60/neo, HL-60/Bcr-Abl, and K562 cells were cultured and passaged as described previously (4, 7). Logarithmically growing cells were exposed to the designated concentrations of either GA or 17-AAG and/or PS341. After these treatments, cells were pelleted and washed free of the drug(s) prior to the performance of the studies described below.

Preparation of S-100 Fraction and Western Analysis of Cytosolic cyt c. Untreated and drug-treated cells were harvested by centrifugation at 1000 × g for 10 min at 4°C. The cell pellets were washed once with ice-cold PBS and resuspended with five volumes of buffer (20 mM HEPES-KOH (pH 7.5), 10 mM KCl, 1.5 mM MgCl2, 1 mM sodium EDTA, 1 mM sodium EGTA, 1 mM DTT, and 0.1 mM PMSF), containing 250 mM sucrose. The cells were homog-
enized with a 22-gauge needle, and the homogenates were centrifuged at 100,000 × g for 30 min at 4°C (S-100 fraction; Refs. 4 and 25). The supernatants were collected, and the protein concentrations of S-100 were determined by Bradford method (Bio-Rad, Hercules, CA). Twenty to 30 µg of the S-100 fraction were used for Western blot analysis of cdc 4 as described previously (4 and 25).

**Immunoprecipitation and Immunoblotting Analyses.** After the designated treatment, cells were lysed in the lysis buffer (1% SDS, 1% Triton X-100, 0.5% deoxycholate, 150 mM NaCl, 2 mM EDTA, 1 mM PMSE, and 10 µg/ml leupeptin) for 1 h, and the nuclear and cellular debris was cleared by centrifugation. Protein G-agarose beads were washed twice with RIPA 1 buffer [50 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% NP 40, and 0.1% deoxycholate] and then incubated with Ab-specific mAb (1 in 100; Santa Cruz) at 4°C for 2 h. After washing the Protein G and the antibody mix with RIPA 1 buffer, 100 µg of total cell lysates were added and incubated overnight at 4°C. The immunoprecipitates were washed three times in RIPA 1 buffer, and proteins were eluted with the SDS sample loading buffer. Proteins were separated by SDS-PAGE as described (24, 25). Immunoprecipitates were examined by Western blot analysis after transfer of proteins to nitrocellulose membranes. Western blot analyses were performed using anti-Hsp90 and anti-Hsp70 antibodies (24, 25).

**Preparation of Detergent-soluble and Insoluble Fractions.** After the designated drug treatments, cells were lysed with TNEVE buffer (50 mM Tris-HCl (pH 7.5), 2 mM EDTA, 100 mM NaCl, 1 mM sodium orthovanadate, 1% NP40 containing 20 µg/ml aprotinin, 20 µg/ml leupeptin, 1 mM PMSE, 25 mM NAF, and 5 µM 1-Nethylmaleimide). NP40 in soluble proteins were solubilized with SDS buffer [2% SDS, 100 mM DTT, 80 mM Tris (pH 6.8), 10% glycerol]. Thirty µg of proteins from the NP40 soluble and insoluble fraction were separated on 7.5% SDS-polyacrylamide gels and analyzed by Western blotting (24, 25, 27).

**Western Analyses of Proteins.** Western analyses of caspase-9, caspase-3, Bid, poly(ADP-ribose) polymerase, XIAP, cIAP, survivin, and β-actin were performed using specific antisera or monoclonal antibodies according to protocols reported previously (24–27). Horizontal scanning densitometry was performed on Western blots by using acquisition into Adobe Photo Shop (Apple, Inc., Cupertino, CA) and analysis by the NIH Image Program (NIH, Bethesda, MD). The expression of β-actin was used as a control.

**Apoptosis Assessment by Annexin V Staining.** After drug treatments, cells were resuspended in 100 µl of staining solution (containing Annexin V fluorescein and propidium iodide in a HEPES buffer; Annexin-V-FLUOS Staining kit; Boehringer-Mannheim, Indianapolis, IN). After incubation at room temperature for 15 min, cells were analyzed by flow cytometry (14). Annexin V binds to those cells that express phosphatidylserine on the outer layer of the cell membrane, and propidium iodide stains the cellular DNA of those cells with a compromised cell membrane. This allows for the discrimination of live cells (unstained with either fluorochrome) from apoptotic cells (stained only with Annexin V) and necrotic cells (stained with both Annexin V and propidium iodide; Ref. 14).

**Morphology of Apoptotic Cells.** After drug treatment, 50 × 10^5 cells were washed with PBS (pH 7.3) and resuspended in the same buffer. Cytoskeleton preparations of the cell suspensions were fixed and stained with Wright stain. Cell morphology was determined by light microscopy. In all, five different fields were randomly selected for counting of at least 500 cells. The percentage of apoptotic cells was calculated for each experiment, as described previously (14, 27).

**Flow Cytometric Analysis of Cell Cycle Status and Apoptosis.** The flow cytometric evaluation of the cell cycle status and apoptosis was performed according to a method described previously method (27). The percentage of cells in the apoptotic sub-G1 as well as G1, S-phase, and G2-M phases were calculated using Multicycle software (Phoenix Flow Systems, San Diego, CA).

**Assessment of Hb Levels.** HL-60/neo, HL-60/Bcr-Abl, and K562 cells were treated with 10 µg/ml of Annexin V-FLUOS Staining kit; Boehringer-Mannheim, Indianapolis, IN). After incubation at room temperature for 15 min, cells were analyzed by flow cytometry (14). Annexin V binds to those cells that express phosphatidylserine on the outer layer of the cell membrane, and propidium iodide stains the cellular DNA of those cells with a compromised cell membrane. This allows for the discrimination of live cells (unstained with either fluorochrome) from apoptotic cells (stained only with Annexin V) and necrotic cells (stained with both Annexin V and propidium iodide; Ref. 14).

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**Assessment of Hb Levels.** HL-60/neo, HL-60/Bcr-Abl, and K562 cells were treated with the designated concentrations of the drugs. Cells were then washed with PBS, and intracellular Hb levels were determined by a method described previously (3) and expressed as µg/µg of cellular protein.

**Akt Kinase Assay.** In untreated and STI571-treated cells, Akt kinase activity was determined by using an immunoprecipitation-kinase assay with reagents provided in a commercially available kit (New England Biolab, Beverly, MA; Ref. 7). Briefly, cell lysates were used to immunoprecipitate Akt using a polyclonal Akt antibody. Immunoprecipitates were then incubated with GSK-3α fusion protein in the presence of ATP and kinase buffer, allowing immunoprecipitated Akt to phosphorylate GSK-3α, which was analyzed by Western blotting using a phospho-GSK-3α/β (serine 219) antibody (27).

**Statistical Analysis.** Significant differences between values obtained in a population of leukemic cells treated with different experimental conditions were determined by paired t test analyses. A one-way ANOVA was also applied to the results of the various treatment groups, and post hoc analysis was performed using the Bonferroni correction method.

**Results.**

**GA and 17-AAG Induce Hb and Apoptosis of HL-60/Bcr-Abl and K562 Cells.** In the present studies, we determined whether the ansamycin GA, or its clinically relevant analogue 17-AAG (28), would lower Bcr-Abl levels and induce differentiation and apoptosis of Bcr-Abl-positive leukemic cells. Fig. 1 demonstrates that treatment with 5 µM GA or 17-AAG induces apoptosis of HL-60/neo > HL-60/Bcr-Abl > K562 cells (P < 0.05). Although 17-AAG induced more apoptosis of HL-60/neo than GA, the drugs had similar apoptotic effects against HL-60/Bcr-Abl and K562 cells (Fig. 1A). A time-dependent increase in apoptosis attributable to 5 µM GA or 17-AAG was observed in K562 cells (Fig. 1B). A dose-dependent (from 0.1 to 50 µM) increase in apoptosis of K562 cells was more obvious after treatment with GA than 17-AAG (Fig. 1C). A similar time and dose-dependent increase in apoptosis attributable to GA or 17-AAG was also observed for HL-60/Bcr-Abl cells (data not shown).

As reported previously, both HL-60/Bcr-Abl and K562 cells express detectable levels of Hb (Fig. 1D). Treatment with 5 µM GA or 17-AAG significantly increased the intracellular levels of Hb (Fig. 1D) and imparted a rusty color to the cell pellet of HL-60/Bcr-Abl and K562 cells (not shown).

Because in previous studies we demonstrated that Bcr-Abl expression inhibits the cytosolic accumulation of cyt c because of apoptotic stimuli, we examined whether GA or 17-AAG-induced apoptosis of HL-60/Bcr-Abl or K562 cells is associated with cytosolic accumulation of cyt c and the subsequent processing of caspase-9 and caspase-3. Fig. 2A demonstrates that treatment with 5 µM 17-AAG induced cytosolic accumulation of cyt c in HL-60/Bcr-Abl and K562 cells, but this was less than in HL-60/neo cells (Fig. 2A). The levels of cyt c were controlled for protein loading (not shown). Treatment with 5 µM GA for 24 h also increased the cytosolic cyt c levels more in HL-60/neo than HL-60/Bcr-Abl and K562 cells (data not shown). Concomitantly, GA and 17-AAG also induced less processing of caspase-9 and caspase-3 in HL-60/Bcr-Abl and K562 cells than in HL-60/neo cells (Fig. 2A). As compared with HL-60/neo, for unclear reasons, HL-60/Bcr-Abl and K562 cells expressed higher levels of caspase-9. Both GA and 17-AAG treatment had no effect on Bcl-xL, Bcl-2, or Bax levels in HL-60/neo, HL-60/Bcr-Abl, or K562 cells (data not shown). Taken together, these findings indicate that GA and 17-AAG partially overcome the resistance and trigger the intrinsic “mitochondrial" pathway of apoptosis.

**GA and 17-AAG Reduce Bcr-Abl Levels and Cause Decreased Binding of Bcr-Abl to Hsp90.** We examined whether GA or 17-AAG-induced apoptosis and differentiation is associated with lowering of Bcr-Abl levels. Fig. 3A demonstrates that exposure to 5 µM GA or 17-AAG reduced Bcr-Abl protein levels in HL-60/Bcr-Abl and K562 cells. As has been described previously (29), GA and 17-AAG also down-regulated the levels of c-Raf in Bcr-Abl-positive cells and the control HL-60/neo cells (Fig. 3A). The decline in c-Raf levels in all cell types and the additional decline in Bcr-Abl levels in HL-60/Bcr-Abl and K562 cells were associated with a reduction in the Akt kinase activity, which was estimated by documenting a decline in the phospho-GSK-3α levels (Fig. 3A). Lowering of c-Raf levels and Akt kinase activity may be promoting GA and 17-AAG-induced apoptosis.
of HL-60/neo cells and contributing toward the proapoptotic influence of the decline in Bcr-Abl levels in GA or 17-AAG-treated HL-60/Bcr-Abl and K562 cells (30). The findings that the effects of GA and 17-AAG on Raf-1 expression and Akt activity are roughly comparable in the three cell lines, despite different apoptotic responses, would appear to argue against a primary role for these genes in determining sensitivity to these agents. It is noteworthy that whereas in HL-60/Bcr-Abl and K562 cells GA and 17-AAG treatment did not significantly alter \( \text{IkB}\alpha \) levels, an ~2-fold increase in \( \text{IkB}\alpha \) levels was observed in HL-60/neo cells. Although we did not directly investigate it, this may also promote apoptosis because of GA or 17-AAG reducing the nuclear localization, and thereby inhibiting the transactivation and antiapoptotic effects of NF-\( \kappa \)B in HL-60/neo cells (5, 7, 26). The mechanism(s) by which GA or 17-AAG induces \( \text{IkB}\alpha \) levels was not determined in the present studies.

Fig. 2. GA and 17-AAG induce the intrinsic pathway of apoptosis in HL-60/neo as well as HL-60/Bcr-Abl and K562 cells. After treatment with 5 \( \mu \)M 17-AAG, S100 fractions were obtained from cell lysates, and the cytosolic accumulation of cyt c was determined by Western blot analysis (A). Alternatively, cell lysates from 17-AAG- or GA-treated cells (5 \( \mu \)M for 24 h) were used to determine the processing of caspase-9 and caspase-3 by Western blot analyses (B; see text).

Fig. 1. GA or 17-AAG induced more apoptosis of HL-60/neo, as compared with HL-60/Bcr-Abl and K562 cells. HL-60/neo, HL-60/Bcr-Abl, and K562 cells were exposed to 5 \( \mu \)M GA or 17-AAG for 72 h (A). Alternatively, K562 cells were treated with 5 \( \mu \)M GA or 17-AAG for 24, 48, or 72 h (B) or exposed to 0.1, 1.0, 5.0, 25.0, or 50 \( \mu \)M GA or 17-AAG for 72 h (C). After these treatments, untreated or treated cells were stained with Annexin V. Positively stained apoptotic cells were quantitated by flow cytometry (see text). GA or 17-AAG increased Hb levels in HL-60/Bcr-Abl and K562 cells (D). After exposure of HL-60/Bcr-Abl and K562 cells to 5 \( \mu \)M GA or 17-AAG for 48 h, intracellular Hb levels (\( \mu \)g/50 \( \mu \)g of protein) were estimated spectrophotometrically (see text). Bars, SD.
17-AAG-mediated decline in Bcr-Abl levels is attributable to degradation by the proteasomes. Fig. 4A shows that cotreatment with the proteasome inhibitor PSC341 significantly inhibited the GA or 17-AAG (5 μM for 24 h)-mediated decline in Bcr-Abl and c-Raf. Fig. 4, B and C, clearly demonstrate that Bcr-Abl and c-Raf proteins, which were protected from degradation by the proteasomes by cotreatment with PSC341, were present only in the detergent (NP40)-insoluble fraction, because they had to be extracted with the SDS buffer prior to detection. Treatment with PSC341 alone for 24 h produced an increased in c-Raf but not in Bcr-Abl levels in both detergent-soluble and -insoluble fractions (Fig. 4, B and C). This may be because of the differences in the half-life of the two proteins in K562 cells. Fig. 4D demonstrates that cotreatment with a concentration of PSC341, which alone is not cytotoxic, significantly inhibited 17-AAG-induced apoptosis of K562 cells (P < 0.01). Similar observations were made with GA-induced apoptosis of K562 and HL-60/Bcr-Abl cells (data not shown).

Discussion

Present studies demonstrate that treatment with GA or its clinically less toxic analogue 17-AAG depletes Bcr-Abl and induces apoptosis and Hb levels in HL-60/Bcr-Abl and K562 cells. These data show for the first time that in these cells, GA and 17-AAG triggered the cytosolic accumulation of cyt c and induced the processing of caspase-9 and caspase-3, thus activating the intrinsic pathway of apoptosis (24, 31). Bcr-Abl TK activity is known to induce Bcl-xL.

Fig. 3. GA and 17-AAG treatments shift the binding of Bcr-Abl from HSP90 to HSP70, which is associated with lowering of Bcr-Abl levels. After treatment with 5 μM GA or 17-AAG for 24 h, immunoblot analyses of Bcr-Abl, Abl, IκBα, c-Raf, or phospho-GSK-3β (A) or of HSP-70 and HSP-90 (B) were performed using specific antibodies (see text). Alternatively, using protein G-agarose beads coated with anti-Abl monoclonal antibody, Bcr-Abl was immunoprecipitated from cell lysates (see text). Immunoprecipitates were immunoblotted with specific anti-HSP90 and HSP70 antibodies (C).

Fig. 4. GA and 17-AAG down-regulate Bcr-Abl, Abl, and c-Raf in HL-60/Bcr-Abl and K562 cells through proteasomal degradation. Cells were treated with 5 μM GA or 17-AAG for 24 h with or without the proteasomal inhibitor PSC341 (50 nM). After these treatments, immunoblot analyses of Bcr-Abl and c-Raf-1 were performed (A). Alternatively, after treatment with the drugs, cells were lysed with the TNSEV buffer to obtain the NP40 (detergent)-soluble fraction. The NP40 (detergent)-insoluble pellet was further treated with SDS-containing buffer to obtain NP40-insoluble fraction. Detergent-soluble (B) or -insoluble (C) fractions were subjected to SDS-PAGE and Western blot analyses using anti-Abl and anti-c-Raf antibodies (see text). β-Actin levels served as the loading control. K562 cells were also treated with 17-AAG (5 μM) or PSC341 (30 nM) alone for 48 h or cotreated with 17-AAG plus PSC341. After these treatments, cells were stained with Annexin V antibody, and the percentage of Annexin V-staining apoptotic cells were determined by flow cytometry (D). Bars, SD.
levels by increasing STAT5 and/or GATA-1 activity (32, 33). In addition, Bcr-Abl may also promote the antiapoptotic effect of Bcl-xL by phosphorylating and inactivating BAD through Akt (30, 34). These effects of Bcr-Abl on Bcl-xL inhibit the mitochondrial release of cyt c into the cytosol attributable to diverse apoptotic stimuli (4). Although Bcl-xL levels in HL-60/Bcr-Abl and K562 cells were not lowered by GA (or 17-AAG), by lowering Bcr-Abl and Akt kinase activity, GA may have reduced the antiapoptotic activity of Bcl-xL. This may have promoted the cytosolic accumulation of cyt c. Similar observations were made after treatment of HL-60/Bcr-Abl and K562 cells with As2O3 (17). As compared with HL-60/neo, HL-60/Bcr-Abl cells expressed higher levels of Hsp70 and caspase-9. Recently, Hsp70 has been shown to inhibit the intrinsic pathway of apoptosis by preventing the recruitment of procaspase-9 to Apaf-1 “apoptosome” (35). In addition, a selective depletion of Hsp70 by the antisense Hsp70 cDNA was shown to induce apoptosis of breast cancer but not breast epithelial cells (36). Therefore, higher Hsp70 expression (in HL-60/Bcr-Abl and K562 cells) may be contributing to Bcr-Abl-mediated resistance to apoptosis downstream to the activity of cyt c. It is possible that in addition to exhibiting lower cytosolic accumulations of cyt c, higher Hsp70 levels in HL-60/Bcr-Abl and K562 cells may be responsible for reduced GA-induced processing of caspase-9 and caspase-3 and apoptosis (35). It should be noted that the difference in cyt c release between HL-60/neo cells and the two Bcr-Abl-expressing cell lines seems out of proportion to the differential degree of apoptosis in these cells induced by GA and 17-AAG. The same is true, although to a lesser extent, in the case of caspase-9 processing. However, activation of caspase-3 appears to be roughly in line with the disparate degree of apoptosis in the three cell lines. One way to explain these findings would be that: (a) there may be a threshold effect for the activity of released cyt c; or (b) the high levels of Hsp70 in Bcr-Abl-overexpressing cells may interfere with the activity of the apoptosome. Thus, the induction of Hsp70 may be an additional antiapoptotic consequence of Bcr-Abl expression in leukemic cells.

GA is known to bind to the ATP/ADP binding pocket in the NH2-terminus region of Hsp90 (18). This blocks the binding of ATP to Hsp90, thereby disrupting its chaperone, protein-protein association with the “client” proteins including Raf-1, v-Src, p185c-erbB-2, and mutant p53 (17, 19, 20). This has been shown to result in the intracellular degradation of these proteins through the proteasomes (17, 22, 29). GA-induced depletion of p185c-erbB-2, Raf, and mutant p53 has also been shown to exert an antiproliferative effect on breast cancer cells (28). Present studies highlight a similar effect of GA-mediated inhibition of the chaperone function of Hsp90 with respect to Bcr-Abl. Our data indicate that treatment with GA or 17-AAG disrupts binding of Hsp90 with Bcr-Abl TK, while promoting its chaperone association with Hsp70 and degradation by the proteasomes. During the preparation of this report, similar findings were also reported by An et al. (37). However, unlike their report, we have shown that cotreatment with the proteasome inhibitor PS341 not only inhibits the 17-AAG (a clinically relevant analogue of GA)-mediated decline in Bcr-Abl levels but also inhibits 17-AAG-induced apoptosis of HL-60/Bcr-Abl and K562. We also demonstrate that by lowering Bcr-Abl levels, 17-AAG and GA, similar to herbinycin A (23), promote erythroid differentiation (Hb induction) in HL-60/Bcr-Abl and K562 cells.

Recent reports have documented several promising strategies that target either the mRNA or protein encoded by the bcr-abl fusion gene, which is pathogenetically responsible for the malignant phenotype of CML and Bcr-Abl-positive adult ALL (9–12). The relatively specific inhibitor of Bcr-Abl TK, STI-571, has been shown to produce a high rate of hematological remissions in CML, but the remissions induced in patients with the blast crisis of CML or Bcr-Abl-positive ALL have not been durable (38, 39). Relapses, despite continuous administration of STI-571, suggest the emergence of STI-571-resistant Bcr-Abl positive leukemic cells. Indeed, a continuous in vitro exposure of Bcr-Abl-positive leukemic cells to STI-571 induced either bcr-abl gene amplification and mRNA and/or increased Bcr-Abl protein levels, resulting in resistance to STI-571 (40, 41). In a recent report, we had demonstrated that treatment with As2O3 alone reduces Bcr-Abl protein levels (14), and a combination of As2O3 plus STI571 is more effective than either agent alone in inducing apoptosis of HL-60/Bcr-Abl and K562 cells (42). In the present studies, our data showing that 17-AAG also lowers Bcr-Abl levels and Akt kinase activity as well as induces apoptosis create the rationale to investigate the cytotoxic effects of 17-AAG plus STI-571 in Bcr-Abl-positive leukemic cells. Collectively, the in vitro findings presented here support the in vivo studies of the combination of 17-AAG plus STI571 against Bcr-Abl-positive human leukemias that are either sensitive or resistant to STI571.

References
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