Gambogic acid is an antagonist of antiapoptotic Bcl-2 family proteins

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Abstract

The natural product gambogic acid (GA) has been reported to have cytotoxic activity against tumor cells in culture and was identified as an active compound in a cell-based high-throughput screening assay for activators of caspases, proteases involved in apoptosis. Using the antiapoptotic Bcl-2 family protein, Bfl-1, as a target for screening of a library of natural products, we identified GA as a competitive inhibitor that displaced BH3 peptides from Bfl-1 in a fluorescence polarization assay. Analysis of competition for BH3 peptide binding revealed that GA inhibits all six human Bcl-2 family proteins to various extents, with Mcl-1 and Bcl-B the most potently inhibited [concentrations required for 50% inhibition (IC_{50}), $< 1\mu mol/L$]. Competition for BH3 peptide binding was also confirmed using a time-resolved fluorescence resonance energy transfer assay. GA functionally inhibited the antiapoptotic Bcl-2 family proteins as shown by experiments using isolated mitochondria in which recombinant purified Bcl-2 family proteins suppress SMAC release *in vitro*, showing that GA neutralizes their suppressive effects on mitochondria in a concentration-dependent manner. GA killed tumor cell lines via an apoptotic mechanism, whereas analogues of GA with greatly reduced potency at BH3 peptide displacement showed little or no cytotoxic activity. However, GA retained cytotoxic activity against *bax*$^{-/-}$*bak*$^{-/-}$ cells in which antiapoptotic Bcl-2 family proteins lack a cytoprotective phenotype, implying that GA also has additional targets that contribute to its cytotoxic mechanism. Altogether, the findings suggest that suppression of antiapoptotic Bcl-2 family proteins may be among the cytotoxic mechanisms by which GA kills tumor cells. [Mol Cancer Ther 2008;7(6):1639–46]

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Introduction

Gambogic acid (GA) is a medicinal compound derived from the gamboges resin of the tree *Garcinia hanburyi*. GA has documented cytotoxic activity against tumor cell lines in culture, with concentrations required for killing 50% of cells (LD_{50}) of ~ 1 μmol/L (1, 2). This natural product also displays antitumor activity in preclinical mouse models involving human tumor xenografts (3–5). In contrast, GA is reportedly well tolerated in mice and rats (2, 4, 6), suggesting that a therapeutic window might be identified at which tumor but not normal cells are killed. It would therefore be interesting to know the cytotoxic mechanism of GA.

The mechanism by which GA kills tumor cell lines involves apoptosis, a cell death processing involving caspase family proteases. In fact, GA was identified as an active compound in a cell-based high-throughput screening assay that measured caspase activation (1). Among the regulators of apoptosis are Bcl-2 family proteins. Humans have six genes encoding distinct antiapoptotic Bcl-2 family proteins: Bcl-2, Bcl-X_L, Mcl-1, Bfl-1, Bcl-W, and Bcl-B (7, 8). These proteins typically localize to intracellular membranes, especially mitochondrial membranes, where they have been shown to block the release of apoptogenic proteins such as cytochrome *c*, SMAC endonuclease G, and AIF (9–11). Several antiapoptotic Bcl-2 family proteins are known to become pathologically overexpressed in human cancers, conferring apoptosis-resistant phenotypes (12–17).

The antiapoptotic proteins are neutralized endogenously by proteins containing an α-helical interaction motif, known as BH3 (7, 18–20). Synthetic BH3 peptides bind antiapoptotic Bcl-2 family proteins with nanomolar affinities, promoting apoptosis (21, 22). Nonpeptidyl compounds have been identified that compete with BH3 peptides for binding to antiapoptotic Bcl-2 family proteins, mimicking BH3 peptides and creating interest in development of these molecules as potential cancer therapeutics (23, 24).

We show here that GA has the ability to compete with BH3 peptides for binding to several antiapoptotic Bcl-2 family proteins *in vitro*. GA also neutralizes the function of these proteins with respect to release of SMAC from isolated mitochondria. The cytotoxic activity of GA, however, appears to include Bcl-2 family-independent mechanisms, suggesting that suppression of Bcl-2 and related proteins represents only one of the cytotoxic mechanisms of this natural product.

Materials and Methods

Compounds

The MicroSource Spectrum Collection Library (Discovery Systems) is a ~2,000-compound collection of mostly pure

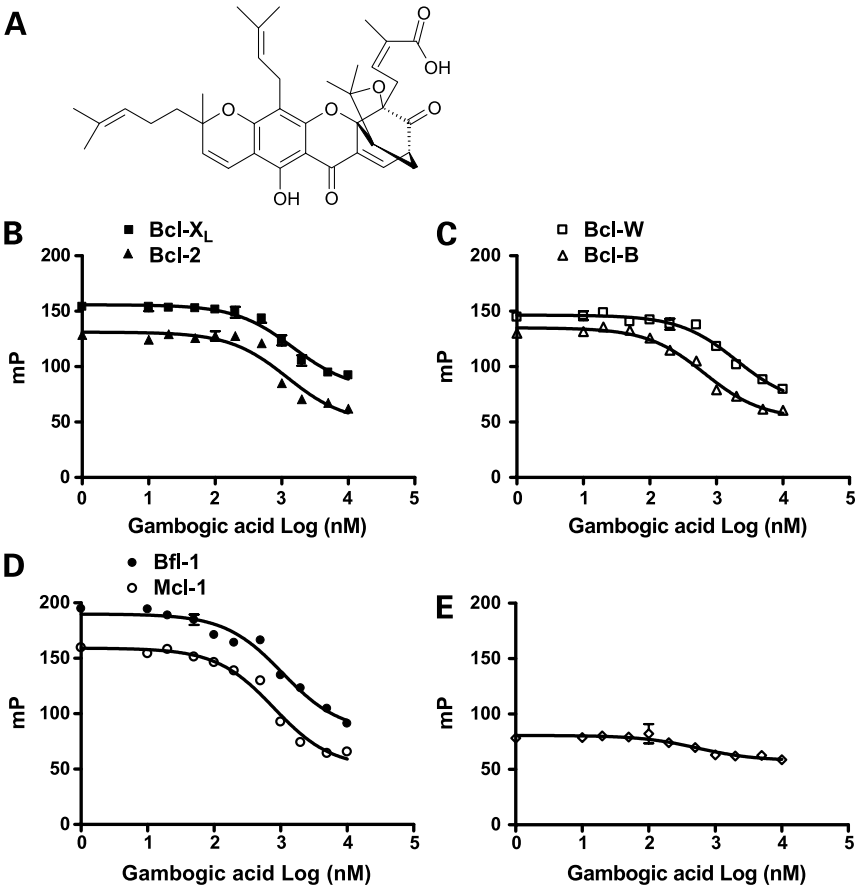

Figure 1. GA inhibits binding of FITC-BH3 peptide to antiapoptotic Bcl-2 family proteins. **A,** structure of GA. **B** to **D,** for peptide competition experiments in FPA mode, 100 nmol/L Bcl-2 family proteins, including Bcl-X_L, Bcl-2, Bcl-W, Bcl-B, Bfl-1, or Mcl-1, were incubated with various concentrations of GA for 2 min in PBS buffer, and 5 nmol/L FITC-conjugated Bid BH3 peptide was added. **E,** GA does not interfere with FITC-Bid BH3 peptide alone, tested as a control. Fluorescence polarization (mP) was measured after 10 min. Interaction of GA with FITC-conjugated Bid BH3 peptide alone was used as a control. Note that background fluorescence polarization for these assays from FITC-BH3 peptide in the absence of Bcl-2 family protein is ~50 to 70 mP (FP_{min}), whereas the maximum fluorescence polarization ranges from ~130 to ~190 mP (FP_{max}), depending on the Bcl-2 family member.

natural products and their derivatives. Compounds were supplied as 10 mmol/L stocks of Me_2SO, stored at -20°C, and thawed immediately before analysis. Aliquots were dissolved to a final concentration of 10 μmol/L for fluorescence polarization assays (FPA). GA was purchased from Calbiochem. All the GA analogues were purchased from MicroSource.

Protein Purification

Glutathione *S*-transferase (GST)-fusion proteins containing Bcl-X_L, Bcl-2, Bcl-W, Bcl-B, Bfl-1, and Mcl-1 lacking their COOH-terminal transmembrane domains (~last 20 amino acids; "ΔTM") were expressed from pGEX4T-1 plasmid in XL-1 Blue cells (Stratagene) as described previously (24). Briefly, cells were grown in 2 L Luria-Bertani with 50 μg/mL ampicillin at 37°C to an $A_{600\ nm}$ of 1.0. IPTG (0.5 mmol/L) was added, and the cultures were incubated at 25°C for 6 h. Cells were then recovered in 20 mmol/L phosphate buffer (pH 7.4), 150 mmol/L NaCl, 1 mmol/L DTT, 1 mmol/L EDTA, and 1 mmol/L phenylmethylsulfonyl fluoride followed by sonication. Cellular debris was sedimented by centrifugation at 27,500 × *g* for 20 min, and the resulting supernatants were incubated with 10 mL glutathione-Sepharose (Pharmacia) at 4°C for 2 h. The resin was washed three times with 20 mmol/L phosphate buffer (pH 7.4), 150 mmol/L NaCl, and 1 mmol/L DTT, and 10 mmol/L reduced glutathione dissolved in 50 mmol/L Tris-HCl (pH 8.0) was used to elute the GST-fusion proteins.

Other recombinant proteins used here, including His_6-Bid and His_6-caspase-8, were expressed and purified using methods similar to prior publications (25).

Fluorescence Polarization Assays

FPAs were done as described previously using various Bcl-2 family proteins and FITC-conjugated Bid BH3 peptide (24, 26). Briefly, Bcl-2 proteins were incubated with 5 nmol/L FITC-Ahx-EDIIRNIARHLAQVGDSMDR in the dark. Fluorescence polarization was measured using an Analyst AD Assay Detection System (LJL Biosystem) in PBS (pH 7.4). IC_{50} determinations were done using GraphPad Prism software.

Competitive Peptide Displacement Assays

Methods for competitive peptide displacement assays were similar to previous publications (24). Briefly, 100 nmol/L GST-Bcl-2 proteins were incubated with the compounds at various concentrations for 5 min at room temperature in PBS. Then, 5 nmol/L FITC-Bid BH3 peptide was added and fluorescence polarization was measured after 10 min. IC_{50} determinations were generated by fitting the experimental data using a sigmoidal dose-response nonlinear regression model with GraphPad Prism software.

Time-Resolved Fluorescence Resonance Energy Transfer Assays

For time-resolved fluorescence resonance energy transfer (TR-FRET) assays, GST-Bcl-X_L and anti-GST-terbium (Invitrogen) were mixed together with the FITC-Bad BH3 peptide in PBS containing 0.005% Tween 20 in 96-well plates in a total volume of 20 μL/well. After incubation at room temperature for 30 min, 2 μL GA-containing solutions were added to the reaction mixtures containing 10 nmol/L Bcl-X_L, 10 nmol/L FITC-Bad BH3 peptide, and 2 nmol/L anti-GST-terbium for 30 min at room temperature. TR-FRET signals were measured with a SpectraMax M5 plate reader (Molecular Devices) using the following settings: excitation at 330 nm, emission for FITC signal at 490 nm, and emission for terbium signal at 520 nm.

Mitochondria Purification and Protein Release Assays

HeLa cells were pelleted by centrifugation and then washed once in HM buffer [10 mmol/L HEPES (pH 7.4), 250 mmol/L mannitol, 10 mmol/L KCl, 5 mmol/L $MgCl_2$, and 1 mmol/L EGTA] containing 1 mmol/L phenylmethylsulfonyl fluoride and a mixture of protease inhibitors (Roche Molecular Biochemicals). The cell pellet was then homogenized in HM buffer by 50 strokes of a Dounce homogenizer using a B-type pestle. The homogenate was centrifuged twice at 600 × *g* for 5 min to remove nuclei and debris. The resulting supernatant was centrifuged at 10,000 × *g* for 10 min, and the resulting mitochondria-containing pellet was washed twice with the HM buffer.

For mitochondrial protein release assays, 10 μL mitochondria (50 μg) were added into a final volume of 50 μL HM buffer containing GA, tBid, or tBid preincubated with GA or Bcl-2 family proteins at 30°C for 15 min. The reactions were further incubated at 30°C for 40 to 60 min, and mitochondria were pelleted by centrifugation and the supernatants were collected, boiled in Laemmli sample buffer, and analyzed by SDS-PAGE/immunoblotting using anti-SMAC antibody (25).

Peptide Synthesis

Peptides were synthesized using Fmoc solid-phase synthesis on an ACT 350 multiple peptide synthesizer. FITC-conjugated Bid were synthesized on Fmoc-alanine Wang resin to give peptides with COOH-terminal carboxyl groups. FITC was linked to the Ahx. The crude peptides were purified with a Gilson high-performance liquid chromatography instrument and analyzed by matrix-assisted laser desorption/ionization time-of-flight mass analysis with an Applied Biosystems Voyager System 6264.

Cell Culture, Transfection, and Apoptosis Assays

HeLa, HL-60, Jurkat, PPC1, and mouse embryonic fibroblast cells were maintained in DMEM (Irvine Scientific) supplemented with 10% fetal bovine serum, 1 mmol/L L-glutamine, and antibiotics. Apoptosis was assessed using staining with Annexin V-FITC and propidium iodide (PI) followed by flow cytometry analysis using FL-1 and FL-3 channels of a flow cytometer (Becton Dickinson; FACSort). Annexin V–positive/PI-negative cells were considered apoptotic.

For caspase assays, cell lysates were prepared and normalized for protein content, and 10 μg aliquots of cell lysates were incubated with 100 μmol/L DEVD-AFC, measuring enzyme activity by the release of AFC fluorescence. Data are reported as relative fluorescence units of product produced per minute per microgram of total protein.

Results

Identification of GA as a Bfl-1-Inhibitory Compound by High-Throughput Screening

We devised a high-throughput screening in which binding of a FITC-conjugated BH3 peptide to recombinant purified Bfl-1 protein is measured by FPA. A library of ~2,000 natural products was screened for suppression of BH3 peptide binding by >50% using this FPA, resulting in ~30 hits, among which was GA.

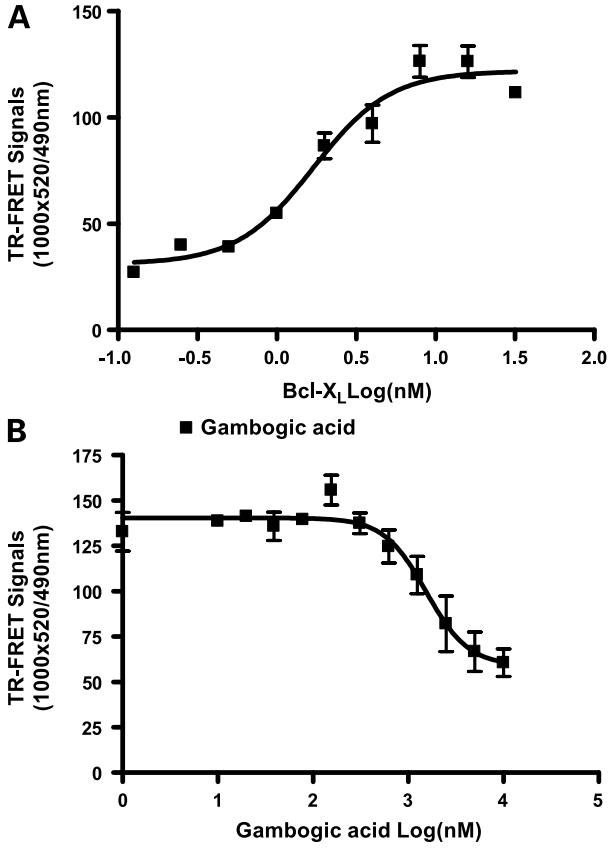

Figure 2. TR-FRET assay confirms that GA competes with BH3 peptide for binding to Bcl-X_L. **A,** binding of GST-Bcl-X_L to FITC-BH3 peptide was assayed by TR-FRET. The final reaction mixtures contain 10 nmol/L FITC-Bad BH3 peptide, 2 nmol/L anti-GST-terbium, and various concentrations of GST-Bcl-X_L proteins in PBS buffer containing 0.005% Tween 20. **B,** analysis of GA competition with BH3 peptide for Bcl-X_L binding by TR-FRET. Reaction mixtures of final 20 μL volume contain 2 μL various concentrations of GA with 10 nmol/L FITC-Bad BH3 peptide, 2 nmol/L anti-GST-terbium, and 10 nmol/L GST-Bcl-X_L protein in PBS buffer containing 0.005% Tween 20. The mixtures are incubated for 30 min at room temperature. TR-FRET signals were measured using excitation at 330 nm, emission for FITC signal at 490 nm, and emission for terbium signal at 520 nm. Data are presented as 520/490 nm ratio (mean ± SD; $n = 3$).

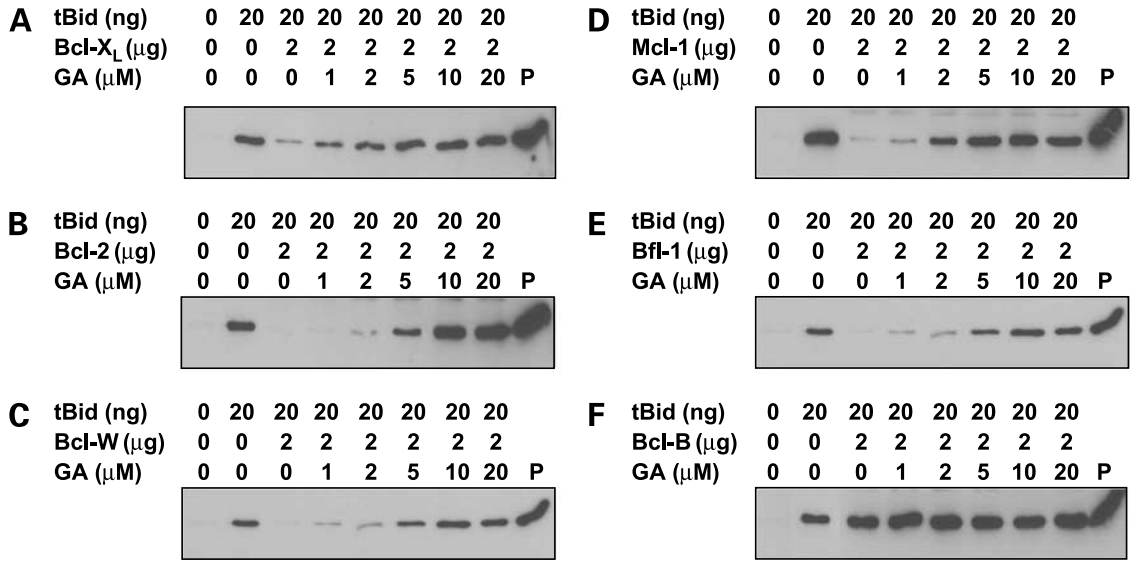

Figure 3. GA neutralizes ability of Bcl-2 family proteins to suppress tBid-induced mitochondrial leakage. **A** to **F,** tBid (20 ng, cleaved by caspase-8) was preincubated with 2 μg purified recombinant antiapoptotic Bcl-2 family proteins, including Bcl-X$_L$ (**A**), Bcl-2 (**B**), Bcl-W (**C**), Bcl-B (**D**), Bfl-1 (**E**), or Mcl-1 (**F**), together with various concentrations of GA for 15 min in HM buffer before adding 50 μg isolated HeLa mitochondria for 1 h at 30°C. Samples were centrifuged to generate supernatants that were analyzed by SDS-PAGE/immunoblotting using anti-SMAC antibody, comparing relative amounts of SMAC released from equivalent amounts of input mitochondria treated with tBid. *Pellet,* intact, untreated mitochondria, provided as a control.

GA Competes with BH3 Peptides for Binding to the Six Antiapoptotic Bcl-2 Family Proteins

The activity of GA against the six human antiapoptotic Bcl-2 family proteins was contrasted using FPA we have established previously (24). GA displaced to various extents FITC-BH3 peptide binding to all six proteins, with apparent IC_{50} values of 1.47 μmol/L for Bcl-X$_L$, 1.21 μmol/L for Bcl-2, 2.02 μmol/L for Bcl-W, 0.66 μmol/L for Bcl-B, 1.06 μmol/L for Bfl-1, and 0.79 μmol/L for Mcl-1. Thus, Mcl-1 and Bcl-B showed the greatest sensitivity (apparent IC_{50}, <1 μmol/L) and Bcl-2 showed the least sensitivity (Fig. 1). Due to solubility, we were unable to increase the GA concentration to achieve complete BH3 peptide displacement for some Bcl-2 family proteins in these assays. Incubating GA with FITC-BH3 peptide in the absence of Bcl-2 family proteins only marginally affected baseline (background) fluorescence polarization ($\sim\Delta$20 mP at concentrations >20 μmol/L), excluding a direct effect of GA on the peptide probe as an explanation for the results (Fig. 1). In contrast to Bcl-2 family proteins, GA did not inhibit in FPA using rhodamine-SMAC peptide binding to BIR3 of XIAP (data not shown) or FITC-ATP binding to Hsp70 (data not shown), thus showing the specificity for antiapoptotic Bcl-2 family proteins.

TR-FRET Confirmation of GA Competition for BH3-Binding Site

To confirm by an independent method the ability of GA to compete with BH3 peptides for binding to Bcl-2 family proteins, we devised a TR-FRET assay for Bcl-X$_L$, in which FITC-Bad BH3 peptide bound to recombinant purified GST-Bcl-X$_L$ is excited by emissions from terbium-conjugated anti-GST antibody. FITC-Bad BH3 peptide bound to GST-Bcl-X$_L$ (but not GST; data not shown) in a concentration and saturable manner, with apparent K_d values of 2 nmol/L (Fig. 2A). GA inhibited FITC-Bad BH3 peptide binding to GST-Bcl-X$_L$, as measured by TR-FRET,

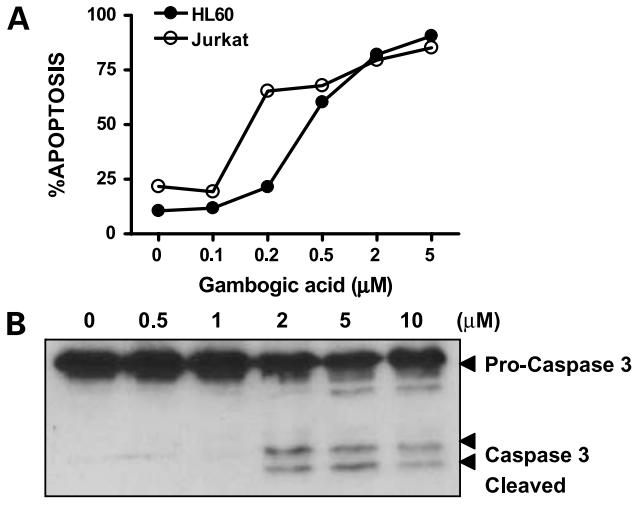

Figure 4. GA induces apoptosis of cancer cells. **A,** GA was used to treat HL-60 (*black symbols*) or Jurkat (*white symbols*) cells at concentrations of 0, 0.1, 0.2, 0.5, 2, and 5 μmol/L (from *left* to *right*). Cells were collected after 20 h and stained with FITC-Annexin V/PI, and the percentage of Annexin V – positive/PI-negative cells was determined. **B,** PPC1 cells were treated with GA at concentrations of 0, 0.5, 1, 2, 5, and 10 μmol/L. After 20 h, the cells were collected and the cell lysates were analyzed by SDS-PAGE/immunoblotting using anti-caspase-3 antibody. The positions of uncleaved proform and cleaved large subunit of the enzyme are indicated.

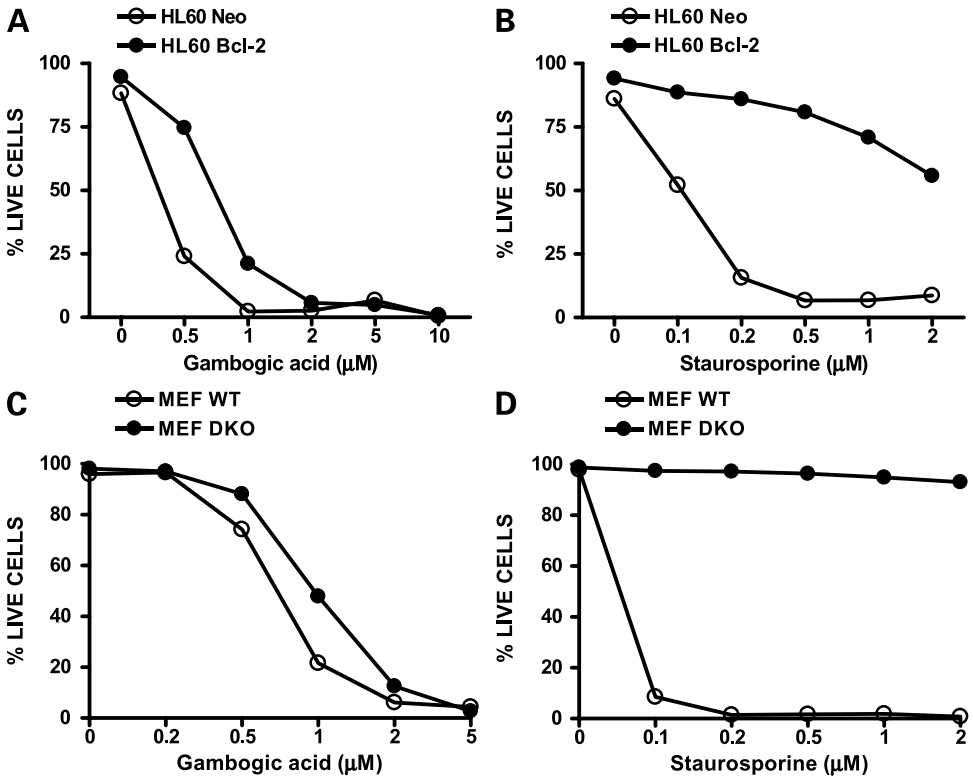

Figure 5. Effects of Bcl-2 overexpression or Bax/Bak double knockout on GA-induced cytotoxicity. **A** and **B,** neo-control or Bcl-2-overexpressing HL-60 cells were treated with (**A**) GA or (**B**) staurosporine at various concentrations. **C** and **D,** mouse embryonic fibroblast cells with wild-type (*WT*; *black symbols*) or $bax^{-/-}bak^{-/-}$ double knockout (*white symbols*) genotypes were treated with (**C**) GA or (**D**) staurosporine at various concentrations. After 8 h, the cells were collected and stained with FITC-Annexin V/PI, and the percentage of live cells was determined (Annexin-negative/PI-negative). Representative of at least three independent experiments.

with apparent IC_{50} values of 1.5 μmol/L (Fig. 2B), thus yielding similar results as those obtained using FPA.

GA Neutralizes Activity of Bcl-2 Family Proteins *In vitro*

An active, NH_2-terminally truncated form of the pro-apoptotic BH3-containing protein Bid (tBid) induces release of apoptogenic proteins such as SMAC from isolated mitochondria *in vitro* (25, 27, 28). Using this assay, we showed that five of the six human antiapoptotic Bcl-2 family proteins negate tBid-induced release of SMAC from isolated mitochondria (Fig. 3). For these five proteins (Bcl-2, Bcl-X_L, Bfl-1, Bcl-W, and Mcl-1), adding GA restored tBid-induced SMAC release in a concentration-dependent manner (Fig. 3). Complete restoration was typically achieved with 5 μmol/L GA, representing ~10:1 molar excess of GA relative to antiapoptotic Bcl-2 family proteins. GA also enhanced tBid-induced release of SMAC from isolated mitochondria when recombinant Bcl-2 family proteins were not added (Supplementary Data),[1] suggesting that it may neutralize endogenous antiapoptotic Bcl-2 family proteins associated with mitochondria.

GA Overcomes Cytoprotection of Bcl-2 in Leukemia Cells

GA has cytotoxic activity against various tumor cell lines in culture and induces apoptosis (1, 2). We confirmed the apoptosis-inducing activity of GA using Jurkat T-cell acute lymphoblastic leukemia and HL-60 acute promyelomonocytic leukemia cell lines using Annexin V/PI staining, counting Annexin V–positive/PI-negative cells as apoptotic (Fig. 4A). The concentration of GA required to induce apoptosis of ~50% of the cells within 20 to 24 h was ~0.2 and ~0.5 μmol/L for Jurkat and HL-60, respectively. GA also induced clearly detectable proteolytic processing of procaspase-3, a marker of apoptosis, at concentrations >2 μmol/L, as determined by immunoblotting using lysates from prostate cancer cell PPC1 treated with GA (Fig. 4B). To assess whether GA affects expression of Bcl-2 family proteins, we did immunoblotting experiments in which HL-60 leukemia cells were treated with caspase inhibitor benozyl-valinyl-alanyl-asparatyl-fluoromethylketone to prevent apoptosis and thus avoid secondary declines in protein expression. Levels of Bcl-2 and Bcl-X_L were not affected by treatment with GA (Supplementary Fig. S2).[1]

We tested the cytotoxic activity of GA against HL-60 cells that had been stably transfected with neo-control or Bcl-2 expression plasmids (29). Overexpression of Bcl-2 reduced the sensitivity of HL-60 cells to GA, shifting the dose-response curve to the right, such that higher concentrations of GA were required to kill the cells (Fig. 5A). Treating HL-60 neo-control and Bcl-2-transfected cells with an upstream activator of the mitochondrial pathway for apoptosis, staurosporine, confirmed that Bcl-2-overexpressing HL-60 cells have a block to apoptosis (Fig. 5B), which GA overcomes. Experiments in which staurosporine and GA

[1] Supplementary material for this article is available at Molecular Cancer Therapeutics Online (http://mct.aacrjournals.org/).

were combined showed that GA overcomes Bcl-2-mediated resistance to staurosporine (Supplementary Fig. S3).[1]

GA-Induced Cytotoxicity Is Only Partly Bcl-2 Dependent

Antiapoptotic Bcl-2 family proteins suppress mitochondria-initiated cell death by inhibiting proapoptotic Bcl-2 members Bax and Bak (10, 20, 30). Consequently, when cells are genetically engineered to lack Bax and Bak, then Bcl-2 and related cytoprotective proteins no longer display an antiapoptotic phenotype (31). Cells doubly deficient in Bax and Bak therefore provide a context for assessing the mechanism of GA-induced toxicity. GA killed transformed mouse embryonic fibroblasts generated from $bax^{+/+}bak^{+/+}$ embryos in a concentration-dependent manner (Fig. 5C). However, GA also killed $bax^{-/-}bak^{-/-}$ mouse embryonic fibroblasts, requiring only ~2-fold higher concentrations of compound to reach the LD_{50}. Treating these mouse embryonic fibroblasts with staurosporine confirmed that Bak/Bak double knockout cells have a profound block to cell death (Fig. 5D), which GA overcomes. We conclude therefore that GA induces cytotoxicity through mechanisms that are only partly dependent on Bcl-2 family proteins.

GA Analogues Show Differential Activity against Bcl-2 Family Proteins, Correlating with Cytotoxicity

We examined the activity of analogues of GA with respect to displacement of Bid BH3 peptide from Bfl-1 *in vitro* (Fig. 6A) and cytotoxic activity (Fig. 6B). In the FPA, dihydro-GA and acetyl-iso-GA displaced BH3 peptide from Bfl-1 with potencies only 3- to 6-fold less than GA (Fig. 6A). In contrast, tetrahydro-GA and garcinolic acid had greatly reduced activity in BH3 displacement assays.

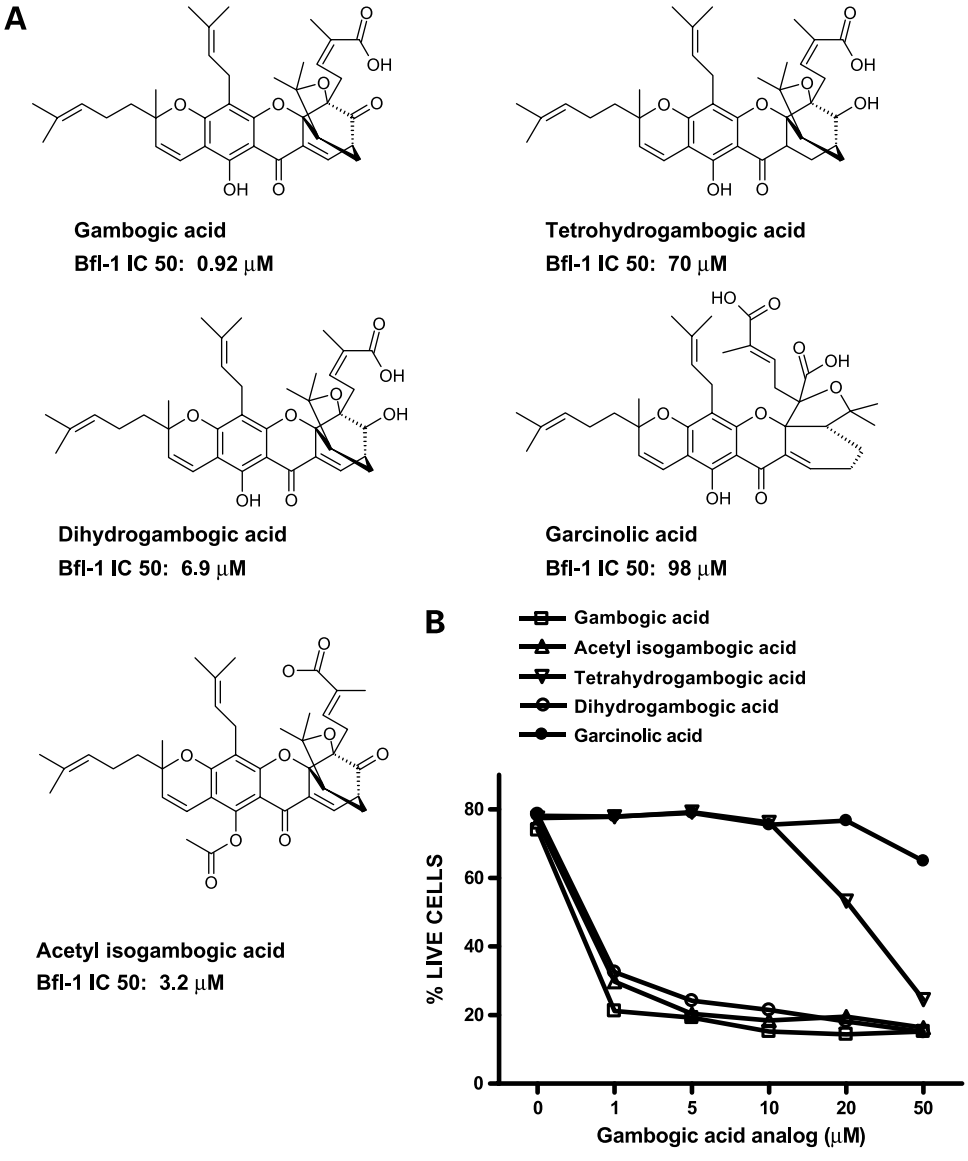

Figure 6. Comparison of cytotoxic activity and BH3 peptide displacement activity of GA and analogues. **A,** structures of GA and GA analogues are depicted, and IC_{50} values from FPA are presented for competitive binding assays using Bfl-1 and FITC-Bid BH3 peptide. **B,** Jurkat cells were treated with GA and analogues at concentrations of 0, 1, 5, 10, 20, and 50 μmol/L. Cells were collected after 20 h and stained with FITC-Annexin V/PI, and the percentages of live cells (Annexin-negative/PI-negative) were determined. Representative of at least three experiments.

In agreement with the BH3 displacement data, dihydro-GA and acetyl-iso-GA exhibited cytotoxic activity against cultured leukemia cells, whereas tetrahydro-GA showed markedly reduced activity (~50-fold less potent) and garcinolic acid was nontoxic (Fig. 6B). Thus, the cytotoxic activity of GA analogues correlates roughly with their ability to compete with BH3 peptides for binding to an antiapoptotic Bcl-2 family member.

Discussion

We show here that the cytotoxic natural product GA competes for BH3 peptide binding sites on several antiapoptotic members of the Bcl-2 family and neutralizes the ability of these proteins to suppress release of apoptogenic proteins from isolated mitochondria. Structure-function analysis of GA using analogues suggested a general correlation between BH3 competition and cytotoxicity activity, but experiments with *bax/bak* double knockout cells suggest GA-induced cytotoxicity is only partially dependent on Bcl-2 family proteins.

GA has been reported to affect other molecular events relevant to cytotoxicity, including inducing increases in Bax protein levels, reducing Bcl-2 protein levels, suppressing transferrin receptor internalization, inhibiting the catalytic activity of human topoisomerase IIα, and modulating the nuclear factor-κB signaling pathway (2, 32–34). GA also reportedly induces G_2-M-phase arrest of dividing cells (35), suggesting that this natural product hits targets involved in cell cycle. Thus, like many natural products, GA may have several targets in mammals, among which are antiapoptotic members of the Bcl-2 family.

The *in vivo* activity of GA and its therapeutic index has been explored previously in rodents (2, 6). Using a prostate cancer xenograft model, for example, it was recently reported that GA effectively inhibited tumor angiogenesis and suppressed tumor growth with few side effects, where GA was delivered by s.c. injection daily for 15 days (4). Also, using a mouse model of glioma, i.v. injection of GA daily for 14 days was reported to significantly reduce tumor volumes with little evidence of side effects (5). Antitumor activity was attributed to induction of apoptosis and to suppression of angiogenesis.

It is interesting to speculate why plants might produce compounds that neutralize Bcl-2 family proteins. Insects, nematodes, and other animal species that eat plants contain evolutionarily conserved antiapoptotic Bcl-2 family proteins (reviewed in ref. 36). Consequently, plants might produce antagonists of these compounds as a mechanism to defend themselves against animal species. In this regard, several natural products from diverse plant species have been shown to competitively displace BH3 peptides and neutralize the activity of antiapoptotic Bcl-2 family proteins, including epigallocatechin gallate from green tea, theaflavins from black tea, and gossypol from cotton seeds (37, 38). These compounds are structurally diverse, but all display <1 μmol/L activity against a variety of human antiapoptotic Bcl-2 family proteins. It remains to be determined whether their affinity is greater for Bcl-2 family members from insects or other lower organisms of the animal kingdom.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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