Identification of Chelerythrine as an Inhibitor of BcXL Function  

Received for publication, March 31, 2003, and in revised form, April 15, 2003  
Published, JBC Papers in Press, April 17, 2003  
DOI 10.1074/jbc.C300138200  

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The identification of small molecule inhibitors of antiapoptotic Bcl-2 family members has opened up new therapeutic opportunities, while the vast diversity of chemical structures and biological activities of natural products are yet to be systematically exploited. Here we report the identification of chelerythrine as an inhibitor of BcXL-Bak BcXL homology (BH) domains binding through a high throughput screening of 107,423 extracts derived from natural products. Chelerythrine inhibited the BcXL-Bak BH3 peptide binding with IC_{50} of 1.5 µM and displaced Bax, a BH3-containing protein, from BcXL. Mammalian cells treated with chelerythrine underwent apoptosis with characteristic features that suggest involvement of the mitochondrial pathway. While staurosporine, H7, etoposide, and chelerythrine released cytochrome c from mitochondria in intact cells, only chelerythrine released cytochrome c from isolated mitochondria. Furthermore BcXL-overexpressing cells that were completely resistant to apoptotic stimuli used in this study remained sensitive to chelerythrine. Although chelerythrine is widely known as a protein kinase C inhibitor, the mechanism by which it mediates apoptosis remain controversial. Our data suggest that chelerythrine triggers apoptosis through a mechanism that involves direct targeting of BcXL family proteins.

Proteins of the BcXL family are central regulators of apoptosis. While the precise molecular mechanisms by which these proteins confer their biological activities remain to be determined, they are thought to act directly on the mitochondria. Members of the BcXL family can be divided into three subfamilies based on several conserved sequence motifs known as BH domains (BH±)  

The Bak BH3 peptide labeled with 125I was from Amersham Pharma Biotech. Reagents and Cell Lines—Human SH-SY5Y and MCF7 cells were maintained as described previously (14). HCT116 cells and FDC-P1 cells were gifts from Bert Vogelstein (Dana Farber Cancer Institute) and David C. Huang (The Walter and Eliza Hall Institute), respectively. LipofectAMINE (Invitrogen) was used for transfections according to the user’s manual, and BcXL stably transfected SH-SY5Y cells were selected with 400 µg/ml hygromycin B (Calbiochem) and maintained in medium containing 100 µg/ml hygromycin B after 2–3 weeks of selection. The peptide protease inhibitor e-VAD-fmk was from Enzyme System Products, Livermore, CA. Staurosporine, H7 (1-(5-isoquinolinesulfonfyl)-2-methylpiperazine), and etoposide were from Sigma, and antimycin A was discovered serendipitously (8). A few other compounds have also been discovered in silico, and they are of diverse structures (9–11).

Natural products cover a molecular diversity not available from synthetic libraries with an unrivaled success rate as drug leads (12). We have, therefore, carried out a large scale high throughput screen of natural product extracts to uncover compounds that would disrupt the interaction between BcXL and the Bak BH3 peptide. Here we report the identification of chelerythrine, which is a natural benzophenanthridine alkaloid and a known protein kinase C inhibitor (13), as an inhibitor of BcXL-Bak BH3 peptide binding. Chelerythrine released cytochrome c (Cyt c) from isolated mitochondria and induced apoptosis in BcXL-overexpressing cells that were completely resistant to staurosporine or etoposide. Chelerythrine thus represents the first BH3 mimetic identified through high throughput screening of natural products.

EXPERIMENTAL PROCEDURES

Reagents and Cell Lines—Human SH-SY5Y and MCF7 cells were maintained as described previously (14). HCT116 cells and FDC-P1 cells were gifts from Bert Vogelstein (Dana Farber Cancer Institute) and David C. Huang (The Walter and Eliza Hall Institute), respectively. LipofectAMINE (Invitrogen) was used for transfections according to the user’s manual, and BcXL stably transfected SH-SY5Y cells were selected with 400 µg/ml hygromycin B (Calbiochem) and maintained in medium containing 100 µg/ml hygromycin B after 2–3 weeks of selection. The peptide protease inhibitor e-VAD-fmk was from Enzyme System Products, Livermore, CA. Staurosporine, H7 (1-(5-isoquinolinesulfonfyl)-2-methylpiperazine), and etoposide were from Sigma, and BcXL-I was from Calbiochem.

Fluorescence Polarization (FP) Assay—The Bak BH3 peptide labeled...
CH₂Cl₂ fraction. The active fraction was fractionated using gradient modified Kupchan solvent partition method (15) to give an active assay buffer (50 mM Tris, pH 8, 150 mM NaCl, and 0.1% bovine serum albumin). 5 mM sucrose, 1 mM EDTA, 50 mM HEPES (pH 7.5), 1 mM Tris (pH 8.0), 10 mM EGTA, and 10 mM HEPES (pH 7.5) at 0.5 mg/ml and treated at room temperature with the indicated compounds for 15 min followed by centrifugation. CytC released into the supernatant was subjected to fractionation by 10% SDS-PAGE followed by Western blotting analysis.

**Flow Cytometry**—For detection of sub-G₁ DNA, cells were washed once, resuspended in 200 μl of phosphate-buffered saline, and fixed in a 50-fold excess of ice-cold 70% ethanol. Cells were recovered by centrifugation at 1000 × g for 5 min at 4 °C, washed, stained with 50 mg/ml propidium iodide for 30 min at room temperature, and analyzed in a FACScan flow cytometer (BD Biosciences). Mitochondrial potential change as measured by JC-1 staining was performed in accordance with the manufacturer’s instructions (Molecular Probes). A minimum of 10,000 cells/sample were analyzed.

RESULTS AND DISCUSSION

Identification of Chelerythrine as an Inhibitor of BclXL and BAX Peptide Interaction—A high throughput screen based on FP (7) was devised to identify compounds that disrupt the interaction between BclXL and the BAX domain of Bak. A total of 107,423 extracts prepared from plants, actinomycetes, fungi, marine invertebrates, and marine bacteria were screened. Twelve extracts were chosen for isolation of active compounds, and the active principle of four extracts from plants was found to be chelerythrine (Fig. 1A). Chelerythrine displaced the fluorescently labeled BAX domain peptide from a recombinant GST-BCLXL fusion protein with IC₅₀ of 1.5 μM (Fig. 1B). Similar concentrations of chelerythrine with GST protein and the labeled peptide produced no significant change in polarization (Fig. 1B).

Chelerythrine Disrupts the Interaction between BclXL and BAX—The ability of chelerythrine to displace the Bak BH3 peptide in the FP assay suggests that it may be able to displace BH3-containing proteins from BclXL. In vitro translated [³⁵S]BAX bound specifically to GST-BclXL immobilized on glutathione beads, and the addition of chelerythrine resulted in a dose-dependent decrease in Bax binding (Fig. 1, C and D). Chelerythrine was, however, unable to disrupt interaction between the Caenorhabditis elegans sex determination proteins (³⁵S)FEM3 and (³⁵S)FEM2 (18) immobilized on glutathione beads (data not shown), suggesting that the action of chelerythrine on Bax and BclXL was specific. The solution structure of Bel-2 has been solved (19), and the data suggest that Bel-2 and BclXL have highly similar three-dimensional structures, including the hydrophobic groove. Interestingly we found that

![Figure 1](https://example.com/fig1.png)

**Figure 1.** Chelerythrine is an inhibitor of BclXL-BAX domain peptide interaction. A, structure of chelerythrine. B, displacement of fluorescein-labeled BAX peptide from GST-BclXL by chelerythrine (filled diamond). Displacement of the BAX peptide is indicated by a decrease in polarization, mf, millipolarization. Polarization readings with BAX peptide and GST protein in the presence of increasing concentrations of chelerythrine are shown with open triangles. C and D, dose-dependent displacement of [³⁵S]BAX from GST-BclXL by chelerythrine. C, GST-BclXL bound on Sepharose-glutathione beads was preincubated with the indicated concentrations of chelerythrine for 30 min prior to the addition of an equal amount (500,000 cpm) of [³⁵S]BAX. GST protein bound on beads was eluted as a negative control. Autoradiographs of [³⁵S]BAX and Coomassie Blue images of GST-BclXL and GST are shown. D, the autoradiograph in C was scanned using a densitometer to quantify the bands, and the relative amount of BAX bound to the beads was determined. CTR, control.

![Figure 2](https://example.com/fig2.png)

**Figure 2.** Chelerythrine mediates apoptosis through the mitochondrial pathway. A, chelerythrine induces mitochondrial depolarization that is partially blocked by ZVAD-fmk. SH-SYSY human neuroblastoma cells were treated with the indicated concentrations of chelerythrine in the presence or absence of the broad spectrum caspase inhibitor ZVAD-fmk (20 μM). After 16 h, cells were harvested, stained with JC-1, and analyzed by flow cytometry. The increases in JC-1 green fluorescence (FL1) indicate the degree of depolarization in the mitochondria. B, chelerythrine-induced DNA fragmentation in SH-SYSY cells is efficiently blocked by ZVAD-fmk. SH-SYSY cells were treated with the indicated concentrations of chelerythrine in the presence or absence of ZVAD-fmk (20 μM), and 48 h later they were fixed and stained with propidium iodide. Samples were analyzed by flow cytometry. Percentages of sub-G₁ DNA are shown. Data are representative of at least three experiments. DNA Con., DNA content.

竜粟泰(Plumbaginaceae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。BclXLとBH3ドメイン間の相互作用を阻害する化合物の高通量スクリーニングは、可溶性BclXLとGH3 Bardoxoma vulgarica(Bomboideae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。BclXLとBH3ドメイン間の相互作用を阻害する化合物の高通量スクリーニングは、可溶性BclXLとGH3 Bardoxoma vulgarica(Bomboideae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。BclXLとBH3ドメイン間の相互作用を阻害する化合物の高通量スクリーニングは、可溶性BclXLとGH3 Bardoxoma vulgarica(Bomboideae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。BclXLとBH3ドメイン間の相互作用を阻害する化合物の高通量スクリーニングは、可溶性BclXLとGH3 Bardoxoma vulgarica(Bomboideae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。BclXLとBH3ドメイン間の相互作用を阻害する化合物の高通量スクリーニングは、可溶性BclXLとGH3 Bardoxoma vulgarica(Bomboideae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。BclXLとBH3ドメイン間の相互作用を阻害する化合物の高通量スクリーニングは、可溶性BclXLとGH3 Bardoxoma vulgarica(Bomboideae)から分離抽出したchelerythrineは、BclXL同定した免疫応答を阻害することが報告されている。
the binding of Bax to Bcl-2 was disrupted by chelerythrine in a dose-dependent manner (data not shown).

Chelerythrine-mediated Apoptosis Exhibits Characteristic Features Similar to Cell Death Induced by Proapoptotic Members of the Bcl-2 Family—Since the mitochondria play a key role in the control of apoptosis and it is the main site where BclXL and Bcl-2 exert their function, we evaluated mitochondrial function in response to chelerythrine with the fluorescent dye JC-1 that allows the analysis of mitochondrial potential change (Δψm). Treatment of human neuroblastoma SH-SY5Y cells (20) with chelerythrine at 2.5 and 5 μM for 16 h induced a substantial decrease in mitochondrial potential as indicated by an increase in JC-1 green fluorescence (Fig. 2, A and B). Chelerythrine-induced mitochondrial potential changes were partially inhibited by the broad spectrum caspase inhibitor ZVAD (Fig. 2, A and B), similar to reports showing that Bax-induced mitochondrial potential change was partially sensitive to caspase inhibition (21). Treatment of SH-SY5Y cells with chelerythrine also induced the appearance of sub-G1 DNA that is indicative of apoptosis (Fig. 2, C and D). The appearance of sub-G1 DNA is totally blocked by the addition of ZVAD (Fig. 2, C and D), which is consistent with the notion that DNA fragmentation is dependent on caspase activation (22). The ZVAD-treated cells without sub-G1 DNA, however, were not viable since they were unable to grow upon replating on fresh tissue culture plates (data not shown). Similar to apoptosis mediated by proapoptotic members of the Bcl-2 family, inhibition of caspases only slows down but does not abrogate the cell death process (23, 24). The change in mitochondrial potential and the appearance of sub-G1 DNA upon chelerythrine treatments were observed in two other cell lines, HCT116, a colon carcinoma cell line, and MCF7, a breast cancer cell line (data not shown), suggesting that the effect is not limited to SH-SY5Y cells.

Chelerythrine Triggers Cytc Release from Isolated Mitochondria—Many death stimuli trigger apoptosis through the release of Cytc from the mitochondrial intermembrane space to activate Apaf-1, thus coupling this organelle to caspase activation. Treatment of SH-SY5Y cells with etoposide, staurosporine, and H7 (20) as well as chelerythrine induced mitochondrial potential change, Cytc release from the mitochondria (Fig. 3, A and B), and the appearance of sub-G1 DNA (data not shown), which are hallmarks of apoptosis. However, if the action of chelerythrine is on BclXL or Bcl-2 on the mitochondria, it should be able to trigger Cytc release directly from isolated mitochondria as observed with proapoptotic Bcl-2 family members (25, 26). To investigate this, mitochondria were isolated from healthy SH-SY5Y cells and subjected to treatment with various death stimuli. Chelerythrine released Cytc from isolated mitochondria in a dose-dependent manner (Fig. 3, C and D). Etoposide and other protein kinase C inhibitors such as H7 and staurosporine were unable to do so (Fig. 3, C and D) even at concentrations exceeding the required amount to induce apoptosis in intact cells (Fig. 3, B–D, and data not shown). The interactions of Bax or Bak with BclXL in the mitochondrial preparation, if any, appeared to be very weak (data not shown).
Chelerythrine Induces Apoptosis in BclXL-overexpressing SH-SY5Y Cells—Overexpression of Bcl-2 or BclXL is able to block cell death induced by many forms of death stimuli, e.g., radiation and most chemotherapeutic drugs (2, 27). The limited concentrations of endogenous factors serving the apoptotic signaling pathway preceding the mitochondria step enable BclXL overexpression to block these signals. On the other hand, if a compound acts directly on BclXL, it should be able to overcome the effect of overexpression of the protein easily since cellular protein concentration, even in a state of overexpression, is limited in comparison to concentrations achievable with small molecular weight compounds. To test our hypothesis, we generated SH-SY5Y cells that overexpress BclXL. In these cells the level of BclXL is greatly enhanced, while other members of the Bcl-2 family such as Bax, Bak, and Bid stay relatively constant with a moderate down-regulation of Bcl-2 level (Fig. 4A). Treatment of BclXL-overexpressing cells with staurosporine up to 1 μM did not induce cell death as indicated by the lack of mitochondrial potential change (Fig. 4B, data not shown) as well as the absence of sub-G1 DNA (Fig. 4C). In contrast, the vector line was very sensitive to staurosporine-induced apoptosis. Near 100% of the cells exhibited mitochondrial potential change, and 80% of the cells contained sub-G1 DNA when only a 100 nM concentration of the drug was added (Fig. 4, B and C). Similarly the apoptotic effects of etoposide (Fig. 4, B and C) and H7 (data not shown) were abolished by BclXL overexpression. Interestingly, although the staurosporine- and etoposide-treated BclXL-overexpressing cells did not undergo apoptosis, they were arrested at the G2 and S phase of the cell cycle, respectively (see the supplemental figure). These observations are consistent with previous reports indicating that cell cycle arrests induced by genotoxic drugs are not affected by BclXL overexpression (28). Overexpression of BclXL was able to confer resistance to the killing effect of chelerythrine at low concentration of up to 2 μM. At higher concentrations, chelerythrine overcame the protective effect of BclXL and induced apoptosis in these cells effectively (Fig. 4D). Similar results were obtained with a Bcl-2-overexpressing, mouse interleukin-3-dependent, promyelocytic cell line, FDC-P1, in which chelerythrine at concentrations higher than 1.25 μM was able to overcome the protective effect of Bcl-2 (data not shown). The data suggest that chelerythrine, unlike staurosporine, H7, and etoposide, induces apoptosis by inhibiting BclXL/Bcl-2 directly.

Enhanced expression of antiapoptotic Bcl-2-related proteins in cancer cells has been implicated in resistance to currently available antineoplastic agents (2, 27). Chelerythrine has been shown to exhibit cytotoxic activity against radiosensitive and chemoresistant squamous carcinoma cells and p53-deficient cells (29). It delays tumor growth in an experimental model with relatively mild toxicity to the animal (29). Our results indicate that chelerythrine may act as a BH3 mimetic that is able to circumvent the upstream antiapoptotic barriers in transformed cells and thus can be explored as a potential anticancer therapeutic.

The inhibitors of BclXL-BH3 interaction identified so far are all proapoptotic in nature. However, the diverse structural differences among these compounds suggest that they may act through multiple mechanisms in affecting the Bcl-2 family proteins. Interestingly BclXL overexpression confers slight protection against chelerythrine- and BH3I-1-induced apoptosis, while it sensitizes the cells toward antimycin A3 (8). The identification of chelerythrine as a novel inhibitor of BclXL-BH3 interaction adds to the repertoire of reagents that are invaluable in defining the molecular mechanisms by which proteins of the Bcl-2 family mediate their functions.

Acknowledgments—We are grateful to Drs. Bert Vogelstein, David C. Huang, and Craig B. Thomson for the generous supply of reagents. We thank Shuang Cao for technical assistance, Juan Jose Castillo (Faculty of Agronomy, University of San Carlos, Guatemala City, Guatemala) for the collection of plants used in this study.

REFERENCES

1. Gross, A., McDonnell, J. M., and Korsmeyer, S. J. (1999) Genes Dev. 13, 1899–1911
2. Cory, S., and Adams, J. M. (2002) Nat. Rev. Cancer 2, 647–656
3. Sattler, M., Liang, H., Nettleship, D., Meadows, R. F., Harlan, J. E., Elwinger, M., Youn, H. S., Shuker, S. B., Chang, B. S., Minn, A. J., Thompson, C. B., and Fesik, S. W. (1997) Science 275, 983–986
4. Chittenden, T., Flemington, C., Houghton, A. B., Ebb, R. G., Gallo, G. J., Elangovan, B., Chinnadurai, G., and Lutz, R. J. (1995) EMBO J. 14, 5589–5596
5. Holinger, E. P., Chittenden, T., and Lutz, R. J. (1999) J. Biol. Chem. 274, 13304–13309
6. Baell, J. B., and Huang, D. C. (2002) Biochem. Pharmacol. 64, 851–863
7. Degtarev, A., Lugovsky, A., Cardone, M., Muller, B., Wagner, G., Mitchison, T. J., and Yuan, J. (2001) Mol. Cell. 18, 539–549
8. Truong, S. P., Kim, K. M., Basanze, G., Giedt, C. D., Simon, J., Zimmerman, J., Zhang, K. Y., and Hockenberg, D. M. (2001) Nat. Cell Biol. 3, 183–191
9. Wang, J. L., Liu, D., Zhang, Z. J., Shan, S., Han, X., Srinivasula, S. M., Croce, C. M., Ahnemri, E. S., and Huang, Z. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 7124–7129
10. Eayedy, I. J., Ling, Y., Naero, K., Tomita, Y., Wu, X., Cao, Y., Guo, R., Li, B., Zhu, X., Huang, Y., Long, Y. Q., Roller, P. P., Yang, D., and Wang, S. (2001) J. Med. Chem. 44, 4313–4324
11. Lugovsky, A. A., Degtarev, A. I., Fahmy, A. F., Zhou, P., Gross, A. J., Yuan, J., and Wagner, G. (2002) J. Am. Chem. Soc. 124, 1234–1240
12. Nielsen, J. (2002)Curr. Opin. Chem. Biol. 6, 297–305
13. Herbert, J. M., Augereau, J. M., Gleye, J., and Maffrand, J. P. (1999) Biochem. Biophys. Res. Commun. 172, 993–999
14. Chan, S. L., Tan, K. O., Zhang, L., Yee, K. S., Ronca, F., Chan, M. Y., and Yu, V. C. (1999) J. Biol. Chem. 274, 32461–32468
15. Pettit, G. R., Kamano, Y., Anayi, R., Herald, C. L., Doubek, D. L., Schmidt, B. K., and Ruddie, J. J. (1983) Tetrahedron 41, 885–994
16. Hanaoka, M., Motonishi, T., and Mukai, C. (1986) J. Chem. Soc. Perkin Trans. 1, 2253–2256
17. Tan, K. O., Tan, K. M., Chan, S. L., Yee, K. S., Bevort, M., Ang, K. C., and Yu, V. C. (2001) J. Biol. Chem. 276, 2802–2807
18. Tan, K. M., Chan, S. L., Tan, K. O., and Yu, V. C. (2001) J. Biol. Chem. 276, 44293–44292
19. Petsos, A. M., Medek, A., Nettleship, D. G., Kim, D. H., Yoon, H. S., Swift, K., Matayoshi, E. D., Oltersdorff, T., and Fesik, S. W. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 3012–3017
20. Ronca, F., Chan, S. L., and Yu, V. C. (1997) J. Biol. Chem. 272, 4425–4426
21. Finucane, D. M., Bosay-Wetzel, E., Waterhouse, N. J., Cotter, T. G., and Green, D. R. (1999) J. Biol. Chem. 274, 2223–2233
22. Enari, M., Sakaihira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., and Nagata, S. (1998) Nature 391, 43–50
23. Xiang, J., Chao, D. T., and Korsmeyer, S. J. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14559–14563
24. Cheng, E. H., Wei, M. C., Weiler, S., Flavell, R. A., Mak, T. W., Lindestad, T., and Korsmeyer, S. J. (2001) Mol. Cell 8, 705–711
25. Cosulich, S. C., Worrall, V., Hedge, P. J., Green, S., and Clarke, P. R. (1997) Curr. Biol. 7, 913–920
26. Narita, M., Shimizu, S., Ito, T., Chittenden, T., Lutz, R. J., Matsuda, H., and Tsujimoto, Y. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 14861–14866
27. Johnson, A. T., Rhee, S. G., and Lowe, S. W. (2002) Cell 108, 153–164
28. Minn, A. J., Rudin, C. M., Boise, L. H., and Thompson, C. B. (1995) Blood 86, 1963–1970
29. Chmura, S. J., Dolan, M. E., Cha, A., Mauceri, H. J., Kufe, D. W., and Weichselbaum, R. R. (2000) Clin. Cancer Res. 6, 737–742
Supplemental figure for Chan et al.

Data from FACS analysis are presented. Vector and BclXL overexpressing SH-SY5Y cell lines were treated with increasing concentrations of the indicated compounds for 48 h. The percentage of cells with Sub-G1 DNA is shown.
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J. Biol. Chem. 2003, 278:20453-20456.
doi: 10.1074/jbc.C300138200 originally published online April 17, 2003

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