

Glyfoline induces mitotic catastrophe and apoptosis in cancer cells

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Glyfoline exhibits cytotoxic activity *in vitro* and antitumor activity in mice bearing murine or human solid tumors, but the underlying mechanisms are unknown. In our study, we found that glyfoline inhibited cell growth and induced accumulation of mitotic cells in human cancer cell lines. Glyfoline induced the appearance of spindle abnormalities, chromosome mis-segregation, multipolar cell division and multiple nuclei, all of which are indicative of mitotic catastrophe. However, glyfoline did not bind to DNA and did not inhibit or stabilize tubulin polymerization, but slightly increased the resistance of mitotic spindles to nocodazole-induced disassembly. In addition, microtubule aster formation was significantly enhanced in the extract prepared from glyfoline-arrested mitotic cells compared to that from synchronized mitotic cells. When Eg5, a mitotic kinesin that plays an essential role in establishing mitotic spindle bipolarity, was inhibited using S-trityl-cysteine in glyfoline-treated cells, formation of spindle multipolarity, multipolar cell division, and multinuclei was significantly reduced. After glyfoline-mediated arrest of cells at mitosis, considerable poly(ADP-ribose) polymerase degradation was induced and the number of annexin V-positive cells significantly increased, indicating that glyfoline ultimately induces apoptosis. Small interfering RNA-mediated silencing of the spindle checkpoint proteins BUBR1 and MAD2 markedly reduced induction of mitotic cell accumulation, but did not affect glyfoline-induced mitotic catastrophe and apoptosis. Thus, glyfoline induces mitotic catastrophe probably by enhancing microtubule aster formation and subsequent apoptosis in cancer cells independently of spindle checkpoint function.

Owing to their broad range of bioactivity, acridine and acridone derivatives have a long history of use in the treatment of human diseases, including parasitic infections and cancers.¹ The anticancer activity of these agents is mainly attributed to the planarity of the aromatic structures, which can intercalate within the double-stranded DNA structure and thereby inter-

fere with DNA replication and transcription.² The use of acridine and acridone derivatives as anticancer chemotherapeutics that target DNA,^{3,4} topoisomerases,⁵ telomerase/telomeres,⁶ protein-kinases,⁷ multidrug resistance,⁸ and hypoxia-selective environments⁹ is also under intensive investigation. Glyfoline (4, 1,6-dihydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one, Fig. 1a), an anti-neoplastic 9-acridone alkaloid first isolated from *Glycosmis citrifolia*, has been synthesized¹⁰ and shows potent antitumor activity against several murine and human tumors both *in vitro* and *in vivo* and is cytotoxic for doxorubicin-resistant human leukemia HL-60 cells.^{10,11} Immunoelectron microscopic analysis of biotinylated glyfoline in nasopharyngeal carcinoma cells revealed that it accumulates at the inner mitochondrial membrane.¹² Nasopharyngeal carcinoma cells treated with glyfoline were arrested at G2/M phase of the cell cycle and underwent apoptotic changes,¹³ indicating that glyfoline-induced damage alters G2/M progression and results in apoptosis. However, the underlying mechanism(s) of its anti-neoplastic activity is uncertain.

Mitotic catastrophe is defined as a type of cell death that occurs during, or shortly after, dysregulated or failed mitosis¹⁴⁻¹⁶ and a process that results from aberrant mitosis and leads to cell death.¹⁷ Typical features of mitotic catastrophe are blocked mitosis, mitotic spindle disorganization, and failed chromosome segregation. Mitotic catastrophe can be induced by DNA damage, disruption of mitotic spindles, prolonged growth arrest, or inhibition of the cyclin-dependent kinase, CHK1, or Aurora kinases.^{14,16} A combination of cell cycle

Key words: glyfoline, mitotic arrest, mitotic catastrophe, microtubule aster formation, apoptosis

Abbreviations: Boc-D-FMK: Boc-Asp(OMe)-fluoromethyl ketone; EtBr: ethidium bromide; HPLC: high-pressure liquid chromatography; PARP: poly(ADP-ribose) polymerase; PBS: phosphate-buffered saline; PI: propidium iodide; PMSE: phenylmethylsulfonyl fluoride; siRNAs: small interfering RNAs; WST-8: methylthiazole tetrazolium; Z-IE(OMe)TD(OMe)-FMK: Z-Ile-Glu(OMe)-Thr-Asp(OMe)-fluoromethyl ketone; Z-IE(OMe)HD(OMe)-FMK: Z-leu-Glu(OMe)-His-Asp(OMe)-fluoromethyl ketone; Z-VAD(OMe)-FMK: Z-Val-Ala-Asp(OMe)-fluoromethyl ketone

Grant sponsor: National Science Council; **Grant number:** NSC95-2311-B-001-059-MY3; **Grant sponsor:** Academia Sinica, Taiwan;

Grant number: AS-96-TP-B06-component project 3

DOI: 10.1002/ijc.24841

History: Received 21 Jun 2009; Accepted 4 Aug 2009; Online 20 Aug 2009

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