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REVIEW ARTICLE

Gossypol: from contraceptive for men to antitumoral activity

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ABSTRACT

It is widely thought that Bcl-2 family proteins regulate commitment to apoptosis primarily through their capacity to control the permeability of the mitochondrial outer membrane permeabilization (MOMP) triggers the release of multiple apoptogenic factors into the cytosol and, thereby apoptosis. Various Bcl-2 family members affect this key event of the apoptotic cascade in different ways, determining their pro- or anti-apoptotic status. The Bcl-2-type proteins inhibit MOMP, thereby preserving cell viability. In contrast, Bax-type proteins and the diverse group of BH3-only proteins facilitate MOMP and thus promote cell death. Recently, several drugs that act as BH3 mimetics have been identified, including Gossypol. This review revisits the proprieties of gossypol family, their using as anticancer agents for cancer therapy.

Keywords: *Apoptosis, Gossypol, Bcl-2, Antitumoral activity*

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INTRODUCTION

Our story concerns the intrinsic or mitochondrial pathway of apoptosis, in which mitochondrial outer membrane permeabilization (MOMP) results in diffusion of proteins from the intermembrane space to the cytosol. Holocytochrome c (cyto c) thus gains access to APAF-1, leading to caspase activation and death. Even without caspase activation, MOMP generally results in cell death. The decision, MOMP or no MOMP, translating into death or survival of the cell, is made by the interactions of the Bcl-2 family proteins, both pro- and antiapoptotic. Of these, Bax and Bak are the proapoptotic "effectors," which are likely to be directly responsible for the permeabilization of the outer membrane. These are antagonized by the actions of the antiapoptotic Bcl-2 proteins (Table 1), including Bcl-2, Bcl-xL, Mcl-1, and Bfl/A1. A third class of Bcl-2 proteins, the BH3-only proteins, appear to make the "thumbs up or thumbs down" decision by regulating the other family members (1). Recently, several drugs that act as BH3 mimetics have been identified (2), including Gossypol. The ability of gossypol to bind to Bcl-2 and Bcl-X_L was discovered using computer-assisted molecular modeling and fluorescence-polarization assays (3). Nuclear magnetic resonance imaging studies have shown gossypol binding to the BH3 binding pocket of Bcl-X_L (3). Gossypol is a polyphenol isolated

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from the seed, roots, and stem of the cotton plant (*Gossypium sp.*) (Figure 1). The substance, a yellow pigment similar to flavonoids, is present in cottonseed oil. In the plant, it acts as a natural defensive agent against predators, provoking infertility in insects. In the 1970s, the Chinese government began researching the use of gossypol as a contraceptive. Their studies involved over 10,000 subjects, and continued for over a decade. They concluded that gossypol provided reliable contraception, could be taken orally as a tablet, and did not upset men's balance of hormones. However, gossypol also had serious flaws. The studies also discovered an abnormally high rate of hypokalemia among subjects. Hypokalemia — low blood potassium levels — is usually the result of kidney malfunction and causes symptoms of fatigue, muscle weakness, and at its most extreme, paralysis. In addition, about 7% of subjects reported effects on their digestive system, and about 12% increased fatigue. Most subjects recovered after stopping treatment and taking potassium supplements. A later study showed that taking potassium supplements during gossypol treatment did not prevent hypokalemia in primates. In 1998, the World Health Organization's Research Group on Methods for the Regulation of Male Fertility recommended that research should be abandoned. In addition to the other side effects, the WHO researchers were concerned about gossypol's toxicity: the toxic dose in primates is less than 10 times the contraceptive dose. This report effectively ended further studies of gossypol as a temporary contraceptive, but research into using it as an alternative to vasectomy continues in Austria, Brazil, Chile, China, the Dominican Republic, and Nigeria (4).

In addition to its contraceptive properties, gossypol has also long been known to possess anti-malarial properties. The ability of gossypol to bind to Bcl-2 and Bcl-X_L it confers anti tumor activity.

Table 1. Classification of Bcl-2-family proteins*

Apoptotic property	Subclass	Protein nomenclature	Domains
Anti-apoptotic	Multidomain	Bcl-2, Bcl-XL, Mcl-1, Bfl/A1, Bcl-w, Bcl-B	BH1, BH2, BH3, BH4
Proapoptotic	Multidomain	Bax, Bak, Bok	BH1, BH2, BH3
	BH3 only (direct activator)	Bid, Bim	BH3
	BH3 (derepressor)	Bad, Bik, Bnip3, Puma, Noxa, Bmf, Hrk, others	BH3

* From Azmi, A. S. and R. M. Mohammad (2009)

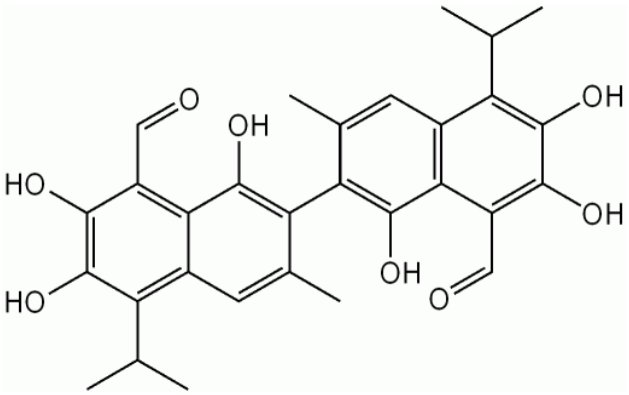

Figure 1. The chemical structure of (–)-gossypol.

There are three isoforms of gossypol, also known as BL-193 or AT-101, which include (-)-BL-193, (+)-BL-193, and (±)-BL-193. The (-)-BL-193 has been shown to be more potent than its either isoforms in its growth-inhibitory effects. Multidimensional nuclear magnetic resonance methods have shown (-)-BL-193 binds with nanomolar affinity to Bcl-2 (320 nmol/L), Bcl-xl (480 nmol/L), and Mcl-1 (180 nmol/L) (5, 6). Single-agent activity of (–)-gossypol has been shown in a variety of cancer cell lines, including chronic lymphocytic leukemia (CLL), prostate and colon carcinoma, and patient-derived multiple

myeloma and diffuse large-cell lymphoma cells (7-11). Furthermore, synergy was seen with dexamethasone in multiple myeloma and with docetaxel in prostate cancer cell lines (12, 13). In addition, (–)-gossypol was shown to be effective with cyclophosphamide and rituximab in a B-cell lymphoma xenograft model (14).

Mechanistically, gossypol has been shown to disrupt Bcl-xl–Bim and Bcl-xl–Bax complexes (9). However, we have recently shown that gossypol-induced apoptosis in a Bax- and Bak-independent manner by converting Bcl-2 into a proapoptotic molecule (15). We found that gossypol, but not other Bcl-2-interacting molecules, induced cyto c release and loss of mitochondrial membrane potential independently of mitochondrial permeability transition pores (mPTP) and Bak/Bax activation. Furthermore, gossypol induced an allosteric change in Bcl-2 in both $bak^{-/-}/bax^{-/-}$ cells and Bcl-2 overexpressing cells. This change in Bcl-2 conformation led to the release of cyto c in the presence of Bcl-2 and Bcl-xL in reconstituted proteoliposomes. Gossypol, also suppresses tumor growth in Bcl-2 overexpressing cells xenografts in nude mice. We conclude that gossypol converts the antiapoptotic molecule Bcl-2 into a proapoptotic molecule that can mediate the release of cyto c and induces apoptosis. Notably, the conversion was accomplished using a mixture of gossypol enantiomers, suggesting that tests should be repeated with (–)-gossypol alone. This is the first study describing a small molecule that can induce Bcl-2 conformational change and links this change to cyto c release. Our results suggest that, in addition of the rheostat balancing of protective Bcl-2 protein to proapoptotic Bcl-2 family proteins, the conformational status of Bcl-2 is also important in determining the fate of the cell. It has also been shown interaction of Bcl-2 with a nuclear orphan receptor may convert Bcl-2 from a protector to a killer molecule via conformational change. Photodynamic therapy and ursodeoxycholic acid also cause a conformational change in Bcl-2 and promotes HA14–1 to bind to Bcl-2.

It has been shown earlier that gossypol could induce oxidative DNA breakage in vitro in the presence of metal ions such as copper (16, 17). In a recent report it has been shown that gossypol induces apoptosis in chronic lymphocytic leukemia through generation of ROS which in turn mediate the release of cytochrome c and thus causing apoptosis (7). In another recent report in reconstituted proteoliposomes, it has been shown that gossypol competes with Bax/Bid BH3 ligands for binding to Mcl-1 hydrophobic grove, thereby providing an explanation for how gossypol restores Bax permeabilizing function in the presence of Mcl-1. No evidence was found indicating that gossypol transforms Mcl-1 into a killer molecule (18). Further work needs to be done to ascertain gossypol's primary mechanism of action. Currently, (–)-gossypol is being evaluated in phase I and II clinical trials as a single agent in B-cell malignancies and prostate cancer, and in combination with other antitumor agents in a variety of hematologic, lymphoid, and solid-tumor malignancies (6) (Details can be found at www.clinicaltrials.gov/ct2/results?term=at101).

APOGOSSYPOLONE (APOG2)

ApoG2 is a derivative of gossypol that was designed by Ascenta in order to reduce the non-specific reactivity and toxicity of gossypol and is currently in the preclinical phase of testing. This modification involved the removal of two reactive aldehyde groups on the polyphenolic rings of gossypol. Current research shows ApoG2 is a potent inhibitor of Mcl-1 and Bcl-2 proteins. ApoG2 has recently been shown that blocks binding of Bim and Bcl-2 and induces apoptosis in lymphoma cell lines with minimal toxicity (19) . Further it has also been shown that Apog2 induces

apoptosis in follicular Small Cleaved Cell Lymphoma model, pre-B-acute lymphoblastic leukemia, mantle cell lymphoma, marginal zone lymphoma, as well as chronic lymphocytic leukemia. Therefore, ApoG2 could potentially be a more effective drug in the lymphoma clinic spanning a greater array of patients (20). More recently, ApoG2 alone or in combination with adriamycin has been shown induces apoptosis in human hepatocellular carcinoma cells (HHC) by downregulating anti-apoptotic proteins Bcl-2, Mcl-1, and Bcl-XL, up-regulating pro-apoptotic protein Noxa, and promoting the activities of caspases-9 and -3. Tumor growth in hepatocellular carcinoma xenograft was inhibited in nude mice when ApoG2 was administered orally without causing damage to the normal tissues (21). ApoG2, also induces apoptosis and suppresses tumor growth in nasopharyngeal carcinoma xenografts (22) .

CONCLUDING REMARKS

New genetic and biochemical approaches have fostered remarkable progress in our understanding of cancer biology during the past decade (23). One of the most important advances has been the recognition that resistance to cell death, particularly apoptotic cell death, is an important aspect of both tumorigenesis and development of resistance to anticancer drugs (23-25). Much recent research on new cancer therapies has therefore focused on devising ways to overcome this resistance and to trigger the cell death of tumor cells. Although the detailed mechanisms underlying tumor cells resistance to apoptosis remain to be characterized, some important components and steps in this process have already been elucidated. For decades, clinicians and basic scientists have been puzzled by the fact that tumor cells simultaneously acquire the capability to escape immune surveillance mechanisms and evade the cytotoxic action of diverse cytotoxic insults, for example, DNA damage (e.g. by irradiation, alkylation, methylation or crosslinking), microtubule destabilization or topoisomerase inhibition. Cancer stem cells concept and new genetic and biochemical approaches in breast cancer indicate also that drug resistance is likely not a primary consequence of acquired genetic alterations selected during or after therapy, but rather inherent to the malignant behavior of cancer cells at diagnosis. Various mechanisms including genetic instability, oncogene overexpression, tumor suppressor down regulation, epigenetic modifications, loss of cell cycle control and impact of tumor microenvironment result in development of tumor resistance to cell death. Understanding these mechanisms at the molecular level provides deeper insight into carcinogenesis, influences therapeutic strategy and might, ultimately, lead to new therapeutic approaches based on modulation of apoptosis sensitivity. The discovery that gossypol can change Bcl-2 from a protector to a killer will be help further elucidate in molecular mechanism of tumor resistance to cell death. This discovery also holds promise for cancer therapy, as overexpression of Bcl-2 or Bcl-xL has been observed in many forms of cancer. Gossypol or ApoG2 represents a promising lead for the development of more potent and specific agents targeting Bcl-2-regulated apoptosis both alone or in combination with other anticancer agents.

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