

Perspective

See related article by Kim et al., p. 782

Benzyl Isothiocyanate: Double Trouble for Breast Cancer Cells

Chinthalapally V. Rao

Abstract

An inverse association between dietary intake of cruciferous vegetables and cancer risk has been established for different types of malignancies, including breast cancer. The anticarcinogenic effect of cruciferous vegetables has been attributed to chemicals with an isothiocyanate (ITC) functional moiety. Research over the past three decades has provided extensive preclinical evidence for the efficacy of various ITCs against cancer in preclinical models. Benzyl isothiocyanate (BITC) is one such compound with the ability to inhibit chemically induced cancer, oncogenic-driven tumor formation, and human tumor xenografts in rodent cancer models. Prior work also has established that BITC has the ability to influence carcinogen metabolism and signaling pathways relevant to tumor progression and invasion. In this issue, Kim and colleagues show that BITC inhibits breast cancer stem cell growth, both *in vitro* and *in vivo*, in association with suppression of the full-length receptor tyrosine kinase RON as well as its activated truncated form (sfRon), both of which seem to drive stemness in breast cancer cells. Overexpression of RON or sfRon prevented the BITC effect. These data complement prior work from this group showing elimination of mammary tumor cells via tumor cell apoptosis by BITC administration. The inhibition of breast cancer stem cells is observed at pharmacologic concentrations of BITC. This perspective briefly reviews epidemiologic evidence, preclinical efficacy data, and molecular and cellular mechanistic attributes of BITC. Critical issues relevant to clinical development of BITC are discussed briefly. *Cancer Prev Res*; 6(8); 760–3. ©2013 AACR.

Introduction

Ancient holy writings emphasized the medicinal value of isothiocyanate (ITC)-rich herbs and plants, apart from their use in cooking, for pickling, as natural toothpaste, and in massage. The ancient Greeks believed that ITC-rich herbs such as mustard had been created by Asclepius, the God of healing, as a gift to mankind. Similarly, sticks from the roots of *Salvadora persica*, commonly known as Miswak sticks and which contain high levels of ITCs, were used for centuries as a traditional method of cleaning teeth (1). Epidemiologic studies suggest that consumption of a wide variety of vegetables and fruits is protective against cancers, and there is a consistent inverse association between the consumption of *Cruciferae* vegetables and risk of cancer at most sites (2, 3). The protective effect of cruciferous vegetables against cancer has been suggested to be due to their relatively high content of glucosinolates, which store ITCs (3–5). For example, broccoli is a rich source of glucoraphanin, a precursor of

sulforaphane (SFN), and garden cress is rich in glucotropaeolin, the precursor of benzyl isothiocyanate (BITC). Although more than 100 structurally different glucosinolate precursors of ITCs have been identified in nature, SFN, allyl isothiocyanate, phenethyl isothiocyanate, and BITC have been studied most extensively for their anticarcinogenic properties and mechanistic actions (5–6).

Even a subtle difference in ITC structure and dose can translate into remarkable divergence in the mechanism of the anticancer effect (3, 7). Existing evidence from preclinical models is mature enough to warrant translation of selective ITCs into human clinical trials. There is growing evidence that the cancer chemopreventive activities of ITCs are complex and can target multiple mechanisms associated with tumor initiation, promotion, progression, and metastasis (3, 4). This perspective report will summarize possible mechanisms and recent novel findings about BITC as a potential chemopreventive agent for human clinical trials.

The cancer chemopreventive potential of BITC has been well established in rodents; wherein, it has the ability to inhibit chemically induced as well as spontaneous cancer development (3, 4, 8, 9). Studies from different laboratories have shown that BITC inhibits chemically induced lung, esophageal, forestomach, urinary bladder, mammary, liver, pancreatic, and colon tumors (8–10). *In vivo* growth of human cancer cells (mammary, pancreatic, and leukemia) implanted into athymic mice is also retarded by BITC administration (11, 12). Dietary administration of BITC for 25 weeks markedly suppressed the incidence and burden

Author's Affiliation: Center for Cancer Prevention and Drug Development, Department of Medicine, Hematology-Oncology Section, PCS Oklahoma Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

Corresponding Author: Chinthalapally V. Rao, Center for Cancer Prevention and Drug Development, 975 Northeast 10th Street, BRC Building II, Room 1203, OUHSC, Oklahoma City, OK 73104. Phone: 405-271-3224; Fax: 405-271-3225; E-mail: cv-rao@ouhsc.edu

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of mammary hyperplasia and carcinoma in mammary tumor virus (MMTV)-*neu* female mice (12). BITC decreased pulmonary metastasis multiplicity and volume of 4T1 murine mammary carcinoma cells injected into the inguinal mammary fat pads of syngeneic female BALB/c mice (13). In addition to the animal studies, a great deal of information exists on *in vitro* anticarcinogenic effects of BITC in a wide variety of tumor cell lines derived from different organ sites (14).

Pharmacokinetic studies suggest that low $\mu\text{mol/L}$ concentrations of BITC are sufficient for tumor inhibitory activities (14). BITC accumulates rapidly in cancer cells and is conjugated with intracellular thiols, particularly glutathione (GSH) and cysteine (3). After ingestion of BITC by human volunteers, the *N*-acetyl cysteine (NAC) conjugate, representing more than 50% of the initial dose, was excreted rapidly in urine, with maximal concentrations evident 2 to 6 hours after dosing (15). Although the biologic effects of NAC conjugates of ITCs are not fully understood, these agents have proven to be effective chemopreventive agents in rodent models (3, 4, 15). Recently, stable reaction products of ITCs with albumin and hemoglobin have been identified (16), and while detailed pharmacokinetic analysis of BITC remains to be established, existing evidence supports association of human physiologic ITC dose levels with cancer chemoprevention effects in rodent models.

Mechanisms of Action of ITCs/BITC

Prevention of cancer initiation by ITCs may involve modulation of carcinogen metabolism through inhibition of phase I enzymes or induction of phase II enzymes, or both (14). BITC and GSH conjugates of BITC were found to inhibit dealkylation of pentoxyresorufin and ethoxyresorufin in liver microsomes, reactions predominantly mediated by cytochrome p450 (CYP)2B1, CYP1A1/1A2, and CYP2E1, as well as by human CYP2B6 and CYP2D6 (3, 8, 17). Interestingly, CYP2B1 is one of the isozymes involved in activation of the tobacco carcinogen 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK; ref. 18). Induction of phase II enzymes is another mechanism by which chemopreventive agents effectively detoxify and eliminate carcinogens before they act as genotoxic agents. Administration of BITC in the diet (0.5%, w/w) for 2 weeks resulted in an increase in activity of the phase II enzyme quinone reductase in the lung, kidney, urinary bladder, and colon of female CD-1 mice (4, 8). The BITC-mediated increase in glutathione *S*-transferase activity in liver and forestomach (19) and the intracellular accumulation GSH in mouse skin papilloma cells correlated with quinone reductase activity (20). In summary, BITC treatment modulates carcinogen metabolism by inhibition of CYP450s and induction of phase II enzymes, leading to suppression of cancer initiation.

Modulatory effects of ITCs on tumor cell proliferation, apoptosis, and autophagy have been studied extensively. Cell-cycle arrest has been shown with BITC treatment in a wide variety of cancer cell lines derived from different organ

sites (21, 22). G_2 -M phase arrest is the most frequently observed cell-cycle change after treatment of cancer cells with BITC, and it is associated with upregulation of many cell-cycle-related genes (23). MCF-10A, a normal mammary epithelial cell line, was significantly more resistant to cell-cycle arrest by BITC. This resistance to cell-cycle arrest may explain partly the relative insensitivity of normal cells and greater selectivity of BITC for tumor cells. The molecular basis for the discrepancy in sensitivity of tumor cells to cytotoxic effects of BITC is still unclear. BITC-mediated G_2 -M phase arrest was blocked significantly by NAC or tiron (24), suggesting that reactive oxygen species (ROS)-dependent activation of extracellular signal-regulated kinase (ERK) may be involved in BITC-induced G_2 -M phase arrest (24). In addition, BITC treatment resulted in disruption of microtubule polymerization with mitotic arrest in A549 lung cancer cells (23).

BITC-induced apoptosis has been documented in cultured (21, 24–26) as well as in xenografted cancer cells (13, 21). Execution of BITC-induced apoptosis likely depends on both mitochondrial and death receptor pathways (21). Although the exact mechanism by which BITC causes apoptosis has been the topic of intense research in the past decade, it is now clear that BITC has the ability to target multiple pathways leading to induction of apoptosis. Key mechanisms in BITC-induced apoptosis include ROS production due to inhibition of mitochondrial respiration, ERK/Akt, and forkhead box protein O1 activation, and modulation of apoptotic and inflammatory proteins (27). The exact relationship between the various BITC-mediated molecular alterations listed earlier and induction of apoptosis is not entirely clear as results differ from laboratory to laboratory. In addition to induction of apoptosis, recent studies show autophagy in response to BITC treatment of cultured breast cancer cells and mice with xenografts (21–23, 27).

Antiangiogenic effects of BITC have been examined by several investigators. Analysis of the vasculature in tumors of mice with MDA-MB-231 xenografts indicated smaller vessel area after BITC treatment compared with control based on immunohistochemistry for the angiogenesis markers CD31 and VEGF receptor 2 (VEGFR2; 12). The BITC treatment caused a significant reduction in the levels of matrix metalloproteinase (MMP)-2 and MMP-9 and downregulated urokinase-type plasminogen activator and plasminogen activator inhibitor-1 in breast cancer cells and xenografts (13). In addition, BITC has been shown to inhibit neovascularization in the rat aorta and chicken chorioallantoic membrane (28), consistent with inhibition of cancer cell migration and invasion. Collectively, these results indicate that BITC has the ability to inhibit several steps necessary for cancer metastasis, including invasion, angiogenesis, and cell migration, by affecting multiple pathways. In addition to these effects, recent evidence shows that BITC-mediated inhibition of mammary cancer development in MMTV-*neu* mice was associated with a marked increase in levels of E-cadherin protein (12), suggesting a possible role of BITC in suppression of the epithelial-mesenchymal transition (EMT).

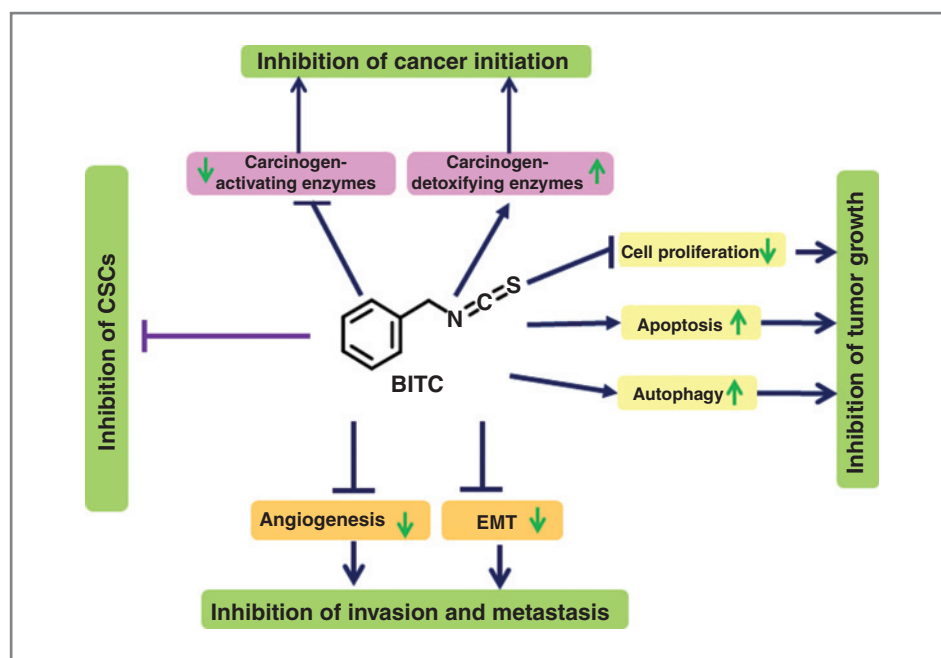


Figure 1. Possible mechanisms of action of BITC as an anticancer agent.

The article by Kim and colleagues (29) in this issue of the *Cancer Prevention and Research* adds a new dimension to the previous mechanistic studies. This study highlights the *in vitro* and *in vivo* potential of BITC to inhibit breast cancer stem cells (bCSC). The authors have shown that a small subset of bCSCs characterized by CD44^{high}/CD24^{low}/ESA⁺ marker expression, aldehyde dehydrogenase-1 (ALDH1) activity, and the ability to form mammospheres were significantly inhibited by BITC. It is noteworthy that concentrations of BITC achievable in plasma significantly decreased mammosphere formation frequency and CD44^{high}/CD24^{low}/ESA⁺ and/or ALDH1⁺ populations in cultured MCF-7 (estrogen receptor-positive) and in SUM159 (triple-negative) breast cancer cells. In an *in vivo* efficacy assay, chronic dietary administration of BITC (3 μ mol BITC/g diet) resulted in a marked decrease in bCSCs in tumors of MMTV-*neu* mice. BITC-mediated inhibition of bCSCs was associated with decreased expression of full-length receptor tyrosine kinase Ron as well as of its truncated activated form (sfRon), which drives stemness in breast cancer cells. Overexpression of sfRon could prevent the BITC effect. The authors have shown that BITC treatment eliminates bCSCs *in vitro* and *in vivo*. The ability of BITC to eliminate both therapy-sensitive epithelial breast cancer cells and therapy-resistant mammary stem cells is notable because the current treatment modalities for triple-negative, therapy-resistant breast cancer are very poor.

Overall, the existing data suggest that BITC is highly effective in suppressing cancer initiation by modulating carcinogen-activating (phase I) and -detoxifying (phase II) enzymes during initiation events of tumor growth. It affects cell-cycle regulation, induction of apoptotic and autophagy events, tumor invasion and metastasis, angiogenesis, and EMT, and it can eliminate CSCs. Thus, BITC, and possibly other ITCs, are multifunctional compounds that may affect

all of the processes of carcinogenesis (Fig. 1). Epidemiologic, preclinical, and mechanistic studies all support the anticancer efficacy of BITC and warrant the clinical development of this and possibly other ITCs for cancer prevention and treatment.

Despite many advances in understanding the cancer preventive effects of BITC, a few issues require further attention. First, the efficacy of BITC or of several synthetic ITCs in inhibiting chemically induced tumors is not unequivocal or absolute. For example, BITC failed to confer protection against lung cancer induced by the tobacco-specific carcinogen NNK during the progression stages of carcinogenesis (30). Similarly, BITC administration was not protective against induction of esophageal tumors by *N*-nitrosomethylbenzylamine in rats (31). Furthermore, BITC has been shown to promote bladder carcinogenesis in rats (32). Even several synthetic isothiocyanates such as phenylhexyl isothiocyanates that were highly effective in suppressing NNK-induced lung tumor formation in mice failed to provide any protective effects against chemically induced esophageal and colon cancers (31, 33). Thus, elucidation of the exact mechanisms involved in the divergent chemopreventive effects requires further investigation. Available data suggest that unanticipated adverse effects were associated with high dosages of the ITCs; however, the adverse effects occurred at doses that are several-fold higher than the normal physiologic doses. In that context, it is noteworthy that the studies by Kim and colleagues (29) showed significant chemopreventive effects at low dose levels corresponding to human exposure levels. A second issue that needs to be addressed is establishment of a formulation of pure BITC suitable for clinical investigation. Third, comprehensive pharmacokinetic data for BITC still are lacking. Finally, suitable biomarker(s) predictive of response are needed for pilot clinical investigations and prior to clinical

trials with cancer incidence as the primary endpoint, which require substantial resources and thousands of subjects.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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