Short Communication

Dietary Isothiocyanate Iberin Inhibits Growth and Induces Apoptosis in Human Glioblastoma Cells

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Abstract. In this study, we evaluated the antiproliferative and proapoptotic effects of the isothiocyanate iberin, a bioactive agent in Brassicaceae species, in human glioblastoma cells. The human glioblastoma cell cultures were treated with different concentrations of iberin and tested for growth inhibition, cytotoxicity, induction of apoptosis, and activation of caspases. Iberin inhibited growth of tumor cells in cell proliferation assays, enhanced cytotoxicity, and induced apoptosis by activation of caspase-3 and caspase-9. Findings from this study could provide a basis for potential usefulness of the diet-derived isothiocyanate iberin as a promising therapeutic micronutrient in the prevention/intervention of brain tumors.

Keywords: iberin, glioblastoma, apoptosis

Isothiocyanates, plant-derived dietary compounds, are promising chemopreventive agents since they are generally non-toxic substances that interfere with the process of cancer development or carcinogenesis. Several natural and synthetic isothiocyanates have demonstrated cancer-preventive properties in animals treated with chemical carcinogens, including polycyclic aromatic hydrocarbons and nitrosamines (1–3). Tumorigenesis is a multistage process with an accumulation of genetic alterations. Suppression of cell proliferation and induction of differentiation and apoptosis during the promotion and progression stages are ideal preventive devices. Glioblastoma is the most common malignant tumor of the adult central nervous system and is hallmarked by high proliferation of tumor cells with increased cellularity and necrosis. Failure to respond to standard therapy including surgery, radiation, and chemotherapy prompts the development of alternative treatment modalities. Recent studies indicate that natural isothiocyanates such as sulforaphane and phenethyl isothiocyanate possess strong antitumor activities both in vitro and in vivo (2, 4–7). There are few studies on the isothiocyanate iberin (sulforaphane sulfoxide analog) (Fig. 1A) in comparison to sulforaphane and phenylethyl isothiocyanate, and its antitumor properties are not thoroughly examined. In the present study, we investigated the effect of the isothiocyanate iberin on the growth of human glioblastoma SNB19 cells. Our findings indicate that isothiocyanate-mediated apoptosis in glioblastoma cells is associated with activation of caspases.

Iberin was isolated from Lesquerella fendleri seedmeal as described previously (8). The human glioblastoma SNB19 cells were cultured in DMEM supplemented with 10% fetal bovine serum, penicillin (100 units/mL), and streptomycin (100 µg/ml) and maintained at 37°C in a 95% air / 5% CO₂ humidified incubator (9). Stock solution of iberin was prepared in DMSO, and an equal volume of DMSO (final concentration was less than 0.1%) was added to controls. Glioblastoma cells were treated with iberin at the indicated concentrations or the equivalent volume of vehicle (DMSO). The antiproliferative effects of iberin were investigated by the widely used MTT cell proliferation assay. To assess cell cytotoxicity, lactate dehydro
enzyme (LDH) leakage was determined in the extracellular cell-culture medium using the CytotoTox 96 Non-Radioactive Cytotoxicity Assay Kit (LDH) from Promega (Madison, WI, USA) according to the manufacturer’s recommended protocol.

Cells were treated with different concentrations of iberin for 24 h and were stained with FLICA (Immunochemistry Technologies Bloomington, MN, USA) following the manufacturer’s instructions. Subsequently the cells were stained with 4’,6-diamino-2-phenylindole (DAPI) to detect the apoptosis induced activation of nuclear fragmentation in situ and examined by fluorescence microscopy. For Western blot analysis, cells were extracted in a buffer solution containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Nonidet P-40, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride, and 1 µg/ml aprotinin on ice for 20 min. A 25-µg protein sample was analyzed by SDS-PAGE, and caspase-3 and caspase-9 protein expression levels were determined by immunoblotting with the following monoclonal antibodies: Caspase-3 (Cell Signaling Technology, Beverly, MA, USA) and Caspase-9 (Cell Signaling Technology). For detection, the ECL detection system (Amersham Biosciences, Buckinghamshire, England) was used according to the manufacturer’s protocol. Equal loading of gels was confirmed by reincubation of the membranes with monoclonal antibody of β-actin (Abcam, Cambridge, MA, USA). Statistical significance of the experimental results was determined by Student’s t-test. For all analyses P<0.05 was accepted as a significant probability level.

The morphological changes by light microscopy in iberin-treated cells are shown in Fig. 1B. We observed cell contraction and nuclear condensation in glioblastoma cells treated with iberin. As shown in Fig. 1C, iberin had a marked dose-dependent effect on SNB19 cell growth. The growth was inhibited by 23%, 38%, and 50% in cells exposed to 1.0, 2.5, and 25 µM iberin, respectively, as compared to the vehicle (DMSO)-treated control cells. An increase in the number of plasma-membrane-damaged cells results in an increase in LDH activity in the culture supernatant. Figure 1D shows the concentration-dependent LDH release from iberin-exposed tumor cells. As shown in Fig. 1D, concentrations as low as 1.0 µM iberin caused a significant increase in LDH release over the DMSO control group (P<0.05), indicating that iberin exposure causes significant damage to the plasma membrane of the tumor cells.

To know whether inhibition of growth by iberin treatment is involved in induction of apoptosis in SNB19 cells, we examined the nuclear morphology of cells with the fluorescent dye DAPI. Within 24 h of treatment with 1 µM iberin, cells clearly exhibited significant morphological changes and chromosomal condensation, which is indicative of apoptotic cell death (Fig. 2A). Such results imply that the cytotoxic action of iberin was due to its ability to induce apoptosis. The detection of activated caspases by application of FLICA was performed with fluorescence microscopy. In the present study a strong correlation was also seen between the percentage of cells labeled with FLICA and those cells labeled with DAPI exhibiting nuclear fragmentation (Fig. 2A). These findings demonstrate that iberin activates induction of apoptosis in human glioblastoma cells (Fig. 2B).
We evaluated caspase-3 by measuring relative levels of activated caspase-3 by Western blotting. As shown in Fig. 3, A and B, the immunoblotting data indicated an increase in cleaved caspase-3, demonstrating the participation of caspase-3 in iberin-induced apoptosis. We also examined the activation of caspase-9 in order to examine the involvement of the mitochondrial pathway in apoptosis induction in iberin-treated cells. As illustrated in Fig. 3, A and C, iberin caused a down-regulation of the procaspase-9 form in SNB19 cells. These data point towards an involvement of the mitochondria-mediated pathway in apoptosis induction by iberin in human glioblastoma SNB19 cells.

Glioblastoma is the most common malignant tumor of the adult central nervous system and relapses with high rates of mortality despite multimodal therapies.
Different epidemiological studies have indicated that diet and cancers are closely associated and people who consume higher amount of fruits and vegetables have a lower risk of various types of cancers (10, 11). Several studies demonstrate that glucosinolates and their main breakdown products, for example, isothiocyanates and indoles, which are present in cruciferous vegetables, exhibit protective activities against cancers (2, 12). Isothiocyanates are known to inhibit the growth of cancer cells, but the mechanisms are still only partially understood. The isothiocyanate iberin has been reported to exhibit certain biological effects (5, 13, 14), but its anticancer mechanism is still elusive. Consequently, the present study was undertaken to shed more light on the mechanisms by which iberin exerts its anti-proliferative properties in cultured human glioblastoma cells. In the present study, for the first time we have demonstrated that iberin inhibits the growth of human glioblastoma SNB19 cells.

Glioblastomas usually present defects in the expression and function of elements of the apoptotic machinery and the identification of novel agents capable of triggering apoptosis in this type of tumor has become an important therapeutic objective. The iberin treatment correlated with major alterations in SNB19 cells that are characteristic of apoptosis including DNA fragmentation and chromatin condensation. Apoptosis was associated with the antiproliferative effect of iberin in SNB19 cells. Apoptosis was mainly induced at a low concentration (2.5 µM). These results are consistent with another study showing that iberin induces apoptosis in leukemia HL60 and its multidrug-resistant sublines (13).

The apoptotic mechanism has been extensively studied and activation of caspase-3 and caspase-9 has been shown to occur in apoptosis. We found that iberin exposure causes an increase in active form of caspase-3 and down-regulation of the procaspase-9 form in human glioblastoma cells. These results suggest that iberin-mediated SNB19 cell death occurs in part through mitochondria-dependent pathways. The possible role of diet in the etiology of brain tumors remains largely unknown. Previous studies indicated a strong association of green vegetables (including consumption of some members of the Brassica genus) with reduced risk of brain cancer development and brain tumor mortality (10). The distribution of phenyl isothiocyanate in mouse brain following the administration of radioactive phenyl isothiocyanate by gavage demonstrates the bioavailability of isothiocyanates to brain cells (15). Our results show that glioblastoma cells are highly sensitive to relatively low concentrations of iberin and it is expected that these levels are likely to be achieved in human plasma through a diet rich in cruciferous vegetables.

In conclusion, we have demonstrated that the cruciferous vegetable glucosinolate derivative iberin is able to inhibit growth of SNB19 cells by inducing apoptosis at low concentrations, and the induction of apoptosis is associated with the mitochondria-mediated apoptotic pathway. Therefore, we speculate that the induction of apoptosis observed in this study may provide a distinct mechanism for the cancer therapeutic and chemopreventive function of iberin, and further studies aimed at the determination of the therapeutic effects of iberin on glioblastoma growth in vivo should provide interesting insights on the potential clinical usefulness of this dietary constituent in the treatment of brain tumors.

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References

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