

Oral Administration of Benzyl-isothiocyanate Inhibits *In Vivo* Growth of Subcutaneous Xenograft Tumors of Human Malignant Melanoma A375.S2 Cells

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Abstract. A number of experiments have demonstrated that benzyl-isothiocyanate (BITC) induces cytotoxic cell death through the induction of apoptosis in various human cancer cell lines. In the present study, we investigated the effects of BITC on the growth of A375.S2 cell xenograft tumors in nude BALB/c mice *in vivo*. The A375.S2 cancer cells were inoculated subcutaneously into the lower flanks of each nude mouse. After cancer cell inoculation, all animals were maintained in the animal room for seven days and all mice produced one palpable tumor. Animals were randomly divided into two groups, each mouse was individually given intraperitoneal injections of BITC (20 mg/kg) or not (control). Results from the *in vivo* experiments indicated that BITC did not significantly affect the body weight of nude BALB/c mice bearing xenograft A375.S2 cell tumors but did significantly decrease the tumor weight.

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Skin cancer, and melanoma in particular, continues to increase at a rate of 4% annually (1), however, the mortality rate of melanoma is not increasing, perhaps due to the earlier detection of tumors (2, 3). Although melanoma represents only 4% of all skin cancers, it causes the greatest number of skin cancer-related deaths (4), and mortality remains high despite early treatment (5). The treatment of melanoma includes surgery, radiotherapy, chemotherapy or radiotherapy combined with chemotherapy. Recently, research has focused on immunomodulatory drugs or immunomodulatory drugs plus molecularly-targeted therapies.

For the past decades, investigators have focused on finding new anticancer agents from plants (6-8). Much evidence has shown that a dietary intake of cruciferous vegetables reduces the risk of many types of cancer (9-12). The isothiocyanate (ITC) functional group of cruciferous vegetables seems to play an important role in their anticancer activity (13). Benzyl isothiocyanate (BITC) has been demonstrated to induce cytotoxic effects (apoptosis) in many human cancer cells such as breast (14, 15), ovarian (16), osteogenic (17), pancreatic (7, 18) and leukemia cells (19). Furthermore, it has been reported that BITC inhibits migration and invasion of colonic (20), gastric (21), breast (22) and pancreatic cancer cells (23) *in vitro*. BITC was also reported to enhance cisplatin-induced cytotoxicity in human HL-60 cells (24).

Much evidence has shown that BITC inhibits chemically-induced cancer in animal models *in vivo* (13, 25). In

particular, BITC acts as a potent inhibitor of mouse tobacco smoke-induced lung carcinogenesis (26) and diethylnitrosamine-induced hepatocarcinogenesis in rats (27). Furthermore, it was reported that BITC inhibited benzo(a)pyrene-induced lung tumorigenesis in A/J mice and mammary carcinogenesis in MMTV-*neu* mice (28, 29). It was also reported that oral administration of BITC to BALB/c mice could inhibit solid tumor growth and lung metastasis of 4T1 mammary carcinoma cells *in vivo* and that BITC has potential as a preventive agent for metastatic breast cancer (30). However, the effect of BITC on skin cancer has yet to be assessed under *in vivo* conditions. Thus, in the present study, we investigated the effects of BITC on tumor growth of human melanoma A375.S2 cells by using a mouse xenograft model.

Materials and Methods

Chemicals and reagents. BITC and dimethyl sulphoxide (DMSO) were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Minimum essential medium (MEM), fetal bovine serum (FBS), L-glutamine, penicillin-streptomycin, and trypsin-EDTA were purchased from Gibco BRL (Grand Island, NY, USA).

Human malignant melanoma A375.S2 cells. The A375.S2 human malignant melanoma cancer cells were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan, ROC). A375.S2 cells were cultured in MEM supplemented with 10% FBS, 2 mM L-glutamine and 1% antibiotics (100 Units/ml penicillin and 100 µg/ml streptomycin) in a humidified atmosphere of 5% CO₂ and 95% air at 37°C. The MEM was changed every two days (31).

BALB/c nude mice. Twenty-six-week-old male BALB/c nude mice, weighing 19-22 g, were obtained from the National Laboratory Animal Center (Taipei, Taiwan, ROC). All animals were maintained in the Laboratory Animal Center of the China Medical University for two weeks and taken care of according to animal guidelines (Affidavit of Approval of Animal Use Protocol) of the China Medical University (Taichung, Taiwan, ROC).

Subcutaneous implantation of A375.S2 cells and treatment of BITC. A375.S2 cancer cells at a density of 1×10^6 in 0.1 ml Dulbecco's PBS were inoculated subcutaneously into the lower flanks of each mouse. Palpable tumors (approx. 20mm³/mouse) were produced after seven days and then animals were randomly divided into two groups with 10 mice each. Group I: all mice were individually given intraperitoneal injections of olive oil only as a control group. Group II: all mice were individually given intraperitoneal injection of vehicle solution containing 20 mg/kg BITC. All the mice were monitored weekly for tumor growth and were treated with the above dose daily for up to 12 days before being weighed and sacrificed (32).

Statistical analysis. All data are reported as the mean±standard deviation (SD). The statistical significance of the differences was analyzed by the Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

Results

BITC affects the subcutaneous implantation of A375.S2 cells in BALB/c nude mice in vivo. Representative animals and their associated tumors at the end of treatment are shown in Figure 1A and B; the body weights of each group are presented in Figure 1C and the representative isolated tumors and weights are shown in Figure 1D and E.

Data from Figure 1A-C indicate that BITC did not significantly affect the total body weight of mice when compared to the controls. Figures 1D and E indicate that BITC significantly reduced the tumor weight when compared to the control, untreated group. Figure 1D and E shows that 20 mg/kg BITC was efficient at retarding growth of A375.S2 cell subcutaneous mouse tumors but did not affect the total body weight compared to untreated tumor-bearing mice.

Discussion

It is well-documented that BITC induces cell death through the induction of apoptosis *in vitro* and it retards chemically-induced cancer production in an animal model. In our previous study, we showed that BITC induced G₂/M phase arrest and apoptosis of A375.S2 human melanoma cells (33) but there is no available information to show whether BITC affects A375.S2 xenograft tumor *in vivo*. The results in Figures 1A-C show that BITC did not significantly affect the body weight of BALB/c nude mice after injection of A375.S2 cells. However, Figures 1D and E indicate that BITC significantly reduced the tumor weight and size of A375.S2 cell tumor in nude mice when compared to the untreated control group.

Our previous studies also showed that BITC induced cell death of WEHI-3 cells *in vitro* and *in vivo* (34). We also found that BITC promoted the activity of macrophage phagocytosis in cells isolated from peripheral blood mononuclear cells (PBMC) and peritoneum (34). Much evidence has been shown that the agent can block cell cycle then induce apoptosis and, thus, can be considered as a new candidate anti-cancer agent (35, 36).

Clinically, many anticancer drugs have been obtained from natural products, such as taxol (37) and genistein (38).

Taken together, and based on our observations, our results indicate that BITC may be promising in cancer therapy.

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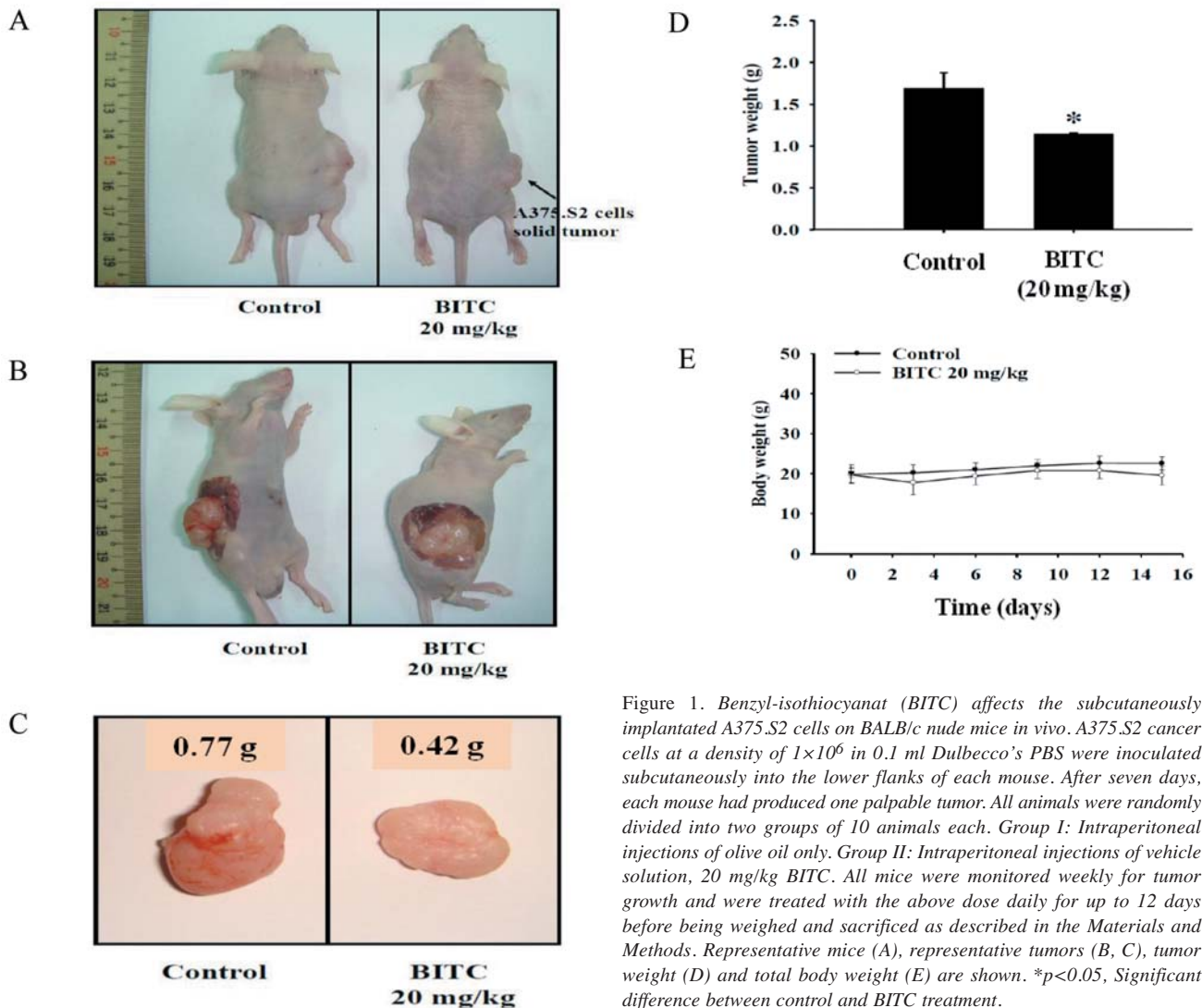


Figure 1. Benzyl-isothiocyanat (BITC) affects the subcutaneously implanted A375.S2 cells on BALB/c nude mice in vivo. A375.S2 cancer cells at a density of 1×10^6 in 0.1 ml Dulbecco's PBS were inoculated subcutaneously into the lower flanks of each mouse. After seven days, each mouse had produced one palpable tumor. All animals were randomly divided into two groups of 10 animals each. Group I: Intraperitoneal injections of olive oil only. Group II: Intraperitoneal injections of vehicle solution, 20 mg/kg BITC. All mice were monitored weekly for tumor growth and were treated with the above dose daily for up to 12 days before being weighed and sacrificed as described in the Materials and Methods. Representative mice (A), representative tumors (B, C), tumor weight (D) and total body weight (E) are shown. * $p < 0.05$, Significant difference between control and BITC treatment.

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