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Intracellular accumulation of structurally varied isothiocyanates correlates with inhibition of nitric oxide production in proinflammatory stimuli-activated tumorigenic macrophage-like cells

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ABSTRACT

In the present study, we analyzed the intracellular accumulation of 6-(methylsulfinyl)hexyl isothiocyanate (6MITC) and its analogs in proinflammatory stimuli-activated J774.1 cells to predict the biological potencies of the ITCs. Our present analyses exhibited that the intracellular accumulation was in the order of 6MITC > **2b** > **2e** ≈ **2c** > **2g** > **2d** > **2f** > **2h**. Investigation of reactivity of the ITCs with glutathione (GSH) in the tumor cells revealed partial inhibition of GSH by the ITCs. Furthermore, the inhibition of nitric oxide (NO) production in the tumor cells was ascribed to the intracellularly accumulated ITCs. The NO suppression was correlated with the inhibition of tumor cell growth. Our present results suggest that the intracellular accumulation of the ITCs can be used to predict their biological potencies, such as inhibition of NO production that was correlated with suppression of tumor cell growth. To the best of our knowledge, this is the first report to predict the biological potency of 6MITC and its analogs with their intracellular accumulation.

1. Introduction

Isothiocyanates (ITCs) occur widely and abundantly as glucosinolates in a variety of vegetables, such as *Brassica* and *Raphanus* species.[1,2] Glucosinolates are hydrolyzed by myrosinase and ITCs are formed by a Lossen rearrangement.[3] Such reactions can be catalyzed by intestinal microflora in humans.[4] There is growing interest in ITCs because of findings from in vitro and in vivo models that they offer possible chemoprotection and chemotherapy against cancer.[3,5,6] ITCs have been reported to induce apoptosis in several cancer cell lines and tested in animal models for various cancer types.[7–11] The antitumor activities of ITCs largely involve modulation of carcinogen metabolism through induction of phase 2 enzymes and/or inhibition of phase 1 enzymes.[12–16] Many standard chemotherapeutic agents have been found in natural sources, and since ITCs are present in the above edible plants and consumed by humans in considerable quantities,[17,18] they may offer preventive and therapeutic effects against cancer.[19]

Wasabi (*Wasabia japonica*) is a member of *Brassica* species and contains comparatively higher levels of ITCs.[20] Its reported physiological functions include enhancement of appetite,[21] activity against microbes,[22] inhibition of platelet aggregation,[20] and suppression of gastric carcinogenesis.[23] A number of analogs of ITCs have been isolated from wasabi root and its characteristic pungent flavor is attributed to 6-(methylsulfinyl)hexyl isothiocyanate (6MITC)[24] that contains methyl sulfoxide group and ITC group linked by the alkyl chain (Fig. 1). 6MITC has also been identified as the major bioactive compound in wasabi and several lines of

Abbreviations: ABC, ATP-binding cassette; GSH, glutathione; IC_{50}, half-maximal inhibitory concentration; IFN-γ, interferon-gamma; iNOS, inducible NO synthase; IRF-1, interferon regulatory factor-1; ITC, isothiocyanate; K_{obs}, Pseudo-first order reaction rate constant; LBS, ligand-binding site; log*P*, logarithm of partition coefficient between 1-octanol and aqueous phases; LPS, lipopolysaccharide; MDR, multidrug resistance; NF-κB, nuclear factor-κB; NO, nitric oxide; NOR4, (±)-*N*-[(*E*)-4-ethyl-2-[(*Z*)-hydroxyimino]-5-nitro-3-hexene-1-yl]-3-pyridine carboxamide; P-gp, P-glycoprotein; PSA, polar surface area; SNAP, *S*-nitroso-*N*-acetyl-DL-penicillamine; 6MITC, 6-(methylsulfinyl)hexyl isothiocyanate.

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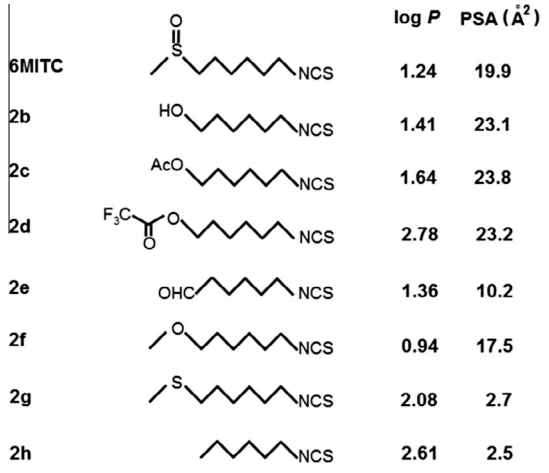

Figure 1. Structures of 6MITC and its analogs used in the present study. The log*P* and PSA values are cited from our previous study.[26]

evidence demonstrated its anticancer properties.[25–27] Administration of 6MITC significantly reduced the number of metastasized melanoma cells in the lungs and inhibited foci formation in a mouse model of pulmonary metastasis.[25] 6MITC also demonstrated significant growth inhibitory activity toward a macrophage-like tumor cell line.[26] Furthermore, 6MITC showed specific suppression for the growth and survival of breast cancer and melanoma cell lines, and its suppression mechanism was considered to involve multiple pathways and might be different from those of other known chemicals.[27] Taken together, these reports suggest that 6-MITC can be utilized as a potential therapeutic option for controlling cancer.

In the present study, in light of the above-described anticancer activity of 6MITC, evaluation of the biological potency of 6MITC and its analogs was investigated (the structures of 6MITC and its analogs are shown in Fig. 1). Mouse reticulum cell sarcoma-derived J774.1 cells were treated with 6MITC and its analogs, and the intracellular accumulation of the tested ITCs was determined. Subsequently, the intracellular accumulation of the ITCs was analyzed with the logarithm of partition coefficient between 1-octanol and aqueous phases (log*P*) and the polar surface area (PSA) values to examine whether there were associations between the intracellularly accumulated ITCs and log*P* or PSA. The reactivity of the ITCs with glutathione (GSH) to form dithiocarbamates was also analyzed to investigate whether there were correlations between the intracellular accumulation of the ITCs and the dithiocarbamate formation. Furthermore, the half-maximal inhibitory concentration (IC_{50}) values of the ITCs for cellular GSH were determined, and the IC_{50} values for cellular GSH were analyzed with the dithiocarbamate formation or the intracellular accumulation of the ITCs to examine whether cellular GSH was correlated with these items. The IC_{50} values of the ITCs for nitric oxide (NO) production by interferon-gamma (IFN-γ)/lipopolysaccharide (LPS)-activated J774.1 cells and the IC_{50} values of the ITCs for tumor cell growth were also determined. The IC_{50} values for NO production were analyzed with the intracellular accumulation of the ITCs or the IC_{50} values for tumor cell growth to investigate whether NO production was associated with these items.

2. Results and discussion

2.1. Intracellular accumulation of the ITCs

J774.1 cells were initially studied for diagnosis and classification of human lymphomas due to the functional similarities in their responses to drugs, and have been considered useful in elucidating mechanisms of drug action and evaluating new chemotherapeutic agents.[28,29] In the present study, the cells were treated with 6MITC and its analogs, and the intracellular accumulation of

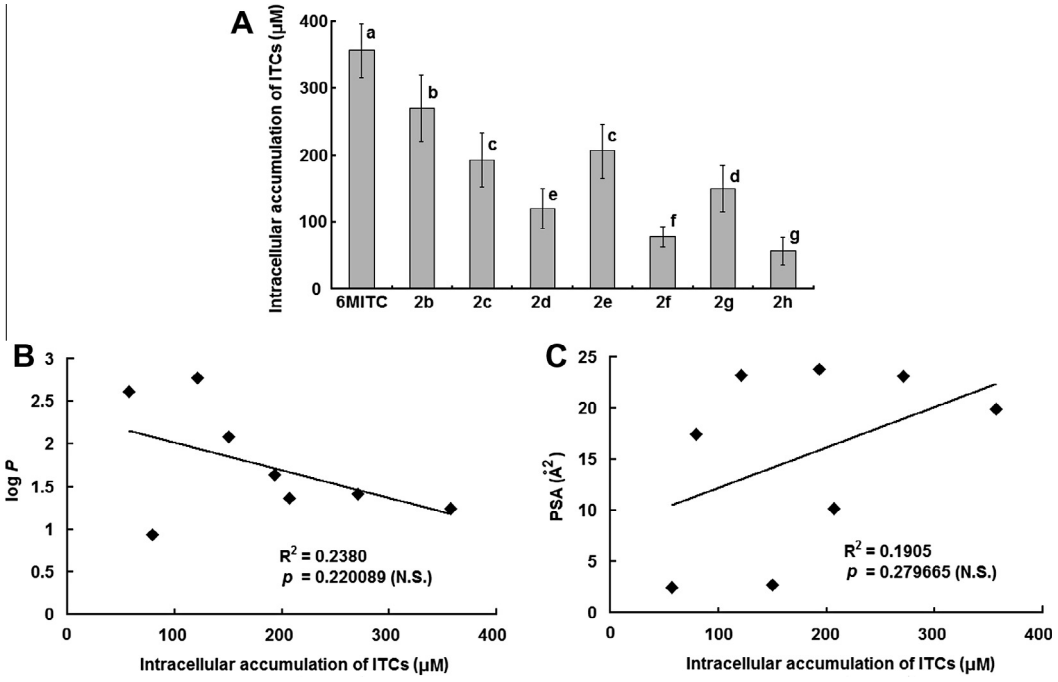

Figure 2. Intracellular accumulation of the ITCs in J774.1 cells. The cells were treated with 6MITC and its analogs, and the intracellular accumulation of the ITCs was determined by a modified cyclocondensation assay. (A) The intracellular accumulation is in the order of 6MITC > **2b** > **2e** ≈ **2c** > **2g** > **2d** > **2f** > **2h**. (B) A linear regression analysis is unable to find a relationship between the intracellular accumulation of the ITCs and the log*P* values. (C) A linear regression analysis is unable to find a relationship between the intracellular accumulation of the ITCs and the PSA values.