Sulforaphane shows remarkable growth inhibition and cytotoxicity against several human leukemia cell lines

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Summary
Sulforaphane, a typical phytochemical, is an isothiocyanate derivative from cruciferous vegetables such as broccoli and its sprout. While sulforaphane protects higher plants from infections by various bio-invaders such as aphids, ticks, bacteria or nematodes, it shows cytotoxicity against various cancer cells in human body. However, the effects of sulforaphane on human leukemia cells are still incompletely elucidated now. In this paper, we examined the effects of sulforaphane on proliferation and viability of some human leukemia cell lines [Eol-1 (eosinophilic leukemia cell line), MOLT4 (T-lymphoblast cell line), RPMI-1788 (B-lymphoblastoid cell line) and U937 (monoblastic leukemia cell line)]. Sulforaphane showed strong cytotoxicity against these cell lines, resulting in inhibition of proliferation in a dose-dependent manner. Our data suggested that sulforaphane may serve as an effective agent for treatment of various types of leukemia.

Key words: sulforaphane, leukemia, cytotoxicity, proliferation

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Introduction

Phytochemicals, higher plant-derived chemical compounds, are responsible for many biological activities. Sulforaphane [1-isothiocyanato-4-(methyl-sulfinyl)butane] is an isothiocyanate compound derived from cruciferous vegetables, i.e. broccoli and its sprouts, and is released by hydrolysis of sulforaphane glucosinolate catalyzed by certain glucosidases\(^1\). In higher plants, sulforaphane protects them from infectious diseases caused by various bio-invaders\(^2\). Recently, in human body, sulforaphane is expected to be an effective carcinogenesis prophylactic drug\(^3\),\(^4\). In brief, sulforaphane shows the various significant physiological functions: such as anti-cancer effects\(^5\)~\(^10\), antioxidant functions\(^11\), anti-*Helicobacter pylori* activity\(^12\), photo-aging prevention\(^13\), prevention of obesity\(^14\), and general health benefits\(^15\). While so many reports regarding the chemopreventive and chemotherapeutic properties of sulforaphane in solid tumors are available, there is little information on those in leukemia cells\(^16\)~\(^23\). Unfortunately, the effects of sulforaphane on human leukemia cells remain to be dissolved still now.

In this paper, we investigated the effects of sulforaphane on proliferation and viability of four human leukemia cells: Eol-1 (eosinophilic leukemia), MOLT4 (T-lymphoblast cell line), RPMI-1788 (B-lymphoblastoid cell line) and U937 (monoblastic leukemia cell line). Here, we revealed that sulforaphane remarkably shows growth inhibition and cytotoxicity against the four leukemia cells in a dose-dependent manner.

Materials and Methods

1. Materials

Sulforaphane (Cayman Chemical, MI, USA), RPMI-1640 culture medium and trypan blue solution (Gibco Laboratories, MD, USA), fetal bovine serum (FBS) (JRH Biosciences, KS, USA) and plasmocin (InvivoGene, CA, USA) were obtained.

2. Cell culture and treatment with sulforaphane

Human eosinophilic leukemia Eol-1 cells (RCB0641) and human monoblastic leukemia U937 cells (RCB0435) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Human B-lymphoblastoid leukemia RPMI-1788 cells (JRCB0035) were provided by JCRB Cell Bank at National Institute of Biomedical Innovation, Health and Nutrition, Japan. T-lymphoblast leukemia MOLT4 cells were kindly offered by Dr. Futoshi Kuribayashi (Kawasaki Medical School, Okayama, Japan). Cells were grown in RPMI-1640 culture medium containing 10% FBS and 5 mg/mL plasmocin as described\(^24\)~\(^27\). Cells (1.0 x 10\(^6\)) in 5 ml of culture medium were incubated in the absence or presence (1, 2 or 5 \(\mu M\)) of sulforaphane at 37°C. Total cells were counted by a hemocytometer under a microscope. Viable cells were counted by the trypan blue dye exclusion method\(^25\).

3. Statistical analysis

Data obtained from proliferation assays and cell viability assays are presented as averages of three separate and independent experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student’s \(t\) test.

Results and Discussion

To study the growth inhibition and cytotoxicity caused by sulforaphane against human leukemia cells, we examined the effects of commercially available sulforaphane on proliferation and viability of the four human leukemia cells.

First, as shown in Fig. 1, the proliferation rates of Eol-1 cells were decreased in a dose dependent manner. In particular, the number of the cells was not mostly increased in the presence of 5 \(\mu M\) sulforaphane. The viability of Eol-1 cells was also decreased in a dose dependent manner, and dramatically reduced at 5 \(\mu M\) sulforaphane (to \(~74%\) at 12 h, to \(~51%\) at 24 h, to \(~35%\) at 36 h
Fig. 1. Influences of sulforaphane on proliferation and viability of Eol-1 cells. (A) Proliferation. Cells (1.0 x 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 μM (filled circles) sulforaphane for upto 48 h. Cell counts were performed by a hemocytometer under a microscope at the indicated times in the graph. Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student’s t test. *, p < 0.05; **, p < 0.01 compared with the data for without sulforaphane. (B) Viability. Cells (1.0 x 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 μM (filled circles) sulforaphane for upto 48 h. Viable cells were counted by the trypan blue dye exclusion method at the indicated times in the graph. Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student’s t test. *, p < 0.05; **, p < 0.01 compared with the data of without sulforaphane at each time point.

Fig. 2. Influences of sulforaphane on proliferation and viability of MOLT4 cells. (A) Proliferation. Cells (1.0 x 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 μM (filled circles) sulforaphane for upto 48 h. Cell counts were performed by a hemocytometer under a microscope at the indicated times in the graph. Data represent the averages of three separate experiments. Statistical differences were calculated using Student’s t test. *, p < 0.05; **, p < 0.01 compared with the data for without sulforaphane. (B) Viability. Cells (1.0 x 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 μM (filled circles) sulforaphane for upto 48 h. Viable cells were counted by the trypan blue dye exclusion method at the indicated times in the graph. Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student’s t test. *, p < 0.05; **, p < 0.01 compared with the data of without sulforaphane at each time point.
Fig. 3. Influences of sulforaphane on proliferation and viability of RPMI-1788 cells. (A) Proliferation. Cells (1.0 \times 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 \mu M (filled circles) sulforaphane for up to 48 h. Cell counts were performed by a hemocytometer under a microscope at the indicated times in the graph. Data represent the averages of three separate experiments. Statistical differences were calculated using Student’s \( t \) test. **, \( p < 0.01 \) compared with the data for without sulforaphane. (B) Viability. Cells (1.0 \times 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 \mu M (filled circles) sulforaphane for up to 48 h. Viable cells were counted by the trypan blue dye exclusion method at the indicated times in the graph. Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student’s \( t \) test. *, \( p < 0.05 \); **, \( p < 0.01 \) compared with the data of without sulforaphane at each time point.

Fig. 4. Influences of sulforaphane on proliferation and viability of U937 cells. (A) Proliferation. Cells (1.0 \times 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 \mu M (filled circles) sulforaphane for up to 48 h. Cell counts were performed by a hemocytometer under a microscope at the indicated times in the graph. Data represent the averages of three separate experiments. Statistical differences were calculated using Student’s \( t \) test. **, \( p < 0.01 \) compared with the data for without sulforaphane. (B) Viability. Cells (1.0 \times 10^6) in 5 ml of culture medium were incubated without (open circles) or with 1 (filled triangles), 2 (filled squares) or 5 \mu M (filled circles) sulforaphane for up to 48 h. Viable cells were counted by the trypan blue dye exclusion method at the indicated times in the graph. Data represent the averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated using Student’s \( t \) test. *, \( p < 0.05 \); **, \( p < 0.01 \) compared with the data of without sulforaphane at each time point.
and to ~14% at 48 h). These results indicated that sulforaphane shows strong cytotoxicity against Eol-1 cells and results in inhibition of proliferation.

Second, as shown in Fig. 2, the proliferation rates of MOLT4 cells were distinctly decreased in a dose dependent manner. Significantly, the number of the cells was dramatically decreased by treatment with 5 \( \mu \text{M} \) sulforaphane. Similarly, the viability of MOLT4 cells was also decreased in a dose dependent manner, and remarkably reduced at 5 \( \mu \text{M} \) sulforaphane (to ~82% at 12 h, to ~61% at 24 h, to ~46% at 36 h and to ~32% at 48 h). These results showed that sulforaphane displays remarkable cytotoxic activity against MOLT4 cells, resulting in inhibition of proliferation.

Next, as shown in Fig. 3, the proliferation rates of RPMI-1788 cells were also clearly decreased in a dose dependent manner. Interestingly, the number of the cells was not increased in the presence of 5 \( \mu \text{M} \) sulforaphane at all. The viability of U937 cells was also markedly decreased in a dose dependent manner, and dramatically reduced at 5 \( \mu \text{M} \) sulforaphane (to ~80% at 12 h, to ~44% at 24 h, to ~24% at 36 h and to ~18% at 48 h). These results revealed that sulforaphane shows noticeable cytotoxicity against RPMI-1788 cells and causes remarkable inhibition of proliferation.

Finally, as shown in Fig. 4, the proliferation rates of U937 cells were decreased in a dose dependent manner as described in our recent paper\(^{27}\). In particular, the number of the cells was dramatically decreased in the presence of 5 \( \mu \text{M} \) sulforaphane. The viability of U937 cells was also decreased in a dose dependent manner, and dramatically reduced at 5 \( \mu \text{M} \) sulforaphane (to ~80% at 12 h, to ~50% at 24 h, to ~22% at 36 h and to ~6% at 48 h). These results demonstrated that sulforaphane displays drastic cytotoxicity and brings about inhibition of proliferation. Our findings also confirmed the previous report in which sulforaphane induces apoptotic cell death in a dose dependent manner\(^{16}\).

Our findings in this study revealed that sulforaphane shows both growth inhibition and cytotoxicity against various types of human leukemia cell lines. We can expect that sulforaphane with strong cytotoxicity against leukemia cells will become an important drug for development of treatment for various types of leukemia.

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