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Growth inhibition, cell-cycle arrest and apoptosis in human T-cell leukemia by the isothiocyanate sulforaphane

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Glucosinolates (GL) can inhibit, retard or reverse experimental multistage carcinogenesis. When brassica plant tissue is broken, GLs are hydrolyzed by the endogenous enzyme myrosinase (Myr), releasing many products including isothiocyanates (ITC). Synthetic ITCs like sulforaphane exert chemopreventive effects against chemically induced tumors in animals, modulating enzymes required for carcinogens' activation/detoxification and/or the induction of cell-cycle arrest and apoptosis in tumor cell lines. To investigate the chemopreventive potential of ITCs while reproducing the circumstances of dietary contact with sulforaphane, we studied proliferation, apoptosis induction and p53, bcl-2 and bax protein expression in Jurkat T-leukemia cells by sulforaphane, the ITC generated *in situ* in a quantitative manner by Myr starting from glucoraphanin (GRA). Jurkat cells were treated with different doses of GRA–Myr mixture. Effects on cell growth or survival were evaluated by counting trypan blue-excluding cells. Cell-cycle progression, apoptosis and expression of p53, bax and bcl-2 proteins were analyzed by flow cytometry. Results were analyzed by two-sided Fisher's exact test. Sulforaphane, but not GRA, caused G_2/M-phase arrest ($P = 0.028$) and increase of apoptotic cell fraction ($P < 0.0001$) in a time- and dose-dependent manner. Necrosis was observed after prolonged exposure to elevated sulforaphane doses. Moreover, it markedly increased p53 and bax protein expression, and slightly affected bcl-2 expression. These findings indicate that sulforaphane but not the native GL GRA can exert both protective and toxic effects inhibiting leukemic cell growth. Sulforaphane therefore deserves study as a potential chemopreventive/chemotherapeutic antileukemic agent.

Introduction

Cancer chemoprevention involves preventing, delaying or reversing carcinogenesis by administering non-toxic synthetic chemicals or chemicals from natural substances before malignancy (1). A number of agents have been reported to be effective against chemical carcinogenesis in several organs, and some are candidates for the inhibition of human cancer development (2–4). The multifactorial nature of the carcinogenic process provides numerous pathways that could be manipulated by these agents in order to inhibit or delay tumor development (5). As a result of the wide variety of possible chemopreventive agents, short-term screening assays are being developed to help identify potentially effective compounds (6).

Edible brassicas (e.g. broccoli and cauliflower) contain substantial quantities of biosynthesized secondary metabolites known as glucosinolates (GLs), as well as myrosinase (Myr) (thioglucoside glucohydrolase; EC 3.2.3.1). When the vegetables are crushed by chopping or chewing, Myr catalyzes a rapid hydrolytic reaction in which the GLs are then converted into isothiocyanates (ITCs) and other products, depending on the chemical structure of GL side chains (7). Many synthetic ITCs exert versatile chemopreventive effects against tumors induced by chemical carcinogens in various animal organs, suggesting that common mechanisms underlie their chemopreventive properties (8,9). Two sets of putative mechanisms have been identified that help to explain the effects of ITCs. The first involves modulation of enzymes that are required for the activation or detoxification of many carcinogens (10,11). In particular, ITCs have been shown to induce the activity of phase II enzymes (e.g. glutathione *S*-transferase, quinone reductase and glucuronosyltransferases) and/or inhibit phase I enzymes (12–14). Recent studies in humans also revealed that a high consumption of brassicas led to an increase in glutathione *S*-transferase activity, supporting the potential role of ITCs in the prevention of human cancer (15). The second set of putative mechanisms involves suppression of tumor development following the initiation of pre-cancerous cells (16). Possible mechanisms of suppression include the deletion of initiated cells from genetically damaged tissue by apoptosis, so that clonal expansion of the lesion is aborted (17). Some constituents of brassicas have already been shown to induce apoptosis or block proliferation in cancer cells *in vitro*. In particular, Huang *et al*. (18) showed that phenylethyl ITC induces p53 transactivation in mouse epidermal cells in a dose- and time-dependent fashion, while Gamet-Payrastre *et al*. demonstrated that sulforaphane induces cell-cycle arrest and apoptosis in HT29 human colon cancer cells (19).

Most of these studies were performed using synthetic ITCs, which are commonly assumed to correspond to major natural constituents of cruciferous vegetables. However, this is not literally so, as sulforaphane only appears as the product of enzymatic degradation of its naturally occurring parent GL, namely glucoraphanin (GRA). Therefore, dietary exposure to sulforaphane, and indeed other ITCs, actually occurs as a result of the hydrolytic breakdown of precursor GLs on ingestion (e.g. by chewing raw vegetables in salads). Although cooking degrades the Myr present in broccoli, sulforaphane may also be produced in the human intestine, where microflora seem to promote the hydrolysis of GLs into ITCs (20).

In order to mimic more closely the circumstances of dietary contact with sulforaphane following consumption of brassicas and to expand our knowledge on the chemopreventive potential

Abbreviations: EB, ethidium bromide; FITC, fluorescein isothiocyanate; FSC, forward scatter; GL, glucosinolate; GRA, glucoraphanin; GSH, Glutathione; ITC, isothiocyanate; Myr, myrosinase; PI, propidium iodide; SSC, side scatter.

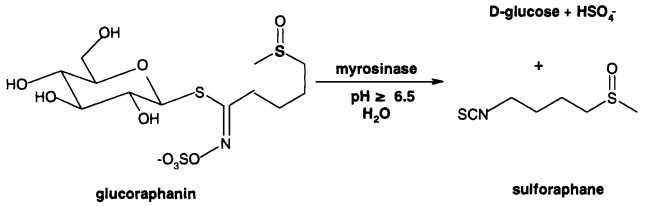

Fig. 1. Production of sulforaphane after enzymatic hydrolysis of GRA.

of ITCs, we decided to study the effects of the mixture of GRA and Myr to produce sulforaphane on cell growth, cell-cycle progression and apoptosis induction in Jurkat cells (i.e. human T-cell leukemia). We also assessed the apoptotic response of Jurkat cells in terms of p53, bcl-2 and bax expression.

We demonstrate for the first time that the ITC sulforaphane generated *in situ* by Myr but not the native precursor GRA induces apoptosis and perturbs cell-cycle progression in Jurkat cells. In this way, it was possible to compare the effect of the native GL GRA and of the ITC sulforaphane. We also found that sulforaphane increases p53 and bax protein level, but does not influence bcl-2.

Materials and methods

Production of GRA, Myr and sulforaphane

GRA was obtained by a recently developed procedure (21) whereby a quantitative yield is produced starting from glucoerucin. Glucoerucin was isolated with a high purity grade from the ripe seeds of rocket (*Eruca sativa* Miller), according to a well-defined protocol (22). The transformation of glucoerucin into GRA is based on the oxidation reaction of sulfides into their corresponding sulfoxides. The semi-synthetic GRA produced was purified according to the method reported by Visentin *et al*. (22). Purity was assessed by HPLC analysis of the desulfo-derivative according to the ISO 9167-1 method (23). The Myr used in the present study was isolated from ripe seeds of white mustards (*Sinapis alba* L.), as reported by Pessina *et al.* (24). The Myr stock solution had a specific activity of ~60 U/mg of soluble protein. One Myr unit was defined as the amount of enzyme able to hydrolyze 1 μmol of sinigrin/min at pH 6.5 and 37°C. Myr solution was stored at 4°C in sterile distilled water until use.

Sulforaphane was generated *in situ* by Myr-catalyzed hydrolysis of GRA (Figure 1). In our experimental conditions of cell culturing (pH 7.4, 37°C), sulforaphane is the only enzymatic breakdown product. The formation of sulforaphane was quantitative, as confirmed by GC-MS techniques (25).

Cell culture

Jurkat T-leukemia cells were grown in suspension and propagated in RPMI 1640 supplemented with 10% heat-inactivated bovine serum, 1% antibiotics (all obtained from Sigma, St Louis, MO). To maintain exponential growth, the cultures were divided every third day by dilution to a concentration of 1×10^5 cells/ml.

Cell treatment

A 4 mM stock solution of GRA was prepared in 0.9% NaCl and stored at 4°C. Samples (0.23 U) of Myr were directly added to 5 ml of the complete cell-culture medium containing increasing concentrations of GRA (3, 10 and 30 μM), prepared by diluting the stock solution with 0.9% NaCl. Exponentially growing Jurkat cells at a concentration of 400 000 cells/ml were subsequently added. The activity of native GRA or Myr was tested by treating the cell cultures with 30 μM GRA in absence of the enzyme or with Myr in absence of GRA, respectively. NaCl (0.9%) was used as control. For each experiment, treatments were done in duplicate and separate cultures were set up for each treatment.

Cytotoxicity test

In separate experiments, the GRA–Myr mixture was added at various concentrations to exponentially growing cells. Cell concentrations were measured as a function of time by counting trypan blue-excluding cells on cell aliquots removed from culture at the designated times (2, 4, 8, 12, 24 and 48 h). Results were calculated as viable cells in GRA–Myr mixture-treated cultures relative to control. ID_{50}, the drug concentration causing cell toxicity by 50% following a 24 h exposure, was calculated by interpolation from dose–response curves (26).

Flow cytometry

Flow cytometry was performed using a FACStar$^+$ flow cytometer (Becton Dickinson, Sunnyvale, CA) equipped with an argon laser (Innova 90; Coherent Radiation, Palo Alto, CA) operating at 488 nm (500 mW) for excitation of the ethidium bromide (EB).

Flow cytometric measurement of cell proliferation

The preparation of samples for measurements of the cell-cycle distribution of nuclei by DNA content was performed according to a two-step method reported elsewhere (27,28). Briefly, cultures were centrifuged for 5 min at 800 *g* and treated with 1 ml of solution I (584 mg/l NaCl, 1000 mg/l Na–citrate, 25 mg/l EB, 10 mg/l RNase and 0.3 ml/l Nonidet P-40). Then, after ~1 h, 1 ml of solution II (15 g/l citric acid, 0.25 M sucrose and 40 mg/l EB) was added and the samples were briefly vortexed. EB fluorescence, forward scatter (FSC) and side scatter (SSC) of nuclei in suspension, measured using a combination of KV550 and OG590 longpass filters (Schott, Mainz, Germany), were recorded for cell nuclei in list mode. For each sample, 10 000 events were registered. The fraction of cells in the different compartments of cell cycle was calculated as described by Schreiber *et al.* (29).

Measurement of annexin V binding by flow cytometry

It has been shown that loss of phospholipid asymmetry of the plasma membrane is an early event of apoptosis (30,31). The annexin V binds to negatively charged phospholipids, like phosphatidylserine. During apoptosis, the cells react to annexin V once chromatin condenses but before the plasma membrane loses its ability to exclude propidium iodide (PI). Hence, by staining cells with a combination of fluorescein isothiocyanate (FITC) annexin V and PI it is possible to detect non-apoptotic live cells, early apoptotic cells and late apoptotic or necrotic cells (32,33).

Cells from exponentially growing cultures were collected at different times. Aliquots of 0.5×10^6 cells were centrifuged (100 *g*) for 5 min and washed with phosphate-buffered saline (PBS: NaCl 145 mM in phosphate buffer 150 mM). The cell pellet was resuspended in 100 μl of labeling solution (ANNEXIN-V-FLUOS, Boehringer Mannheim, Mannheim, Germany) containing 2 ml annexin V labeling reagent and 0.1 μg PI (Sigma) and incubated for 10–15 min, as per manufacturer's instructions. Immediately after adding 0.1 ml of incubation buffer (10 mM HEPES/NaOH, 140 mM NaCl, 5 mM $CaCl_2$), green (FITC) and red (PI uptake) fluorescence of individual cells was measured with a FACStar$^+$ flow cytometer (Becton Dickinson), using 488 nm excitation and a 530 nm bandpass filter for FITC detection and a filter >590 nm for PI detection. For each sample, 10 000 events were registered. Electronic compensation was required to exclude overlapping of the two emission spectra.

Flow cytometric evaluation of p53, bax and bcl-2 proteins

Determination by flow cytometry of different proteins involved in the apoptotic process offers rapid and objective quantification of protein expression (34–37). Under the same exposure conditions described above, 1×10^6 cells were fixed and permeabilized by a commercially available kit, namely Leucoperm solution A and B (Serotec, Oxford, UK). They were then incubated with 10 μl of each antibody, i.e. FITC p53 (35 μg/ml; Novocastra, Newcastle, UK) against phosphorylated and non-phosphorylated protein, FITC bcl-2 (200 μg/ml; Serotec) and bax (200 μg/ml; Santa Cruz Biotechnology, Santa Cruz, CA) or isotype-matched negative control (Serotec). The cells were washed and incubated with 10 μl of the FITC-labeled secondary antibody for the bax-stained cells (5 μg/ml; Serotec). Finally, the cells were washed and resuspended in 0.2 ml of 1% paraformaldehyde. The cells were then analyzed to quantify FITC binding by flow cytometry. From each sample 10 000 cells were analyzed and non-specific binding was excluded by gating around those cells which were labeled by the isotype negative control antibodies.

Statistical analysis

All data are the mean ± SD of two experiments. Fisher's exact test was adopted for statistical evaluation of the results. A *t*-test was used for the analysis of protein level meaurements. All *P* values are two-sided. Precise *P* values are given. All statistical analyses were performed in GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego, CA.

Results

Treatment with sulforaphane causes time- and dose-dependent reduction in cell number

Figure 2 shows the growth curve of Jurkat cells at different time points and after treatment with different concentrations

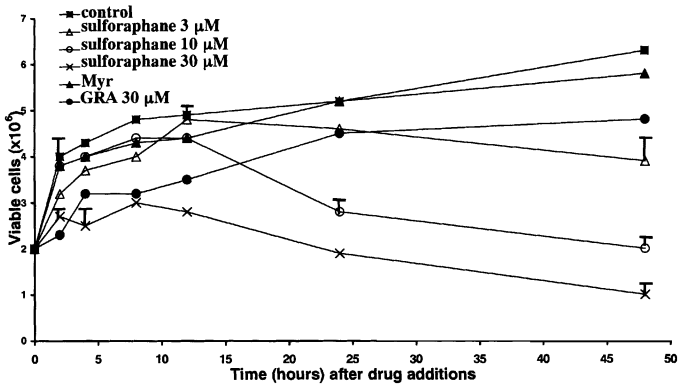

Fig. 2. The effect of sulforaphane on cell viability. The GRA–Myr mixture was added at the appropriate concentrations to exponentially growing cultures of Jurkat cells. Aliquots were removed from drug-treated and untreated control cultures after different time and stained with trypan blue. Data are means of duplicate experiments. When they do not appear, deviation bars are smaller than the symbol size.

of sulforaphane. When the harvested cells were counted, the numbers of control cells continued to increase, whereas the numbers of treated cells decreased in a time- and dose-dependent manner. The maximum effect was registered after 48 h of treatment and at a concentration of 30 μM sulforaphane. In fact, the number of cells decreased from ~6 to 1×10^6. These changes appear to be irreversible, persisting when the treated cells were recultured in sulforaphane-free medium (data not shown). Only a slight decrease in the number of viable cells in cultures treated with Myr or GRA 30 μM was observed. The ID_{50}, as measured by the number of viable cells in cultures 24 h after the addition of GRA plus Myr, was seen at a concentration of ~15 μM.

Sulforaphane but not GRA induces cell-cycle arrest

To elucidate the rate at which sulforaphane altered cell-cycle progression, cell-cycle progression was determined at several time points. Table I reports the effects of sulforaphane (and of GRA and Myr) on the cell-cycle distribution of Jurkat cells. The effect of sulforaphane was time dependent. The immediate effects (8 h), observed at drug concentrations of 10–30 μM, appeared primarily as an increase in the proportion of cells in the G_2/M phase of the cell cycle (from ~17 to 24%) accompanied by a slight compensatory decrease in G_1 phase cells. Longer exposure (24 h) to sulforaphane concentrations of 3, 10 and 30 μM led to a further decrease in the proportion of G_1 cells, while the percentage of cells in G_2/M phase increased from 18 to 30%. Prolonged (48 h) exposure of Jurkat cells to sulforaphane appeared as a decrease in G_1-phase cells (from 44 to 33%), a loss of S-phase cells (from 44 to 38%) and a marked increase in the proportion of G_2/M-phase cells (from 12 to 25%). All these effects were particularly apparent following treatment with sulforaphane at concentrations of 10 and 30 μM.

No effect on cell-cycle progression was detected in cell cultures treated with enzyme only. A very slight effect was observed with GRA after 48 h of treatment.

Sulforaphane but not GRA induces apoptosis in a time- and dose-dependent fashion

Apoptosis was observed at the highest dose of sulforaphane and at late stages during the treatment (at 24 and 48 h). It manifested by appearance of cells with decreased DNA content, identified in the DNA content distributions as cells with DNA

Table I. Effects of sulforaphane (and of GRA and Myr) on cell-cycle progression with respect to the control[a]

Treatment	8 h			24 h			48 h		
	G_0/G1 (mean ± SD)	S (mean ± SD)	G_2/M (mean ± SD)	G_0/G1 (mean ± SD)	S (mean ± SD)	G_2/M (mean ± SD)	G_0/G1 (mean ± SD)	S (mean ± SD)	G_2/M (mean ± SD)
Control	37.24 ± 2.30	45.91 ± 3.67	16.84 ± 2.60	38.22 ± 2.30	44.12 ± 2.02	17.65 ± 1.26	44.26 ± 2.0	44.17 ± 1.26	11.56 ± 3.21
Sulforaphane 3 μM	39.33 ± 1.36	42.36 ± 2.44	18.31 ± 2.07	37.28 ± 0.78	43.18 ± 0.01	19.52 ± 0.78	39.66 ± 2.38	46.03 ± 4.96	14.32 ± 0.78
Sulforaphane 10 μM	33.49 ± 3.22	42.33 ± 3.67	24.18 ± 1.07	26.08 ± 3.67	43.99 ± 0.42	29.92 ± 4.09	42.09 ± 2.44	37.76 ± 2.38	20.15 ± 1.98
Sulforaphane 30 μM	33.56 ± 4.09	43.28 ± 1.32	23.16 ± 0.23	31.87 ± 4.96	42.90 ± 2.60	25.23 ± 2.38	33.33 ± 2.44	41.50 ± 2.01	25.17 ± 1.30[b]
GRA 30 μM	36.29 ± 2.07	44.39 ± 0.44	19.32 ± 2.38	40.38 ± 2.07	43.62 ± 0.86	15.99 ± 2.93	51.66 ± 2.91	37.96 ± 0.86	10.38 ± 0.01
Myr	36.32 ± 0.11	47.64 ± 1.07	17.44 ± 0.79	38.86 ± 3.30	44.34 ± 2.22	16.80 ± 1.07	45.11 ± 0.18	43.84 ± 0.67	10.99 ± 0.92

[a]At the indicated time, distribution of the cells in G_0/G_1, S and G_2/M phase was analyzed by flow cytometry as described in Materials and methods. Results are expressed as the percentage of total cells. Data represent means of duplicate experiments.
[b]$P = 0.028$ with respect to the control.

Table II. Incidence of apoptosis as concurrently detected by two methods in Jurkat cells treated with different doses and at different times with sulforaphane[a]

Time (h)	Concentrations of sulforaphane (μM)	% Apoptosis	
		Sub-G_1 DNA (mean ± SD)	Annexin (mean ± SD)
8	Control	1.00 ± 0.14	1.00 ± 0.00
	3	1.50 ± 0.71	1.00 ± 0.00
	10	1.85 ± 0.21	1.50 ± 0.71
	30	2.00 ± 0.00	5.50 ± 2.12
24	Control	1.15 ± 0.21	2.00 ± 0.00
	3	1.00 ± 0.00	3.00 ± 1.41
	10	2.85 ± 0.21	4.00 ± 1.41
	30	8.50 ± 0.71[b]	16.00 ± 4.24[c]
48	Control	1.00 ± 0.00	3.50 ± 0.71
	3	1.00 ± 0.00	2.00 ± 0.00
	10	2.5 ± 0.71	4.00 ± 0.00
	30	38.00 ± 0.00[d]	32.00 ± 0.71[d]

[a]At the indicated time, total cells were treated as described in Materials and methods. The DNA content (sub-G_1 peak) and the binding of annexin V–FITC to the plasma membrane were analyzed by flow cytometry.
[b]$P = 0.035$, [c]$P = 0.001$, [d]$P < 0.0001$ with respect to the control.

values below that of G_1 cells ('sub-G_1 cells'). Thus, as an example, after 8 h of treatment with 30 μM sulforaphane, only 2% of apoptotic cells were found. This fraction significantly increased to 8 and 38% after 24 and 48 h of treatment, respectively (Table II).

The proportion of cells reactive with the annexin V–FITC conjugate indicates that target cells start to die as late as 24 h after effector and target mixing by an apoptosis-related mechanism. Thus, after 8 h of treatment of Jurkat cells with 30 μM sulforaphane, the incidence of annexin V^{pos} and PI^{neg} cells—i.e. apoptotic cells—was 5% (*vs* 1% in the control). They increased to 16% after 24 h and 32% after 48 h (Table II). Membrane permeabilization, revealed by PI uptake (annexin V^{pos} – PI^{pos}), presented the same kinetics (data not shown). There was only 3% of annexin V^{pos}/PI^{pos} cells after 8 h of treatment. This fraction increased to 14 and 30% after 24 and 48 h of treatment, respectively. No effects on apoptotic event induction were detected in cell cultures treated with GRA or with Myr (data not shown).

Sulforaphane modulates p53 and bax protein expression, but does not affect bcl-2 protein expression

To examine the effect of sulforaphane on levels of p53, bcl-2 and bax, cells were treated for 48 h with sulforaphane 30 μM. Apoptotic cells were gated, based on their changed FSC and SSC distribution, and p53, bcl-2 and bax proteins were then quantified. Figure 3 shows histograms of p53, bcl-2 and bax protein levels following incubation without compound or sulforaphane. p53 and bax expression were found to be increased in treated cells relative to the untreated cells. Typically, p53 and bax protein levels showed ~7- and 3-fold increase with regard to the control, respectively. bcl-2 protein level was only slightly affected.

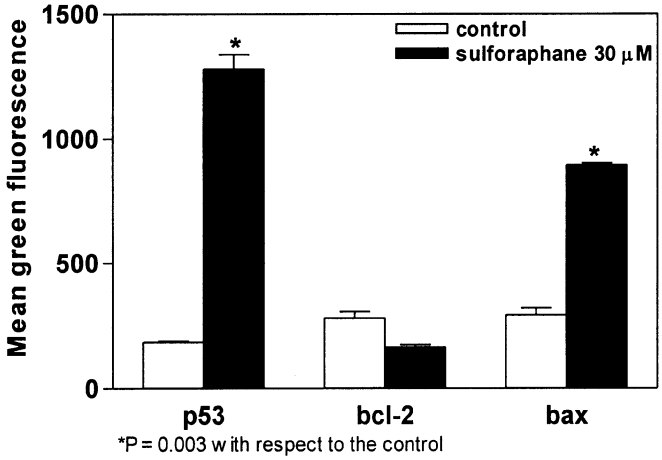

Fig. 3. Representative histograms showing p53, bcl-2 and bax protein level following 48 h culture in the presence of no compound and sulforaphane (30 μM), respectively. When they do not appear, deviation bars are smaller than the symbol size.

Discussion

What this study adds to our knowledge is the first demonstration that sulforaphane, generated from GRA by Myr hydrolysis but not its precursor GL, is able to induce cell-cycle arrest and apoptosis or necrosis in a leukemic cell line. The bioactivity of GRA was observed only in the presence of Myr, thereby demonstrating that the hydrolytic product sulforaphane is the biologically active agent, according to the evidence that ITCs are ~1000-fold more potent than the precursors (38).

Treatment of Jurkat cells with generated sulforaphane resulted in a potent inhibition of cell growth *in vitro*. Flow cytometric DNA analysis of treated cultures revealed that complete derangement of cell-cycle progression was responsible for the inhibition of tumor-cell growth. Examination of the Jurkat cells after a 48 h incubation with various doses of sulforaphane revealed a marked increase in the percentage of cells in the G_2/M cell-cycle phase. Such behavior is in line with the recent finding that synthetic sulforaphane is capable of causing cell-cycle arrest in the G_2/M phase in the HT29 colon cancer cell line (19).

Moreover, simultaneous analysis of cell-cycle progression (sub-G_1 peak) and loss of phospholipid asymmetry of the plasma membrane in sulforaphane-treated cultures revealed a strictly dose- and time-dependent increase in the percentage of Jurkat cells bearing the flow cytometric characteristics of apoptosis. The latter became apparent after 24 h of incubation. Taken together, these observations imply that the growth inhibition of Jurkat cells produced by sulforaphane (as generated *in situ* from the GRA–Myr mixture) results from a combination of apoptosis and of cell-cycle derangements in which G_2/M arrest is a key event. The simultaneous appearance of G_2/M arrest and apoptosis clearly indicates that cell death is a primary direct effect due to the hydrolytic product of GRA, and not to activation of apoptotic pathways as a consequence of cells' inability to overcome growth arrest and proceed through the cell cycle.

Interactions between *p53*, *bax*, *bcl-2* and its associated proteins have been postulated to form one pathway of apoptosis (39,40). We used flow cytometric analysis to evaluate the levels of p53, bcl-2 and bax proteins, after treatment with GRA plus Myr. We found that the generated sulforaphane increased p53 and bax protein levels, whereas bcl-2 levels remained unchanged. These observations support the hypothesis that induction of *p53* expression may be an important step during the sulforaphane-induced apoptosis. Apoptosis is activated by many oxidative agents (41), and Payen *et al.* (42) indicated a sulforaphane-mediated production of reactive

oxygen species. Moreover, Zhang (43) demonstrated that ITCs, and sulforaphane among these, are taken by cells predominantly through GSH conjugation reactions in cells. Sulforaphane may induce oxidative stress by reacting with and depleting the intracellular GSH pool, which may then induce apoptosis. The molecular mechanism may be mediated by the c-Jun N-terminal kinase, as demonstrated for other ITCs (44) and, therefore, by p53 known to be a substrate of this kinase (45). Otherwise, Bonnesen *et al.* (46) demonstrated that sulforaphane is not associated with DNA damage in colon cell lines, as assessed by comet assay. *bax* has been found to be up-regulated during apoptosis in studies on several other cell types (47,48). *p53* is a direct transcriptional activator of the bax gene (48). So in the presence of increased *p53* expression, it is scarcely surprising that *bax* expression is also affected. The induction of apoptosis recorded in this study seems to be dependent on p53 gene. This is contrary to a recent report (19) where sulforaphane-induced HT29 cell death is not associated with a change in p53 protein expression but is accompanied by an over expression of bax. On the other hand, Huang *et al.* (18) demonstrated an essential role of p53 in the induction of apoptosis by phenylethyl ITC in a mouse epidermal cell line. However, it should be borne in mind that many of the genes and proteins thought to be involved in the apoptotic process may have cell- and injury-type-dependent roles (36).

The present study also demonstrates for the first time that high doses of sulforaphane are capable of inducing necrosis. In the Jurkat cells studied by us, sulforaphane induced necrosis after prolonged exposure (48 h) to the highest concentration (30 μM) investigated. This suggests that very high levels of exposure to produced sulforaphane can cause some form of intensive cell toxicity.

We conclude that the sulforaphane produced *in situ* is able to exert G_2/M phase cell-cycle arrest and produce apoptotic death (and at very high doses even necrosis) of Jurkat tumor cells. The end result of these effects is potent inhibition of the leukemic cell growth. We propose that sulforaphane could be tested as a potential chemopreventive and chemotherapeutic agent, also in the light of its effects on xenobiotic metabolism that were detected in a range of concentrations similar to that used in this study. In particular, sulforaphane is able to induce *in vitro* phase II detoxifying enzymes at 5 μM, with a maximal activity at 50 μM and a decrease of activity at 100 μM (49). Experimental investigations into ways in which sulforaphane could be employed in selective chemoprotective strategies for subjects affected by pre-neoplastic lesions could also be worthwhile. Further in-depth *in vitro* studies have to be performed to compare the activity of sulforaphane on different cell types. *In vivo* studies may then lead to clinical trials to investigate the therapeutic potential of sulforaphane.

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