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Anticarcinogenic Effects of Glycoalkaloids from Potatoes against Human Cervical, Liver, Lymphoma, and Stomach Cancer Cells

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Methods were devised for the isolation of large amounts of pure α-chaconine and α-solanine from Dejima potatoes and for the extraction and analysis of total glycoalkaloids from five fresh potato varieties (Dejima, Jowon, Sumi, Toya, and Vora Valley). These compounds were then evaluated in experiments using a tetrazolium microculture (MTT) assay to assess the anticarcinogenic effects of (a) the isolated pure glycoalkaloids separately, (b) artificial mixtures of the two glycoalkaloids, and (c) the total glycoalkaloids isolated from each of the five potato varieties. All samples tested reduced the numbers of the following human cell lines: cervical (HeLa), liver (HepG2), lymphoma (U937), stomach (AGS and KATO III) cancer cells and normal liver (Chang) cells. The results show that (a) the effects of the glycoalkaloids were concentration dependent in the range of 0.1−10 μg/mL (0.117−11.7 nmol/mL); (b) α-chaconine was more active than was α-solanine; (c) some mixtures exhibited synergistic effects, whereas other produced additive ones; (d) the different cancer cells varied in their susceptibilities to destruction; and (e) the destruction of normal liver cells was generally lower than that of cancer liver cells. The decreases in cell populations were also observed visually by reversed-phase microscopy. The results complement related observations on the anticarcinogenic potential of food ingredients.

KEYWORDS: Potatoes; glycoalkaloids; analysis; anticarcinogenic effects; human cancer cells; MTT assay; microscopy

INTRODUCTION

In a previous study (*1*), we reported that seventeen individual potato, tomato, and eggplant glycoalkaloids and some of their hydrolysis products (metabolites) inhibited the growth of human colon (HT29) and liver (HepG2) cancer cells in an in vitro assay. Because consumption of potatoes results in the ingestion of a mixture of glycoalkaloids in varying ratios, additional studies are needed to address the interactions of glycoalkaloids when consumed as mixtures. Depending on the variety, potatoes may contain the glycoalkaloids α-chaconine and α-solanine at concentration ratios of α-chaconine to α-solanine ranging from ∼1.2:1 to ∼2.4:1 (*2*, *3*). It was therefore of interest to extend the studies against different human cancer cell lines of combinations of these two glycoalkaloids present in potato varieties consumed by humans.

The main objective of this study was therefore to determine the reduction in human cervical, liver, lymphoma, and stomach (gastric) cancer cells by pure potato glycoalkaloids extracted from one potato variety and mixtures of glycoalkaloids extracted from five different commercial potato varieties widely consumed in Korea and Japan. For comparison, we also evaluated artificial mixtures containing varying ratios of the isolated α-chaconine and α-solanine to assess possible additive and synergistic anticarcinogenic effects.

MATERIALS AND METHODS

Materials. Toya potatoes were obtained from an experiment station of Kobe University, Kobe, Japan. Dejima, Jowon, Sumi, and Vora Valley potatoes were obtained from the National Institute of Highland Agriculture, Pyeong Chang, Korea. α-Chaconine and α-solanine were isolated from Dejima potatoes as described below. HPLC grade acetonitrile, methanol, and analytical grade KH_2PO_4, NaH_2PO_4, and NH_4OH were obtained from commercial sources. Before use, the solvents were filtered through a 0.45 μM membrane filter (Millipore, Bedford, MA) and degassed with an ultrasonic bath. Activated aluminum oxide and anisaldehyde were obtained from Kant Chemical

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Co. (Tokyo, Japan). Silica-coated TLC plates were purchased from Merck (Darmstadt, Germany). All other compounds came from Sigma (St. Louis, MO).

Human cervical HeLa, hepatoma (liver) HepG2, lymphoma U937, and stomach (gastric) AGS and KATO III cancer cells and normal human liver Chang cells were obtained from American Type Culture Collection (ATCC, Rockville, MD) and from the Korean Cell Line Bank (KCLB, Seoul, Korea), respectively. The cells were maintained in an MEM medium supplemented with 10% of fetal bovine serum, 50 units/mL of penicillin, and 50 mg/mL of streptomycin, at 37 °C in a 5% CO_2 incubator. Cell culture reagents were obtained from GibcoBRL (Life Technologies, Cergy-Pontoise, France). Each sample was dissolved in DMSO (2 mg/200 μL) and stored at −4 °C.

Isolation and Analysis of Glycoalkaloids from Potatoes. Dejima potato tubers purchased from a local market in Daegu, Korea, were stored at 20−25 °C for 2 months to stimulate glycoalkaloid biosynthesis. The cortex layer and sprouts (∼5 mm of peripheral tissue) were peeled off the whole potatoes and then chopped with a knife. The cortex layer and sprout mixtures (500 g) were weighed, blended in a homogenizer with 2% acetic acid in methanol, and filtered through a Toyo no. 2 filter paper in a Büchner funnel. The resulting residue was rinsed three times with 50 mL of 5% acetic acid in water. The washings were combined with the original filtrate. The filtrate was transferred to a 500-mL Erlenmeyer flask to which was added 20 mL of concentrated NH_4OH to precipitate the glycoalkaloids. The solution was placed in a 70 °C water bath for 50 min and then refrigerated overnight. The precipitate was collected by centrifugation at 18000*g* for 10 min at 1 °C and washed twice with a 2% solution of NH_4OH. The pellet was dried at 30 °C under reduced pressure and then freeze-dried.

Preparative isolation of α-chaconine and α-solanine was performed by chromatography on an aluminum oxide column. Crude glycoalkaloid (606 mg) was dissolved in 10 mL of water-saturated butanol. The butanol solution was then applied to the column (30 × 1.5 cm). The compounds were eluted with water-saturated butanol at a flow rate of 0.5 mL/min controlled with a Hitachi L-600 pump. The eluate was collected in 5-mL fractions, which were examined by TLC (*3*−*5*) for detection of α-chaconine and α-solanine. Fractions with the elution positions corresponding to α-chaconine and α-solanine standards were combined and evaporated to dryness. These were then characterized further as described elsewhere (*2*, *3*).

For the extraction from fresh potatoes, each cortex layer (∼5 mm of peripheral tissue) from three uniform-size fresh tubers was peeled and chopped with a knife. After weighing, each cortex preparation (∼10 g) was blended in a homogenizer with 100 mL of 2% acetic acid in methanol, and the resulting mixture was concentrated to 2−3 mL with the aid of a rotary evaporator. The concentrate was dissolved in 40 mL of 0.2 N HCl. The total glycoalkaloids were then immediately precipitated with concentrated NH_4OH. The ammonia was dissipated and, after centrifugation at 18000*g* for 10 min, the resulting pellet was dissolved in methanol and then dried with a rotary evaporation.

The glycoalkaloid samples obtained from Dejima, Toya, Jowon, Sumi, and Vora Valley potato peel weighed 2.25, 1.16, 2.39, 1.68, and 1.25 mg, respectively. Solutions of these samples in DMSO were used to study the reduction in microculture tetrazolium (MTT) activity as a measure of cancer cell death as described below. The potato extracts were also analyzed for α-chaconine and α-solanine content.

Microculture Tetrazolium Assay. The [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) assay that differentiates dead from living cells was adapted from the literature (*6*). The following reagents and instruments were used: MTT reagent, 5 mg/mL in phosphate buffered saline, protected from light, and stored at 20 °C; MEM cell medium (containing 10% fetal bovine serum, 1% penicillin−streptomycin); microplate reader (Bio-Rad Co., Hercules, CA). The assay was carried out as follows: cell lines were seeded into a 96-well microplate (1×10^4 cells/well) and incubated for 24 h. Next, cells were treated with three concentrations of each of the test compounds for 48 h. The MTT (final concentration = 0.5 mg/mL) was then added to each well. After 4 h of incubation at 37 °C, 200 μL of DMSO was added to each well. The optical density (OD) was then read at a wavelength of 540 nm. The decrease in OD measures the extent of decrease in the number of cancer cells exposed to the glycoalkaloids.

Microscopy of Untreated and Treated Cancer Cells. The procedure was adapted from the literature (*7*). The cancer cells in the microplate reader (1×10^6 cells/well) were treated for 48 h with 0, 0.1, 0.5, or 1.0 μg/mL of α-chaconine or α-solanine. The 96-well plates were each washed with 500 μL of cold phosphate-buffered saline, and the cells were then fixed for ∼40 min with 200 μL of cold trichloroacetic acid. Next, the cells were washed three times with water and dried at room temperature. The cells were then stained in each well with 250 μL of 0.2% of sulforhodamine B in a 1% acetic acid and photographed at ×400 magnification with the aid of a Leica microscope (Heidelberg, Germany).

Statistical Methods. The optical densities were transformed by natural logarithms prior to analyses of variance to stabilize the variance among compounds and concentrations. One-way analyses of variance (ANOVA) were used along with Dunnett's one-tailed test for decreases from the control. For the experiments with multiple compounds, the analyses were run separately for each concentration, and the control was included for each run. Isobole diagrams were used to establish additive, antagonistic, and synergistic effects of mixtures of the two glycoalkaloids.

RESULTS AND DISCUSSION

Preparative Extraction and Analysis of Glycoalkaloids. The glycoalkaloids of the cortex layer and attached sprouts of Dejima potatoes were extracted with 2% acetic acid in water. From 500 g of potatoes, 2.27 g of crude glycoalkaloids was recovered, which corresponds to 0.45% of the original weight. The crude extract (606 mg) was chromatographed on an aluminum oxide column. The two glycoalkaloids from the individual fractions were then characterized by HPLC (**Figure 1**). Recoveries from the crude mixture were 150.1 mg of α-chaconine (fractions 15−22) and 184.4 mg of α-solanine (fractions 30−40). We did not observe the formation of any new compounds as a result of the brief exposure of the glycoalkaloids to 0.2 N HCl used to facilitate isolation.

The peels of five Korean fresh potato varieties (Dejima, Jowon, Sumi, Toya, and Vora Valley) were extracted with 2% acetic acid. **Table 1** shows the content of α-chaconine and α-solanine analyzed by HPLC. These samples containing mixtures of both glycoalkaloids were evaluated for their abilities to inhibit cancer cell growth.

Table 1 shows that the α-chaconine content of the cortex (peel) of the five potato varieties ranged from 0.44 μmol/g (377 μg/g) for Sumi potatoes to 1.72 μmol/g (1466 μg/g) for the Dejima cultivar. The corresponding range for α-solanine was from 0.26 μmol/g (136 μg/g) to 0.71 μmol/g (614 μg/g). The ratios of α-chaconine to α-solanine varied from 1.06 for Vora Valley potatoes to 2.83 for the Sumi cultivar.

Anticarcinogenic Effects of α-Chaconine and α-Solanine. **Figure 2** depicts anticarcinogenic effects against one normal and three cancer cell lines by three concentrations (0.1, 1, and 10 μg/mL corresponding to 0.1174−11.74 nmol/mL) of α-chaconine and α-solanine isolated from Dejima potatoes. Inhibition values tended to converge at the highest dose, particularly for α-chaconine.

These results show that the susceptibilities to destruction vary with both the nature of the glycoalkaloid and the type of cancer cells. In all cases, α-chaconine was more active than α-solanine. The two compounds share the same aglycon but differ in the nature of the carbohydrate side attached to the aglycon (**Figure 1A**). α-Chaconine has a branched bis(α-L-rhamnopyranosyl)-β-D-glucopyranose known as chacotriose and α-solanine has a branched α-L-rhamnopyranosyl-β-D-glucopyranosyl-β-galactopyranose known as solatriose. Thus, the chacotriose side chain may have a greater adverse effect on cells than does the

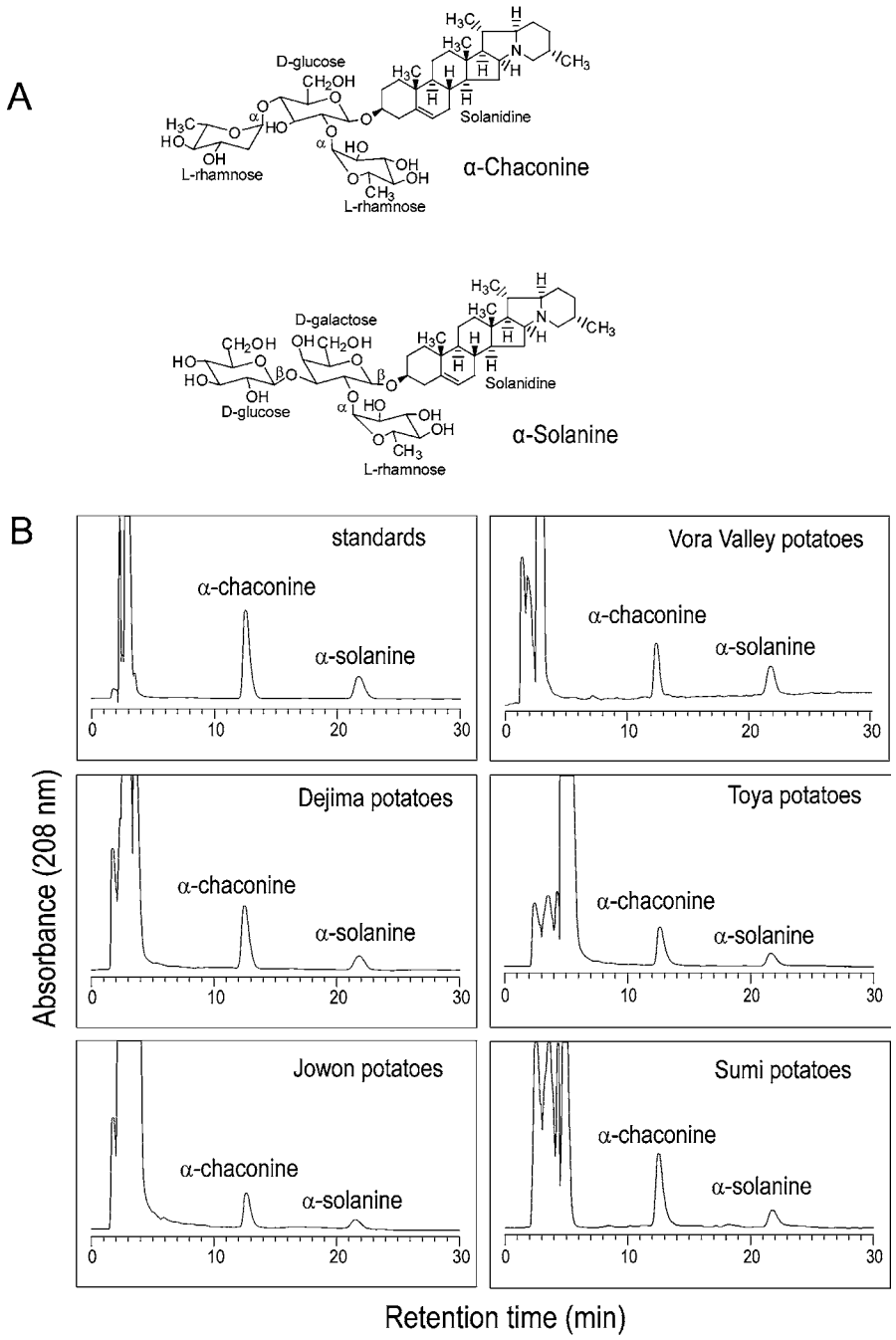

Figure 1. (**A**) Structures of α-chaconine and α-solanine. (**B**) HPLC chromatograms of α-chaconine and α-solanine standards and of extracts from five commercial potato varieties evaluated in this study.

Table 1. α-Chaconine and α-Solanine Content of the Cortex Layer of Five Varieties of Potato Tubers Determined by HPLC ($n = 3$)

potato variety	α-chaconine (*A*) (μmol/g)	α-solanine (*B*) (μmol/g)	*A* + *B*	*A*/*B*
Dejima	1.72 ± 0.037	0.71 ± 0.039	2.43	2.43
Jowon	0.97 ± 0.082	0.46 ± 0.018	1.43	2.13
Vora Valley	0.71 ± 0.016	0.67 ± 0.037	1.39	1.06
Toya	0.64 ± 0.013	0.37 ± 0.004	1.01	1.75
Sumi	0.44 ± 0.011	0.16 ± 0.003	0.60	2.83

solatriose side chain, possibly because of its greater affinity to and disruption of cell membranes (*8*).

The growth inhibition determined by the MTT assay was confirmed visually by microscopy of treated and untreated cancer cells, illustrated in **Figure 3**. This figure strikingly depicts the concentration-dependent decrease in the number of AGS gastric and HepG2 human cancer cells following exposure to α-chaconine and α-solanine. Both the MTT assay and microscopy show that α-chaconine was more effective in killing the cancer cells than was α-solanine.

A Journal reviewer suggested that the MTT assay actually measures a decrease in mitochondrial activity of the cells that may reflect a decrease in cell proliferation but that equally likely reflects toxicity leading to loss of cell viability. The disappearance of large numbers of cancer cells shown in the photographs of **Figure 3** is consistent with an anticarcinogenic mechanism involving loss of cell viability as a result of toxicity.

Additive and Synergistic Effects of α-Chaconine and α-Solanine. In a previous study we found that the ratio of α-chaconine to α-solanine in potato peel, flesh, and whole

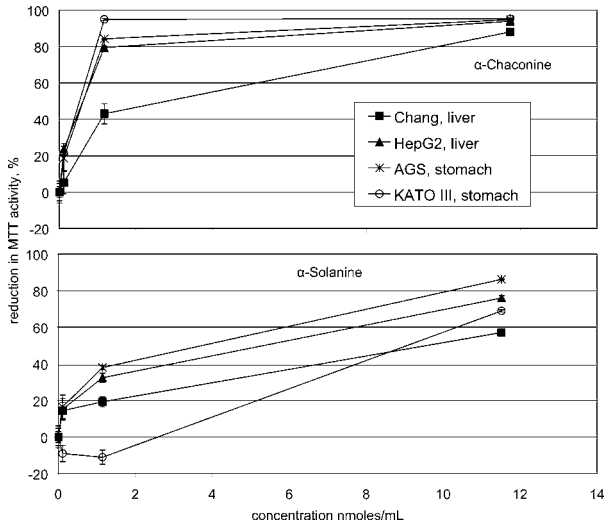

Figure 2. Anticarcinogenic effects against human Chang normal liver cells, HepG2 liver cancer cells, and AGS and Kato III stomach cancer cells each exposed to 0.1174, 1.174, and 11.74 nmol/mL solutions of α-chaconine and to 0.1152, 1.152, and 11.52 nmol/mL solutions of α-solanine isolated from the cortex of Dejima potatoes.

potatoes of eight potato cultivars ranged from 1.2:1 to 2.6:1 (*2*). In this study, the ratio in the peel ranged from 1.06:1 to 2.83:1 (**Table 1**). In addition, previous studies showed that some combinations of the two glycoalkaloids can act synergistically in lysing (disrupting) cell membranes (*9*, *10*); that is, the biological effects of the mixtures were greater than the predicted additive effects of individual compounds. It was therefore of interest to find out whether this is also true for the destruction of cancer cells.

In an exploratory study designed to test effects of mixtures on growth inhibition, the AGS gastric and HepG2 liver cells were tested with seven different solutions: two solutions of 10 μg/mL (11.74 and 11.52 nmol/mL) of pure α-chaconine and α-solanine, respectively, isolated from Dejima potatoes and five solutions containing both compounds in proportions ranging from 1:9 to 9:1. **Table 2** shows the observed effects. The data were also plotted as an isobole diagram, a statistical tool designed to establish the existence of additive, antagonistic, and synergistic interactions (see legend to **Figure 4**).

The data in **Table 2** and **Figure 4** indicate that the following proportions of α-chaconine to α-solanine exhibited synergism against the HepG2 liver cells: 0.1:09, 0.3:0.7, and 0.5:05. The 0.7:0.3 combination was an additive effect, and the interaction with the 0.9:0.1 mixture was antagonistic. For the AGS gastric cancer cells, the effects with the 0.1:0.9, 0.3:0.7, 0.5:0.5, and 0.9:0.1 mixtures were additive and that with the 0.7:0.3 mixture, antagonistic.

The demonstration of synergism deserves additional comment. The calculated statistical parameters in **Table 2** are correct because single degree of freedom contrasts were used to compare the appropriate weighted averages of the pure compounds with the averages of the mixtures. Comparisons were made with the diagonal lines of **Figure 4**, which represent the weighted averages of the pure compounds, not with the horizontal lines that would be drawn as the means of pure compounds. Moreover, it may not always be possible to predict effects of mixtures from effects of individual compounds, possibly because α-chaconine and α-solanine may compete for receptor sites on cell surfaces. Such competition could result in either antagonistic, additive, or synergistic effects. The actual mixtures have to be tested to demonstrate interactive effects.

These results suggest that certain combinations of the two glycoalkaloids that acted synergistically may offer therapeutic or perhaps preventive advantages, as suggested for mixtures of tea catechins (*11*). This exploratory study with a few mixtures merits extension with many more combinations of α-chaconine and α-solanine.

Anticarcinogenic Effects of Glycoalkaloids from Five Potato Varieties. The survival of different cancer cell lines at 25, 50, and 100 μg/mL (29, 58, and 117 nmol/mL) of total glycoalkaloid samples consisting of mixtures of α-chaconine and α-solanine varied with both concentration and potato variety (**Table 3**). These aspects are examined for each cell line and the five varieties on the basis of the data listed in the table.

HeLa Cervical Cancer Cells. At 29 nmol/mL, the extracts from Jowon and Vora Valley varieties did not inhibit cell growth, whereas the percentage inhibition of the other three varieties ranged from 46.9 (Sumi) to 72.4 (Dejima). At 58 nmol/mL, the percent inhibition ranged from 27.4 (Vora Valley) to

Table 2. Individual and Joint Inhibitory Effects of α-Chaconine and α-Solanine Isolated from the Cortex of Dejima Potatoes on the Growth of AGS Gastric and Hep2 Liver Human Cancer Cells

cell line	glycoalkaloid	concn μg/mL	concn nmol/mL	cell growth (OD ± SE, $n = 3$)	t[a]	p value	reduction in MTT activity (%)
AGS	α-chaconine	10	11.74	0.066 ± 0.003			94.9
	α-solanine	10	11.52	0.175 ± 0.003			86.6
	α-chaconine + α- solanine	5 + 5	11.63	0.108 ± 0.004	–0.09	0.93	91.7
	α-chaconine + α-solanine	3 + 7	11.59	0.138 ± 0.008	–1.07	0.3	89.5
	α-chaconine + α-solanine	7 + 3	11.67	0.100 ± 0.004	–2.27	0.015	92.3
	α-chaconine + α-solanine	9 + 1	11.72	0.069 ± 0.003	1.07	0.3	94.7
	α-chaconine + α-solanine	1 + 9	11.54	0.168 ± 0.003	–1.18	0.25	87.1
	control	0	0	1.309 ± 0.037			
HepG2	α-chaconine	10	11.52	0.082 ± 0.001			89.7
	α-solanine	10	11.74	0.167 ± 0.007			79.0
	α-chaconine + α- solanine	5 + 5	11.63	0.088 ± 0.002	6.95	<0.0001	88.9
	α-chaconine + α-solanine	3 + 7	11.59	0.094 ± 0.002	8.47	<0.0001	88.2
	α-chaconine + α-solanine	7 + 3	11.67	0.087 ± 0.002	3.62	0.002	89.0
	α-chaconine + α-solanine	9 + 1	11.72	0.085 ± 0.001	0.76	0.46	89.3
	α-chaconine + α-solanine	1 + 9	11.54	0.098 ± 0.001	10.15	<0.0001	87.6
	control	0	0	0.791 ± 0.061			

[a] The t tests give comparisons to the corresponding weighted averages of the pure compounds. Positive values indicate positive synergism. A negative value of t is indicative of antagonism.

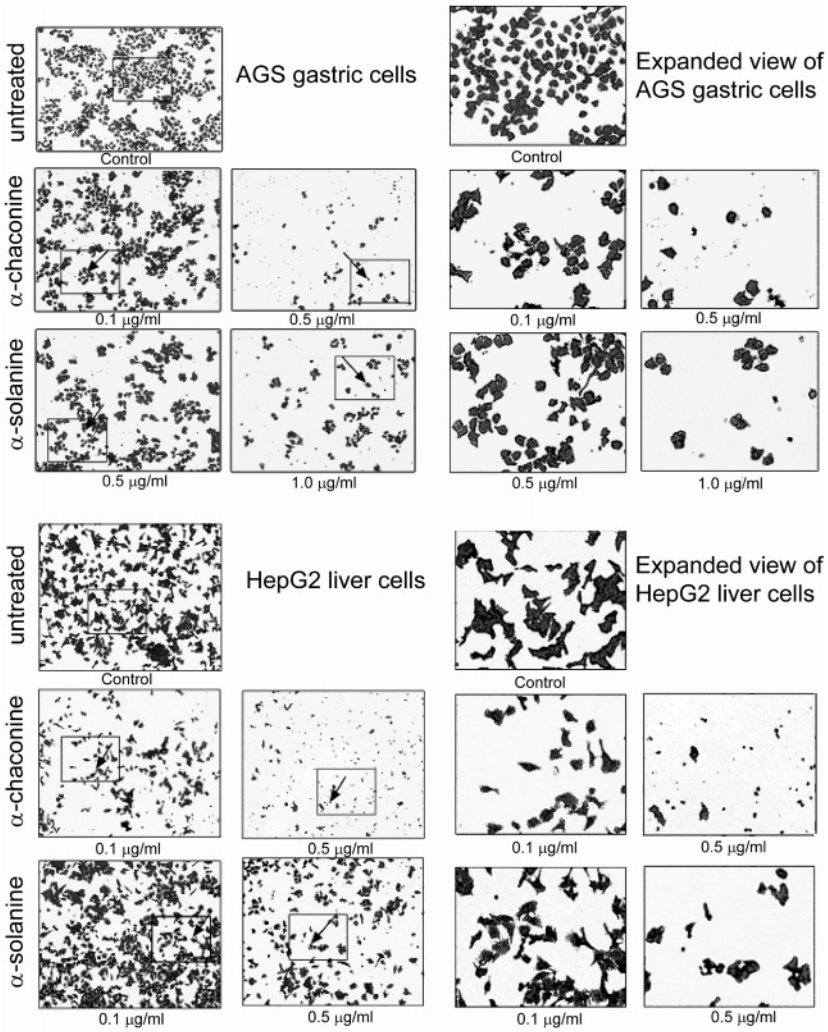

Figure 3. Phase-contrast microscopy showing disappearance of AGS stomach and HepG2 liver human cancer cells induced by α-chaconine and α-solanine. Note that the concentrations used for α-chaconine (0.1 and 0.5 μg/mL or 0.117 and 0.587 nmol/mL) are different from the ones for α-solanine (0.5 and 1.0 μg/mL or 0.576 and 1.152 nmol/mL).

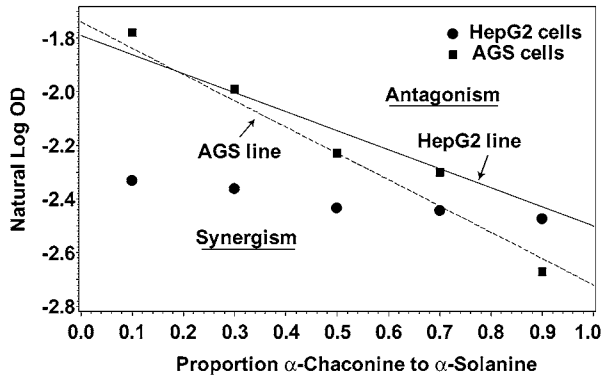

Figure 4. Isobole diagram for determining additive, antagonistic, and synergistic effects of the joint action of α-chaconine and α-solanine on HepG2 (liver) and AGS (stomach) human cancer cells. The treatment means (log scale) were plotted along with the two lines (solid line, HepG2 cells; broken line, AGS cells) representing the two cell types that connect the means for the pure compound treatments. Data points on the respective lines indicate additive effects; those above, antagonism; and those below, synergism.

82.8 (Toya) and that at 117 nmol/mL from 61.2 (Sumi) to 84.2 (Vora Valley). We have no explanation for the differential effect at the 29 nmol/mL dose.

HepG2 Liver Cancer Cells. At 29 nmol/mL, the percent inhibition ranged from 35.9 (Sumi) to 73.7 (Toya); at 58 nmol/mL, from 43.9 (Sumi) to 83.8 (Toya); and at 117 nmol/mL, from 58.2 (Sumi) to 83.8 (Toya).

U937 Lymphoma Cells. At 29 nmol/mL, the percent inhibition ranged from 71.2 (Dejima) to 84.6 (Toya); at 58 nmol/mL, from 79.6 (Dejima) to 84.4 (Vora Valley, Toya); and at 117 nmol/mL, from 78.3 (Dejima) to 84.3 (Toya, Vora Valley).

AGS Gastric Cancer Cells. At 29 nmol/mL, the percent inhibition ranged from 54.5 (Vora Valley) to 74.7 (Toya); at 58 nmol/mL, from 58.4 (Sumi) to 75.1 (Jowon); and at 117 nmol/mL, from 60.1 (Sumi) to 77.8 (Vora Valley).

Kato III Gastric Cancer Cells. At 29 nmol/mL, the percent inhibition ranged from 23.0 (Sumi) to 65.2 (Toya); at 58 nmol/mL, from 38.8 (Sumi) to 71.0 (Vora Valley); and at 117 nmol/mL, from 43.6 (Sumi) to 72.8 (Vora Valley).

Chang Normal Liver Cells. At 29 nmol/mL, the percent inhibition ranged from 33.0 (Sumi) to 66.3 (Dejima); at 58 nmol/mL, from 53.8 (Sumi) to 71.8 (Jowon); and at 117 nmol/mL, from 69.6 (Sumi) to 74.3 (Vora Valley).

The data show that all five potato extracts were active against the five cancer cell types. Anticarcinogenic potency was influenced by the nature of the cancer cells, the concentration of glycoalkaloids, and the ratio of α-chaconine to α-solanine present in the extracts (**Table 1**). It should also be noted that

Table 3. Inhibitory Effects of Mixtures of α-Chaconine and α-Solanine Isolated from Five Varieties of Potatoes on the Growth of HeLa Cervical, HepG2 Liver, U937 Lymphoma, AGS and KATO III Stomach Human Cancer Cells and on Chang Normal Human Liver Cells

cell line	potato variety	glycoalkaloid concn		cell growth[a] (OD ± SE, $n = 3$)	cell growth inhibition (%)
		μg/mL	nmol/mL		
HeLa (cervical)	control	0	0	0.442 ± 0.040	
	Dejima	25	29	0.167 ± 0.028bc	62.2
	Jowon	25	29	0.611 ± 0.018a[b]	no inhibition
	Sumi	25	29	0.235 ± 0.026b	46.9
	Toya	25	29	0.122 ± 0.006c	72.4
	Vora Valley	25	29	0.591 ± 0.047a[b]	no inhibition
	Dejima	50	58	0.077 ± 0.004b	82.5
	Jowon	50	58	0.091 ± 0.016b	79.5
	Sumi	50	58	0.191 ± 0.044a	56.7
	Toya	50	58	0.076 ± 0.002b	82.8
	Vora Valley	50	58	0.321 ± 0.025a[d]	27.4
	Dejima	100	117	0.078 ± 0.002b	82.3
	Jowon	100	117	0.072 ± 0.002b	83.6
	Sumi	100	117	0.171 ± 29a	61.2
	Toya	100	117	0.079 ± 0.003b	82.0
	Vora Valley	100	116	0.070 ± 0.002b	84.2
HepG2[c] (liver)	control	0	0	0.444 ± 0.031	
	Dejima	25	29	0.276 ± 0.023a	37.7
	Jowon	25	29	0.207 ± 0.010a	53.3
	Sumi	25	29	0.285 ± 0.026a	35.9
	Toya	25	29	0.117 ± 0.019b	73.7
	Vora Valley	25	29	0.185 ± 0.012a	58.3
	Dejima	50	58	0.095 ± 0.014b	78.7
	Jowon	50	58	0.080 ± 0.002b	82.1
	Sumi	50	58	0.249 ± 0.009a	43.9
	Toya	50	58	0.072 ± 0.001b	83.8
	Vora Valley	50	58	0.074 ± 0.001b	83.3
	Dejima	100	117	0.084 ± 0.004b	81.1
	Jowon	100	117	0.076 ± 0.002b	82.9
	Sumi	100	117	0.185 ± 0.003a	58.2
	Toya	100	117	0.072 ± 0.002b	83.8
	Vora Valley	100	116	0.076 ± 0.005b	82.9
U937[d] (lymphoma)	control	0		0.491 ± 0.039	
	Dejima	25	29	0.142 ± 0.010a	71.2
	Jowon	25	29	0.081 ± 0.006b	83.6
	Sumi	25	29	0.108 ± 0.004a	78.1
	Toya	25	29	0.075 ± 0.003b	84.6
	Vora Valley	25	29	0.077 ± 0.005b	84.4
	Dejima	50	58	0.100 ± 0.005a	79.6
	Jowon	50	58	0.081 ± 0.008ab	83.5
	Sumi	50	58	0.088 ± 0.002ab	82.1
	Toya	50	58	0.077 ± 0.001b	84.4
	Vora Valley	50	58	0.076 ± 0.004b	84.4
	Dejima	100	117	0.106 ± 0.003a	78.3
	Jowon	100	117	0.083 ± 0.006b	83.0
	Sumi	100	117	0.078 ± 0.002b	84.1
	Toya	100	117	0.077 ± 0.001b	84.3
	Vora Valley	100	116	0.077 ± 0.004b	84.3
AGS[e] (stomach)	control	0	0	0.318 ± 0.007	
	Dejima	25	29	0.116 ± 0.009ab	63.6
	Jowon	25	29	0.122 ± 0.021ab	61.8
	Sumi	25	29	0.141 ± 0.016a	55.6
	Toya	25	29	0.081 ± 0.003b	74.7
	Vora Valley	25	29	0.145 ± 0.014a	54.5
	Dejima	50	58	0.088 ± 0.009b	72.3
	Jowon	50	58	0.079 ± 0.005b	75.1
	Sumi	50	58	0.132 ± 0.005a	58.4
	Toya	50	58	0.081 ± 0.005b	74.7
	Vora Valley	50	58	0.111 ± 0.014ab	65.0
	Dejima	100	117	0.084 ± 0.008b	73.5
	Jowon	100	117	0.080 ± 0.006b	74.9
	Sumi	100	117	0.127 ± 0.007a	60.1
	Toya	100	117	0.076 ± 0.003b	76.0
	Vora Valley	100	116	0.071 ± 0.001b	77.8

Table 3 (Continued)

cell line	potato variety	glycoalkaloid concn μg/mL	glycoalkaloid concn nmol/mL	cell growth[a] (OD ± SE, $n = 3$)	cell growth inhibition (%)
Kato III (stomach)	control	0	0	0.265 ± 0.012	
	Dejima	25	29	0.133 ± 0.008a	49.7
	Jowon	25	29	0.194 ± 0.021a[b]	26.6
	Sumi	25	29	0.204 ± 0.010a[b]	23.0
	Toya	25	29	0.092 ± 0.008a	65.2
	Vora Valley	25	29	0.159 ± 0.055a	40.1
	Dejima	50	58	0.097 ± 0.008ab	63.5
	Jowon	50	58	0.118 ± 0.019ab	55.3
	Sumi	50	58	0.162 ± 29a	38.8
	Toya	50	58	0.077 ± 0.008b	70.8
	Vora Valley	50	58	0.077 ± 0.008b	71.0
	Dejima	100	117	0.099 ± 0.007b	62.7
	Jowon	100	117	0.093 ± 0.005b	65.0
	Sumi	100	117	0.149 ± 0.016a	43.6
	Toya	100	117	0.082 ± 0.006b	69.1
	Vora Valley	100	116	0.072 ± 0.003b	72.8
Chang normal	control	0	0	0.278 ± 0.037	
	Dejima	25	29	0.094 ± 0.009b	66.3
	Jowon	25	29	0.126 ± 0.023ab	54.5
	Sumi	25	29	0.186 ± 0.005a[d]	33.0
	Toya	25	29	0.141 ± 0.009ab	49.3
	Vora Valley	25	29	0.171 ± 0.017a	38.5
	Dejima	50	58	0.081 ± 0.003b	70.8
	Jowon	50	58	0.078 ± 0.006b	71.8
	Sumi	50	58	0.128 ± 0.014a	53.8
	Toya	50	58	0.075 ± 0.003b	73.1
	Vora Valley	50	58	0.084 ± 0.014b	69.9
	Dejima	100	117	0.084 ± 0.002a	69.9
	Jowon	100	117	0.076 ± 0.002ab	72.6
	Sumi	100	117	0.084 ± 0.001a	69.6
	Toya	100	117	0.076 ± 0.004ab	72.5
	Vora Valley	100	116	0.071 ± 0.002b	74.3

[a] Means ($n = 3$) followed by the same letter within a concentration are not significantly different (Tukey's test, $p = 0.05$). The nmol/mL values for the five potato varieties were calculated from the μg/mL values by taking into account the molecular weights of α-chaconine (852.07) and α-solanine (868.07) and their ratios shown in **Table 2**. [b] Not significant < control (Dunnett's test, $p = 0.05$). [c] All significant < control (Dunnett's test, $p < 0.05$). [d] All significant < control (Dunnett's test, $p < 0.0001$). [e] All significant < control (Dunnett's test, $p < 0.001$).

both individual glycoalkaloids and mixtures of the two glycoalkaloids isolated from potatoes were in most cases more effective against the HepG2 liver cancer cells than against the normal Chang liver cells (**Figure 2**; **Table 3**). Other normal human cell lines were not available to us for this study.

Significance for the Human Diet. Glycoalkaloid-containing potatoes and potato products are widely consumed. For example, the daily per capita intake of glycoalkaloids from potatoes in the United Kingdom is estimated to be ~14 mg (*12*). Although the glycoalkaloid concentration of most commercial potatoes is usually below a safety guideline of 200 mg/kg of fresh potatoes, the concentration can increase substantially on exposure of potatoes to light and as a result of mechanical injury (reviewed in ref *13*). A recent study showed that oral consumption of mashed potatoes with a total glycoalkaloid content of ~200 mg/kg equivalent did not induce acute systemic effects in human volunteers (*14*). However, the safety of glycoalkaloids for humans is still being debated (*12*, *15*−*19*).

Because it is difficult to translate results from cell assays to in vivo effects, the observed destruction of a broad range of cancer cells by glycoalkaloids suggests the need for animal and human experiments designed to confirm the in vitro data by corresponding effects in vivo. We are also challenged to ascertain whether the concurrent consumption of dietary glycoalkaloids, which may exert their effects by disrupting cell membranes (*20*) and enhancing the immune system (*21*, *22*), will increase the effectiveness of other anticarcinogenic food ingredients, which may act by different mechanisms. These ingredients include anthocyanins from pigmented rice brans (*23*−*25*), capsaicinoids from peppers (*26*), catechins from teas (*27*), indole-carbinols from cruciferous vegetables (*28*), protease inhibitors and the peptide lunasin from soybeans (*29*, *30*), and tomatine from tomatoes (*1*).

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