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Tomatine-Containing Green Tomato Extracts Inhibit Growth of Human Breast, Colon, Liver, and Stomach Cancer Cells

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Tomato plants (*Lycopersicon esculentum*) synthesize the glycoalkaloids dehydrotomatine and α-tomatine, possibly as a defense against bacteria, fungi, viruses, and insects. Six green and three red tomato extracts were investigated for their ability to induce cell death in human cancer and normal cells using a microculture tetrazolium (MTT) assay. Compared to untreated controls, the high-tomatine green tomato extracts strongly inhibited the following human cancer cell lines: breast (MCF-7), colon (HT-29), gastric (AGS), and hepatoma (liver) (HepG2), as well as normal human liver cells (Chang). There was little inhibition of the cells by the three low-tomatine red tomato extracts. Cell death induced by the pure glycoalkaloids dehydrotomatine and α-tomatine isolated from green tomatoes and characterized by HPLC, GC, and GC-MS, as well as their respective aglycones tomatidenol and tomatidine, was also evaluated. α-Tomatine was highly effective in inhibiting all of the cell lines. Dehydrotomatine, tomatidenol, and tomatidine had little, if any, effect on cell inhibition. The results show that the susceptibility to destruction varies with the nature of the alkaloid and plant extract and the type of cancer cell. These findings extend related observations on the anticarcinogenic potential of glycoalkaloids and suggest that consumers may benefit by eating not only high-lycopene red tomatoes but also green tomatoes containing glycoalkaloids. Possible mechanisms of the anticarcinogenic and other beneficial effects and the significance of the cited observations for breeding improved tomatoes and for the human diet are discussed.

KEYWORDS: HPLC; GC-MS; tetrazolium assay; green tomatoes; red tomatoes; cancer cells; growth inhibition; dehydrotomatine; α-tomatine; tomatidenol; tomatidine; dietary significance

INTRODUCTION

Using a microculture tetrazolium (MTT) in vitro assay, we previously screened 17 glycoalkaloids and metabolites for inhibitory effects against human cancer cells. The commercial tomato glycoalkaloid tomatine (a ∼10:1 mixture of α-tomatine and dehydrotomatine) was found to be a strong inhibitor of growth for both human colon and liver cancer cell lines, as evidenced by the dose-dependent inhibition of HT29 colon cancer cells at levels ranging from 38.0 to 81.5% and that of human HepG2 cancer cells at levels from 46.3 to 89.2% (*1*). The susceptibility of human liver cancer cells to tomatine was higher than was the case with the commercial anticancer drug doxorubicin.

In a long-term study, we also showed that feeding of 2000 ppm of commercial tomatine and 224 ppm of the multiorgan carcinogen dibenzo[*a*,*l*]pyrene (DBP) to rainbow trout resulted in reduced incidences of liver and stomach tumors by 41.3 and 36.3%, respectively, as compared to the incidence of tumors observed with DBP alone (*2*). The tomatine-containing diets did not induce changes in mortality, fish weights, liver weights, or tissue morphology. No adverse pathological effects in the tissues of the fish on the tomatine diets were observed.

Other investigators have found that *Solanum* glycoalkaloids have varied biological effects. Immunization with a molecular aggregate containing tomatine as an adjuvant protected mice against malarial infection (*3*). Tomatine also induced T-cell-mediated regression of murine lymphoid experimental tumors, EG7-Ova (*3*), and acted as an anti-inflammatory agent by blocking NF-κB and JNK signaling in mouse macrophages (*4*). The aglycone tomatidine decreased multidrug resistance of human cancer cells to chemotherapy agents, thus increasing their therapeutic value (*5*). An ointment containing the glycoalkaloids solamargine and solasonine was found to be a safe treatment for skin cancer (*6*). Tomatine is also reported to exhibit antibiotic activities against microorganisms (*7*–*9*).

It was therefore of interest to extend these studies of compounds active against different human cancer cells to tomatine-rich green and tomatine-poor red tomatoes. The main objective of this study was therefore to determine the reduction in human breast, colon, stomach (gastric), and liver cancer cells by nine

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Table 1. Tomatoes Used in the Present Study

sample	tomato type	variety	color	length (mm)	width (mm)	weight (g)/fruit	ammonia precipitate (mg/100 g of fruit)
1	mini	Sancheri Premium[a]	green	13.2 ± 0.8	11.7 ± 1.3	1.1 ± 0.1	494.1
2	mini	Sancheri Premium[a]	green	21.2 ± 0.8	17.8 ± 0.4	4.1 ± 0.4	23.3
3	mini	Sancheri Premium[a]	green	26.7 ± 0.8	22.3 ± 0.8	7.6 ± 0.9	21.1
4	mini	Sancheri Premium[a]	red	25.7 ± 0.8	25.3 ± 0.8	10.8 ± 0.5	16.4
5	mini	Yoyo[a]	green	23.3 ± 1.5	21.7 ± 0.8	6.3 ± 0.7	8.7
6	normal	Chobok Power[b]	green	20.2 ± 1.2	23.2 ± 1.2	6.8 ± 1.1	22.2
7	normal	Chobok Power[b]	red	58.0 ± 6.0	60.0 ± 0.0	137.0 ± 11.2	13.5
8	normal	Rokusanmaru[b]	green	23.3 ± 0.8	23.0 ± 0.6	7.9 ± 0.8	37.2
9	normal	Rokusanmaru[b]	red	51.2 ± 2.3	58.0 ± 4.3	97.8 ± 10.4	7.7

[a] Sakata Seed Co. (Osaka, Japan). [b] Cho Won Seed Co. (Seoul, Korea).

alkaloid isolates of four tomato varieties, red and green, modeled after a similar study with potato glycoalkaloids (*10*). For comparison, we also evaluated cell deaths induced by pure dehydrotomatine and α-tomatine isolated from green tomatoes and their respective aglycones, tomatidenol and tomatidine. To our knowledge, this is the first reported attempt to compare anticarcinogenic activities of pure tomato glycoalkaloids and aglycones to activities of tomatine-rich tomato fruit extracts.

MATERIALS AND METHODS

Materials. Tomato fruits were obtained from Gangwondo Agricultural Research and Extension Center, Gangwondo, Korea (**Table 1**). Tomatine (a ~10:1 mixture of α-tomatine and dehydrotomatine) and the aglycone tomatidine were purchased from Sigma (St. Louis, MO). Pure dehydrotomatine and α-tomatine were isolated from Sigma tomatine by multiple collections of eluates from the HPLC column described below. The aglycone tomatidenol was prepared by hydrolytic removal of the sugar side chain from dehydrotomatine, as described previously (*11*). HPLC grade acetonitrile, methanol, and analytical grade KH_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. All other compounds came from Sigma (St. Louis, MO). Breast (MCF-7), colon (HT-29), liver (HepG2), and stomach (AGS) 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). 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.

Tomato Extraction. Each tomato fruit sample consisted of three uniform-size fresh fruits combined and chopped with a knife. After weighing, each sample (3−41 g) was blended in a homogenizer with 2% acetic acid in methanol (100 mL). The resulting mixture was concentrated to 2−3 mL with the aid of a rotary evaporator. The concentrate was dissolved in 0.2 N HCl (40 mL) and centrifuged at 18000*g* for 5 min at 5 °C. The residue was rinsed twice with 0.2 N HCl (10 mL) and then centrifuged again. Concentrated NH_4OH (20 mL) was added to the supernatant to precipitate the glycoalkaloids. The basic solution was placed in a 65 °C water bath for 50 min and then refrigerated overnight. The precipitate was collected after centrifugation at 18000*g* for 10 min at 5 °C and washed twice with 2% NH_4OH. The ammonia was dissipated, and the resulting pellet was dried at 30 °C under reduced pressure and then dissolved in 2% acetic acid in methanol (2 mL) and centrifuged at 18000*g* for 10 min at 5 °C. An aliquot of the supernatant (50 μL) was injected directly into the HPLC for α-tomatine/dehydrotomatine analysis. A second aliquot of the supernatant (1 mL) was dried and weighed. This dry fraction was used for MTT analysis described below. All extractions and precipitations were done in triplicate.

Analysis of Tomato Glycoalkaloids and Aglycones. HPLC was carried out on a Hitachi liquid chromatograph model 665-II equipped with a Shimadzu UV−vis detector (model SPD-10Avp, Kyoto, Japan) set at 208 nm. Column temperature was controlled with a Shimadzu CTO-10Asvp thermometer. Chromatogram peak areas were integrated with a Hitachi D-2500 chromatointegrator. An Inertsil NH_2 column [5 μm, 4.0 × 250 mm (GL Science Inc., Tokyo, Japan)] was used to analyze dehydrotomatine and α-tomatine. The mobile phase was acetonitrile and 20 mM KH_2PO_4 (23:77, v/v). The flow rate was 1.0 mL/min at a column temperature of 30 °C. Three separate analyses were carried out with each sample.

We used two methods to identify dehydrotomatine and α-tomatine: (a) Retention times on HPLC peaks of pure dehydrotomatine and α-tomatine were compared to corresponding peaks from the tomato extracts, and (b) samples from each peak, collected several times from the HPLC column, were then acid hydrolyzed into sugars and aglycone. The sugars were converted to trimethylsilyl ester derivatives. Individual compositions and molar ratios of sugars were determined by gas−liquid chromatography (GC). Sugars and aglycones were determined by gas chromatography−mass spectrometry (GC-MS) as described in detail in previous publications (*11*−*14*).

Quantification of dehydrotomatine and α-tomatine was accomplished with the aid of a Hitachi model D-2500 chromatointegrator by comparing the HPLC peak area from the sample to the peak area of known amounts of pure dehydrotomatine and α-tomatine isolated from tomato fruits.

MTT Assay for Growth Inhibition of Cells. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay that differentiates dead from living cells was adapted from the literature (*1*, *10*, *15*, *16*). 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). Cell lines were seeded into a 96-well microplate (1×10^4 cells/well) and incubated for 24 h. Next, cells were treated with several concentrations of each of the test compounds for 48 h (10 and 50 μg/mL for pure compounds and 10, 50, and 100 μg/mL for tomato precipitates). The MTT solution (0.1 mg/mL) was then added to each well. After 4 h of incubation at 37 °C, DMSO (200 μL) was added to each well. The absorbance (A) was then read at a wavelength of 540 nm. The decrease in absorbance in the assay measures the extent of decrease in the number of viable cells following exposure to the test substances calculated by using the following formula:

$$\%\text{inhibition of cells} = \frac{A_{\text{test substance}}}{A_{\text{control}}} \times 100$$

Statistical Analysis. Inhibitory concentration at 50% (IC_{50}) values were calculated by constructing a four-parameter logistic curve using the values from the previous calculation, percent inhibition of cells, with the aid of SigmaPlot 11 (Systat Software, Inc., San Jose, CA). The IC_{50} was extrapolated from the graph at 50% of cell inhibition. Comparison of the resultant values was accomplished by calculating the Spearman correlation coefficient, also using SigmaPlot 11. The IC_{50} was compared to a number of variables in the tomatoes: tomato size, ripeness, yield of precipitate, and alkaloid content. The closer the absolute of the coefficient is to the value of 1, the stronger the correlation. A negative coefficient indicates an inverse relationship and a positive coefficient, a direct relationship. All reported correlations were significant, with p values of < 0.05.

RESULTS

Analytical Aspects. We previously reported that the tomato glycoalkaloid referred to as tomatine consisted of a ~10:1 mixture

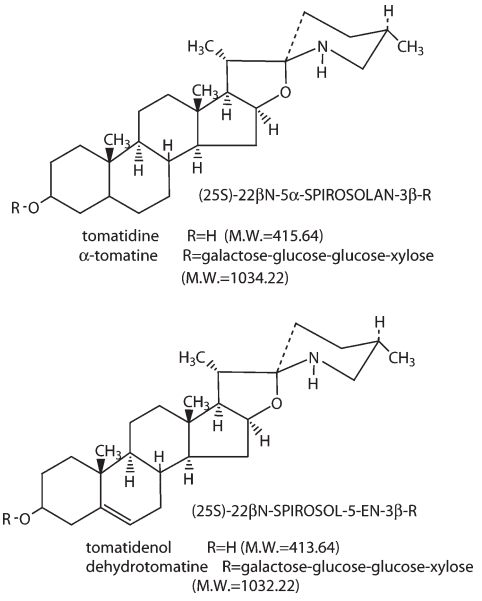

Figure 1. Structures of dehydrotomatine, α-tomatine, tomatidenol, and tomatidine evaluated in the present study.

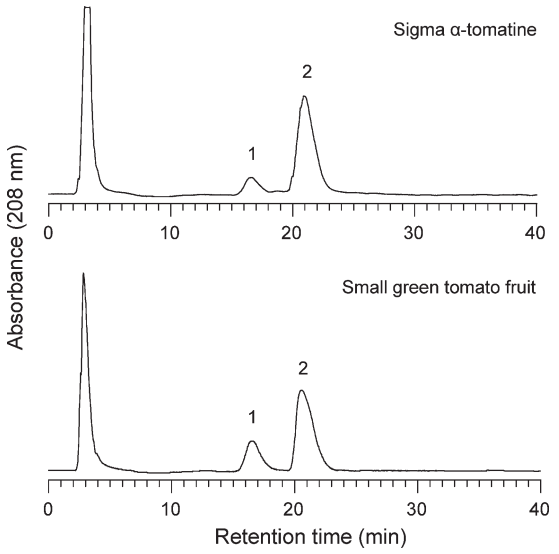

Figure 2. HPLC chromatograms of standard Sigma tomatine and an extract from a small green tomato: column, Inertsil NH_2 (5 μm, 4.0 × 250 mm); column temperature, 30 °C; mobile phase, acetonitrile/20 mM KH_2PO_4 (23:77, v/v); flow rate, 1 mL/min. Peaks: 1, dehydrotomatine; 2, α-tomatine.

of α-tomatine and dehydrotomatine (**Figures 1** and **2**) (*14*, *17*). The structure of dehydrotomatine is different from that of α-tomatine, in that the former molecule has a double bond in the steroidal ring B of the aglycone. Note that both tomato glycoalkaloids have the same tetrasaccharide side chain, lycotetraose. α-Tomatine has lycotetraose attached to the aglycone tomatidine, whereas dehydrotomatine has lycotetraose attached to the aglycone tomatidenol.

Figure 1 also shows that hydrolytic removal of the sugar side chains from dehydrotomatine and α-tomatine results in the formation of the aglycones tomatidenol and tomatidine, respectively. **Figure 2** shows that dehydrotomatine eluted from the HPLC column at 17.5 min, well separated from α-tomatine's elution time of 21.0 min.

Glycoalkaloids in Tomatoes. Table 1 lists the tomatoes used in this study, including descriptive data such as size and yield of extracted precipitate. **Table 2** shows the content of both dehydrotomatine and α-tomatine of these tomatoes per unit of fresh weight, as percent of the precipitate, and per unit fruit. **Table 2** shows that (a) the dehydrotomatine content of the six green tomatoes (in mg/100 g of fresh wt) ranges from 0.89 (sample 3) to 8.05 (sample 1), a 9.0-fold variation from lowest to highest value; (b) the corresponding range for α-tomatine is from 5.75 (sample 3) to 31.40 (sample 1), a 5.5-fold variation; (c) the sums of the concentrations of the two glycoalkaloids range from 6.64 (sample 3) to 39.45 (sample 1), a 5.9-fold variation; and (d) the total glycoalkaloid content per tomato fruit (in μg) ranges from 434.0 (sample 1) to 1110.4 (sample 4), a 2.56-fold for variation. Note that samples 3 and 1 are the same variety, but at different stages of ripeness (7.6 vs 1.1 g/fruit, respectively). It is well-known that tomatine decreases during the ripening process (*18*). The cited data show that both individual and total amounts of glycoalkaloids in green tomatoes vary widely, but less so when calculated in terms of concentration per fruit. Samples 1−4 are the same variety at different stages of ripeness. **Table 2** shows that although the glycoalkaloid content per unit weight of fruit decreases as the fruit grows, the values per unit fruit remain about the same, until the fruit turns to a red color, at which time the tomatine was completely degraded.

The data on the ammonia precipitates in **Table 2** show a large variation in both yield and glycoalkaloid content. We expected that the precipitate would largely consist of glycoalkaloids, as appears to be the case for sample 5. However, although the red tomatoes contained measurable amounts of precipitate, these precipitates contained no detectable amounts of α-tomatine or dehydrotomatine. Surprisingly, green tomato sample 1 yielded a high amount of precipitate, which contained only 8% tomatine. This sample was also the smallest of the tomatoes. The Spearman statistical correlation between tomato size and precipitate yield per fresh weight was significant at (−0.80). The correlation between tomato size and glycoalkaloid content per unit of fresh weight of fruit was even greater at (−0.87). When we rank the maturity of the fruits by taking the percentage of the projected final weight (average weight of a typical ripe fruit of the given variety), the correlation to glycoalkaloid content per unit of fresh weight of fruit equals (−0.91). It appears that size, maturity, and glycoalkaloid content are well correlated.

Inhibition of Cell Growth. The widely used MTT assay measures the decrease in mitochondrial activity of cells, which in turn may reflect a decrease in cell proliferation (*10*) or cell viability. **Tables 3** and **4** show inhibitory effects in terms of IC_{50} values against one normal Chang liver cell line and four cancer cell lines (AGS stomach, HepG2 liver, HT-29 colon, and MCF-7 breast) by nine tomato extracts, two glycoalkaloids (dehydrotomatine and α-tomatine) and two aglycones (tomatidenol and tomatidine).

Table 3 shows that all of the cell lines were inhibited by pure α-tomatine. Although the structure of dehydrotomatine is similar to that of α-tomatine, dehydrotomatine induced weak inhibition compared to that observed with α-tomatine. Tomatidenol inhibited the HT-29 cell line at moderate levels, but had no effect on the other cell lines. Tomatidine had a weak inhibitory effect on the Chang, HepG2, and MCF-7 cell lines, and no effect on the other cell lines.

Figure 3 shows a scatter plot that relates the tomatine levels of the green tomato extracts to cell inhibition. The plot shows that the Chang, AGS, and HepG2 cells were rapidly inhibited at low α-tomatine concentrations. Somewhat higher concentrations of α-tomatine were needed to consistently inactivate the HT-29 and

Table 2. Dehydrotomatine and α-Tomatine Contents of Fresh Tomatoes Listed in **Table 1**

tomato sample	dehydrotomatine (mg/100 g)	α-tomatine (mg/100 g)	sum in ammonia precipitate (dehydrotomatine + α-tomatine) (mg/100 g)	sum in ammonia precipitate[a] (wt %)	dehydrotomatine + α-tomatine (μg/tomato fruit)	other components in precipitate[b] (mg/100 g of fruit)
1	8.05 ± 1.49	31.40 ± 1.97	39.5	8	434	455
2	2.54 ± 0.70	10.80 ± 0.69	13.3	57	547	10
3	0.89 ± 0.04	5.75 ± 0.29	6.6	31	504.6	15
4	nd[c]	nd	nd	0	nd	16
5	1.30 ± 0.06	8.30 ± 0.07	9.6	110	604.8	0
6	4.80 ± 0.20	11.53 ± 1.11	16.3	74	1110.4	6
7	nd	nd	nd	0	nd	14
8	1.74 ± 0.04	9.36 ± 0.32	11.1	30	876.9	26
9	nd	nd	nd	0	nd	8

[a] Sum of α-tomatine and dehydrotomatine divided by total precipitate yield, shown in **Table 1**. [b] Weight of precipitate minus weight of dehydrotomatine + α-tomatine. [c] nd, not detected.

Table 3. Inhibition (IC_{50}, Micrograms per Milliter) of Two Glycoalkaloids (α-Tomatine and Dehydrotomatine) and Two Aglycones (Tomatidenol and Tomatidine) against Chang, AGS, HepG2, HT-29, and MCF-7 Cells Determined by the MTT Assay

cell line	α-tomatine	dehydrotomatine	tomatidenol	tomatidine
Chang	0.21	341	nd[a,b]	253
AGS	0.03	578	nd[b]	nd[b]
HepG2	43	739	nd	199
HT-29	0.03	262	94.5	nd
MCF-7	5.07	403	nd	287

[a] nd, IC_{50} not determined. Growth inhibition not responsive at concentration tested. [b] Low concentrations initially promoted growth.

MCF-7 cells. Although there was a strong correlation between α-tomatine content of the extracts and IC_{50} values (Spearman Correlation = −0.80), we also observed some activity in the red tomato extracts that contained no α-tomatine. At low concentrations, some of the extracts (particularly the ones with low wt % tomatine + dehydrotomatine in the precipitate) and pure tomatidenol and tomatidine caused an initial increase in cell growth, followed by inhibition of growth at higher concentrations (**Tables 3** and **4**, footnote *b*). Below, we examine for each cell line the inhibitory activities of the pure compounds and tomato extracts in terms of IC_{50} (the lower the number, the greater the activity).

Chang Normal Liver Cells. **Table 4** shows that the IC_{50} values for the nine tomato extracts ranged from 0.7 (sample 6) to 31.4 (sample 1) for green tomatoes. The IC_{50} for red tomatoes 4 and 7 were 152 and 153, respectively. IC_{50} values for this normal cell line were consistently higher than those for AGS and HepG2 cancer cell lines. Of the parameters defined, the IC_{50} for this cell line was most highly correlated (−0.93) with the micrograms of glycoalkaloid per unit fruit. The IC_{50} values of the extracts on the Chang cell line were highly correlated with the corresponding values on the AGS and HepG2 cell lines (0.95 and 0.88, respectively).

AGS Stomach Cancer Cells. **Table 4** shows that the IC_{50} values for the nine tomato extracts ranged from 0.3 (ample 6) to 11.4 (sample 1) for green tomatoes. The red tomato extract from sample 7 moderately inhibited the cells, whereas the other two red tomatoes were inactive. The IC_{50} value for α-tomatine of 0.03 was exceptionally low, showing that these cells are highly susceptible to inhibition by this glycoalkaloid. Tomatidenol and tomatidine were inactive against this cell line. The IC_{50} values for this cell line was also highly correlated (−0.93) with the micrograms of glycoalkaloid per unit fruit.

HepG2 Liver Cancer Cells. **Table 4** shows that the IC_{50} values for the six green tomato extracts ranged from 0.2 (sample 6) to 12.3 (sample 1). The red tomato extract from sample 9 was moderately inhibitory, and the other two red tomatoes were inactive. The IC_{50} value for α-tomatine of 43 is about 200 times higher (the activity is lower) than that observed with the AGS cells. Tomatidine was weakly active against this cell line and tomatidenol, inactive. These data show that HepG2 liver cancer cells are highly susceptible to inhibition by some of the green tomato extracts as well as the pure compounds. The IC_{50} for this cell line was also highly correlated (−0.95) with the micrograms of glycoalkaloid per unit fruit.

HT-29 Colon Cancer Cells. **Table 4** shows that the IC_{50} values for the nine tomato extracts ranged from < 0.1 (samples 2 and 5) to 170 (sample 1) for green tomatoes and from 50 (sample 7) to inactive (sample 4) for the red tomatoes. α-Tomatine was highly active against this cell line, similar in activity to that observed against the AGS cell line. Results for this cell line were more variable than for the others. For example, the green extract sample 3, which was highly active in the Chang, AGS, and HepG2 lines, showed no inhibition, whereas the red extract sample 7, which was weakly active or inactive in those same lines, showed moderate inhibition. This cell line was the only one to respond to tomatidenol and had the lowest dehydrotomatine IC_{50} of the five evaluated cell lines. We found no statistical correlation between the IC_{50} values of these cells and any of the other measured parameters. It should also be noted that this cell line was strongly inhibited by extract sample 5, which consisted of nearly 100% glycoalkaloid.

MCF-7 Breast Cancer Cells. **Table 4** shows that the IC_{50} values for the nine tomato extracts ranged from < 0.1 (sample 5) to 377 (sample 1) for the green tomatoes and from 40.6 (sample 7) to inactive (sample 4) for the red tomatoes. The IC_{50} for this cell line was most highly correlated (−0.73) with the percent of glycoalkaloid in the precipitate. The IC_{50} values for MCF-7 and HT-29 correlate well (0.88) with each other, but there was no significant correlation with the other three cell lines.

DISCUSSION

The tabular data and the figures show that (a) the green tomato extracts were active against all cancer cell lines; (b) the red tomato extracts exhibited low and variable activities; (c) pure α-tomatine was highly active against all cancer cells; (d) dehydrotomatine exhibited low activities against all cell lines; and (e) the aglycones tomatidine and tomatidenol showed moderate activity against only some of the cell lines.

With some exceptions, the data also show that cell growth inhibition correlates with α-tomatine content of the extracts. The green tomato precipitates are more inhibitory than the red ones. We do not know whether other components in the extracts may be responsible for variable results, perhaps even stimulating cell growth. Such components could include triterpenoid glycosides,

Table 4. Inhibition (IC_{50}, Micrograms per Milliliter) by Nine Green and Red Tomato Extracts against Chang, AGS, HepG2, HT-29, and MCF-7 Cells Determined by the MTT Assay

	tomato extract								
cell line	1 (Sancheri, small green)	2 (Sancheri, medium green)	3 (Sancheri, large green)	4 (Sancheri, ripe red)	5 (Yoyo, green)	6 (Chobok Power, small green)	7 (Chobok Power, ripe red)	8 (Rokusanmaru, small green)	9 (Rokusanmaru, ripe red)
Chang	31.4	4.8	1.7	152	2.9	0.7	153[b]	1.4	nd[a,b]
AGS	11.4	2	1.4	nd	1.7	0.3	135[b]	1.2	nd[b]
HepG2	12.3	3.2	1	nd	0.8	0.2	nd[b]	0.9	148[b]
HT-29	170[b]	<0.1	nd[b]	nd[b]	<0.1	5.4	50	1.3	163[b]
MCF-7	377[b]	0.33	241[b]	nd[b]	<0.1	18.7	40.6	9	265[b]

[a] nd, IC_{50} not determined. Growth inhibition not responsive at concentration tested. [b] Low concentrations initially promoted growth.

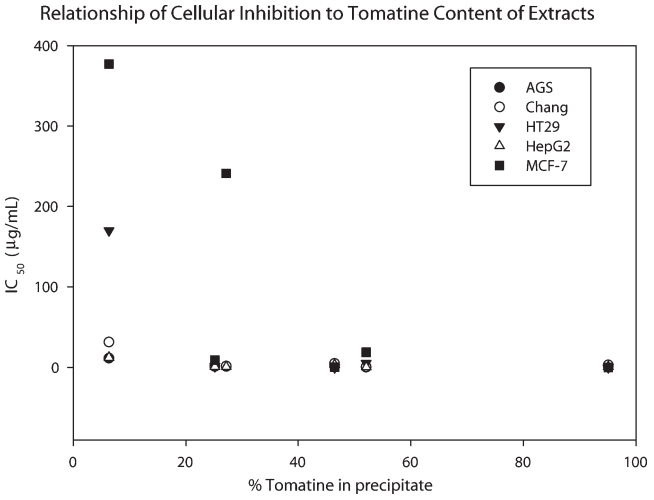

Figure 3. Scatter plot of the α-tomatine content (%) of the tomato extracts versus inhibition (IC_{50}) of five cell lines.

recently reported to be present in large amounts in immature tomatoes (*19*), and esculentin, a glycoalkaloid present in red, but not in green, tomatoes (*20*).

Chemoprevention Mechanisms. Mechanisms responsible for the anticarcinogenic effects of tomato glycoalkaloids differ from mechanisms proposed for lycopene present in red tomatoes. To place our findings in perspective, we will summarize reported biological effects of tomatine and tomatidine.

Because binding of tomatine to cholesterol may be relevant to the mechanism of inhibition of carcinogenesis, we also briefly summarize some of the reported findings that explored this possibility. Tomatine alone and tomatine-rich green tomato diets reduced both dietary cholesterol bioavailability and endogenous cholesterol (*21*, *22*). Despite its ability to disrupt cell membranes in vitro (*23*), orally consumed tomatine does not induce toxicity, presumably because it forms an insoluble complex with cholesterol in the digestive tract, which is then eliminated in the feces. Tomatine inhibited active transport by increasing the general permeability of membranes of the surface of averted rat jejunal sacs (*24*) and removed cholesterol from mucosal cells as well as the output of cholesterol into the lymph (*25*). Unlike tomatine, tomatidine did not induce cell membrane disruptions in fungi and yeasts (*26*). Tomatine and tomatidine exhibited weak inhibition of the Hedgehog (Hh) signaling pathway in the embryonic Zebra fish developmental assay (*27*). Such inhibition appears to be associated with developmental defects.

Physicochemical studies visually demonstrated the morphological changes observed during the formation of 1:1 tomatine−cholesterol complexes that aggregate at the water−air interface (*28*, *29*). Complex formation involves side-by-side stacking of one tomatine next to one sterol molecule.

The cited observations suggest that the mechanism(s) of the chemopreventive effect of tomatine may be the result of multiple molecular events including formation of complexes with cholesterol, potentiation of the immune system, and direct destruction of cancer cells via disruption of cell membranes, reviewed in ref (*8*). Because tomatine induced antigen-specific cellular immunity in mice, it possesses remarkable potential as a vaccine adjuvant for infectious diseases as well as for cancer immunotherapy (*3*). By stimulating the immune system, tomatine-rich green tomatoes may also protect against lethal infections by foodborne pathogens such as *Salmonella*, as has been reported for potato glycoalkaloids (*30*).

Significance for Tomato-Based Diets. The present study was designed to find out whether tomatine-rich green tomatoes (*18*) have the potential to ameliorate carcinogenesis apart from the effects of lycopene, present in red tomatoes. To achieve this objective, we isolated glycoalkaloid-containing fractions from extracts of six green and three red tomato samples from four varieties and then determined the ability of solutions of the isolates to inhibit growth of human cancer cells. For comparison, we also evaluated two pure tomato glycoalkaloids and their aglycones.

Red tomatoes contain numerous health-promoting ingredients, including antioxidative carotenoids (lycopene, β-carotene, lutein), anthocyanins, phenolic compounds (caffeic acid, chlorogenic acid), and flavonoids (kaempferol, naringenin, quercetin) as well as the vitamins A, B, and C (*31*) and lectins (*32*). Unlike green tomatoes, red tomatoes contain high levels of lycopene and very low amounts of glycoalkaloids (*14*, *18*, *33*). A review of most clinical trials with fresh and processed red tomato products suggests a synergistic action of lycopene with other nutrients in lowering biomarkers of oxidative stress and carcinogenesis (*34*). In addition, on the basis of the observed suppression of COX-2 enzymes by tomato phenolics (chlorogenic acid, caffeic acid, myricetin, naringenin, condensed tannins), it is likely that these tomato ingredients also contribute to the chemoprevention of cancer (*35*). We did not analyze for any of these red tomato ingredients in the extracts or supernatants of the ammonia precipitates.

Because our data show that tomatine also inhibited growth of normal liver cells, a key consideration for the use of pure tomatine and of high-tomatine tomatoes in cancer prevention and treatment should be the ratio of effective preventive or therapeutic to toxic dose. As mentioned earlier, no apparent toxic effects were noted in rainbow trout following oral consumption for up to 9 months. The nontoxicity of tomatine is reinforced by the fact that Peruvians consume without deleterious effects high-tomatine red tomatoes that have evolved not to degrade tomatine during maturation (*8*, *36*, *37*). However, it has been suggested that the Peruvians may have adapted to the consumption of high-tomatine tomatoes by developing a mechanism to metabolize the tomato glycoalkaloids. It may also be possible to directly deliver tomatine to diseased cells without affecting normal ones (*38*).

The cited information on anticarcinogenic and other beneficial effects of tomatine suggests the desirability of developing new varieties of tomatine-rich red tomatoes. These tomatoes would contain two classes of anticarcinogenic compounds: (a) tomato glycoalkaloids that stimulate the immune system, form complexes with cholesterol, and disrupt membranes of cancer cells; and (b) antioxidative carotenoids and phenolic compounds that may act by suppressing free radicals that damage DNA by mechanisms discussed elsewhere (*39*). This objective could be accomplished by breeding high-tomatine tomatoes into commercial lines or by suppressing the genes that govern the formation of enzymes that degrade tomatine during postharvest ripening of green to red tomatoes. Because they operate by different mechanisms, both lycopene and tomatine of such newly developed red tomatoes may act additively or synergistically against human disease.

As noted elsewhere (*40*, *41*), plant breeders could also create high-tomatine potatoes by crossing high-tomatine-containing accessions of the wild potato *Solanum acaule* with cultivated *Solanum tuberosum* varieties. In the meantime, consumers may benefit from eating both lycopene-rich red and tomatine-rich freshly harvested or commercially available green tomatoes.

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LITERATURE CITED

(1) Lee, K. R.; Kozukue, N.; Han, J. S.; Park, J. H.; Chang, E. Y.; Baek, E. J.; Chang, J. S.; Friedman, M. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. *J. Agric. Food Chem.* **2004**, *52*, 2832–2839.

(2) Friedman, M.; McQuistan, T.; Hendricks, J. D.; Pereira, C.; Bailey, G. S. Protective effect of dietary tomatine against dibenzo[*a*,*l*]pyrene (DBP)-induced liver and stomach tumors in rainbow trout. *Mol. Nutr. Food Res.* **2007**, *51*, 1485–1491.

(3) Morrow, W. J. W.; Yang, Y.-W.; Sheikh, N. A. Immunobiology of the tomatine adjuvant. *Vaccine* **2004**, *22*, 2380–2384.

(4) Chiu, F. L.; Lin, J. K. Tomatidine inhibits iNOS and COX-2 through suppression of NF-κB and JNK pathways in LPS-stimulated mouse macrophages. *FEBS Lett.* **2008**, *582*, 2407–2412.

(5) Lavie, Y.; Harel-Orbital, T.; Gaffield, W.; Liscovitch, M. Inhibitory effect of steroidal alkaloids on drug transport and multidrug resistance in human cancer cells. *Anticancer Res.* **2001**, *21*, 1189–1194.

(6) Punjabi, S.; Cook, L. J.; Kersey, P.; Marks, R.; Cerio, R. Solasodine glycoalkaloids: a novel topical therapy for basal cell carcinoma. A double-blind, randomized, placebo-controlled, parallel group, multicenter study. *Int. J. Dermatol.* **2008**, *47*, 78–82.

(7) Thorne, H. V.; Clarke, G. F.; Skuce, R. The inactivation of herpes simplex virus by some glycoalkaloids. *Antiviral Res.* **1985**, *5*, 335–343.

(8) Friedman, M. Tomato glycoalkaloids: role in the plant and in the diet. *J. Agric. Food Chem.* **2002**, *50*, 5751–5780.

(9) Simons, V.; Morrissey, J. P.; Latijnhouwers, M.; Csukai, M.; Cleaver, A.; Yarrow, C.; Osbourn, A. Dual effects of plant steroidal alkaloids on *Saccharomyces cerevisiae*. *Antimicrob. Agents Chemother.* **2006**, *50*, 2732–2740.

(10) Friedman, M.; Lee, K. R.; Kim, H. J.; Lee, I. S.; Kozukue, N. Anticarcinogenic effects of glycoalkaloids from potatoes against human cervical, liver, lymphoma, and stomach cancer cells. *J. Agric. Food Chem.* **2005**, *53*, 6162–6169.

(11) Friedman, M.; Kozukue, N.; Harden, L. A. Structure of the tomato glycoalkaloid tomatidenol-3-β-lycotetraose (dehydrotomatine). *J. Agric. Food Chem.* **1997**, *45*, 1541–1547.

(12) Friedman, M.; McDonald, G. M. Acid-catalyzed partial hydrolysis of carbohydrate groups of the potato glycoalkaloids α-chaconine in alcoholic solutions. *J. Agric. Food Chem.* **1995**, *43*, 1501–1506.

(13) Friedman, M.; McDonald, G.; Haddon, W. F. Kinetics of acid-catalyzed hydrolysis of carbohydrate groups of potato glycoalkaloids α-chaconine and α-solanine. *J. Agric. Food Chem.* **1993**, *41*, 1397–1406.

(14) Kozukue, N.; Han, J. S.; Lee, K. R.; Friedman, M. Dehydrotomatine and α-tomatine content in tomato fruits and vegetative plant tissues. *J. Agric. Food Chem.* **2004**, *52*, 2079–2083.

(15) Friedman, M.; Mackey, B. E.; Kim, H.-J.; Lee, I.-S.; Lee, K.-R.; Lee, S.-U.; Kozukue, E.; Kozukue, N. Structure−activity relationships of tea compounds against human cancer cells. *J. Agric. Food Chem.* **2007**, *55*, 243–253.

(16) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res.* **1988**, *48*, 589–601.

(17) Friedman, M.; Levin, C. E. Dehydrotomatine content in tomatoes. *J. Agric. Food Chem.* **1998**, *46*, 4571–4576.

(18) Kozukue, N.; Friedman, M. Tomatine, chlorophyll, β-carotene and lycopene content in tomatoes during growth and maturation. *J. Sci. Food Agric.* **2003**, *83*, 195–200.

(19) Yamanaka, T.; Vincken, J. P.; de Waard, P.; Sanders, M.; Takada, N.; Gruppen, H. Isolation, characterization, and surfactant properties of the major triterpenoid glycosides from unripe tomato fruits. *J. Agric. Food Chem.* **2008**, *56*, 11432–11440.

(20) Iijima, Y.; Fujiwara, Y.; Tokita, T.; Ikeda, T.; Nohara, T.; Aoki, K.; Shibata, D. Involvement of ethylene in the accumulation of esculeoside A during fruit ripening of tomato (*Solanum lycopersicum*). *J. Agric. Food Chem.* **2009**, *57*, 3247–3252.

(21) Friedman, M.; Fitch, T. E.; Yokoyama, W. E. Lowering of plasma LDL cholesterol in hamsters by the tomato glycoalkaloid tomatine. *Food Chem. Toxicol.* **2000**, *38*, 549–553.

(22) Friedman, M.; Fitch, T. E.; Levin, C. E.; Yokoyama, W. H. Feeding tomatoes to hamsters reduces their plasma low-density lipoprotein cholesterol and triglycerides. *J. Food Sci.* **2000**, *65*, 897–900.

(23) Blankemeyer, J. T.; White, J. B.; Stringer, B. K.; Friedman, M. Effect of α-tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin. *Food Chem. Toxicol.* **1997**, *35*, 639–646.

(24) Gee, J. M.; Wortley, G. M.; Johnson, I. T.; Price, K. R.; Rutten, A. A. J. J. L.; Houben, G. F.; Penninks, A. H. Effects of saponins and glycoalkaloids on the permeability and viability of mammalian intestinal cells and on the integrity of tissue preparations in vitro. *Toxicol. in Vitro* **1996**, *10*, 117–128.

(25) Blumer, A.; Watt, S. M.; Simmonds, W. J. Homeostasis of mucosal cholesterol in the small intestine of the rat. *Lipids* **1984**, *19*, 721–727.

(26) Simons, H. L.; Severs, N. J. Plasmalemmal vesicles and the effects of sterol-binding agents in rabbit aortic endothelium. *J. Cell Sci.* **1986**, *83*, 141–153.

(27) Lipinski, R. J.; Dengler, E.; Kiehn, M.; Peterson, R. E.; Bushman, W. Identification and characterization of several dietary alkaloids as weak inhibitors of Hedgehog signaling. *Toxicol. Sci.* **2007**, *100*, 456–463.

(28) Stine, K. J.; Hercules, R. K.; Duff, J. D.; Walker, B. W. Interaction of the glycoalkaloid tomatine with DMPC and sterol monolayers studied by surface pressure measurements and Brewster angle microscopy. *J. Phys. Chem. B* **2006**, *110*, 22220–22229.

(29) Walker, B. W.; Manhanke, N.; Stine, K. Comparison of the interaction of tomatine with mixed monolayers containing phospholipid, egg sphingomyelin, and sterols. *Biochim. Biophys. Acta Biomembr.* **2008**, *1778*, 2244–2257.

(30) Gubarev, M. I.; Enioutina, E. Y.; Taylor, J. L.; Visic, D. M.; Daynes, R. A. Plant derived glycoalkaloids protect mice against lethal infection with *Salmonella typhimurium*. *Phytother. Res.* **1998**, *12*, 79–88.

(31) Dorais, M.; Ehret, D. L.; Papadopoulos, A. P. Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. *Phytochem. Rev.* **2008**, *7*, 231–250.

(32) De Mejía, E. G.; Prisecaru, V. I. Lectins as bioactive plant proteins: a potential in cancer treatment. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 425–445.

(33) Friedman, M.; Levin, C. E. α-Tomatine content in tomato and tomato products determined by HPLC with pulsed amperometric detection. *J. Agric. Food Chem.* **1995**, *43*, 1507–1511.
(34) Basu, A.; Imrhan, V. Tomatoes versus lycopene in oxidative stress and carcinogenesis: conclusions from clinical trials. *Eur. J. Clin. Nutr.* **2007**, *61*, 295–303.
(35) Shen, Y. C.; Chen, S. L.; Zhuang, S. R.; Wang, C. K. Contribution of tomato phenolics to suppression of COX-2 expression in κB cells. *J. Food Sci.* **2008**, *73*, C1–10.
(36) Rick, C. M.; Uhlig, J. W.; Jones, A. D. High α-tomatine content in ripe fruit of Andean *Lycopersicon esculentum* var. *cerasiforme*: developmental and genetic aspects. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12877–12881.
(37) Rick, C. M. The tomato. *Sci. Am.* **1978**, *239*, 77–87.
(38) Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol.* **2007**, *2*, 751–760.
(39) Tang, F. Y.; Shih, C. J.; Cheng, L. H.; Ho, H. J.; Chen, H. J. Lycopene inhibits growth of human colon cancer cells via suppression of the Akt signaling pathway. *Mol. Nutr. Food Res.* **2008**, *52*, 646–654.
(40) Kozukue, N.; Yoon, K.-S.; Byun, G.-I.; Misoo, S.; Levin, C. E.; Friedman, M. Distribution of glycoalkaloids in potato tubers of 59 accessions of two wild and five cultivated *Solanum* species. *J. Agric. Food Chem.* **2008**, *56*, 11920–11928.
(41) Kozukue, N.; Misoo, S.; Yamada, T.; Kamijima, O.; Friedman, M. Inheritance of morphological characters and glycoalkaloids in potatoes of somatic hybrids between dihaploid *Solanum acaule* and tetraploid *Solanum tuberosum*. *J. Agric. Food Chem.* **1999**, *47*, 4478–4483.

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