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Effect of feeding solanidine, solasodine and tomatidine to non-pregnant and pregnant mice

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Abstract

The aglycone forms of three steroidal glycoalkaloids—solanidine (derived by hydrolytic removal of the carbohydrate side chain from the potato glycoalkaloids α-chaconine and α-solanine), solasodine (derived from solasonine in eggplants) and tomatidine (derived from α-tomatine in tomatoes)—were evaluated for their effects on liver weight increase (hepatomegaly) in non-pregnant and pregnant mice and on fecundity in pregnant mice fed for 14 days on a diet containing 2.4 mmol/kg of aglycone. In non-pregnant mice, observed ratios of % liver weights to body weights (%LW/BWs) were significantly greater than those of the control values as follows (all values in % vs matched controls ± S.D.): solanidine, 25.5 ± 13.2; solasodine 16.8 ± 12.0; and tomatidine, 6.0 ± 7.1. The corresponding increases in pregnant mice were: solanidine, 5.3 ± 10.7; solasodine, 33.1 ± 15.1; tomatidine, 8.4 ± 9.1. For pregnant mice (a) body weight gains were less with the algycones than with controls: solanidine, −36.1 ± 14.5; solasodine, −17.9 ± 14.3; tomatidine, −11.9 ± 18.1; (b) litter weights were less than controls: solanidine, −27.0 ± 17.1; solasodine, −15.5 ± 16.8; tomatidine, no difference; (c) the %LTW/BW ratio was less than that of the controls and was significant only for solasodine, −8.7 ± 13.7; and (d) the average weight of the fetuses was less than the controls: solanidine, −11.2 ± 15.2; solasodine, −11.4 ± 9.4; tomatidine, no difference. Abortion of fetuses occurred in five of 24 pregnant mice on the solanidine and none on the other diets. To obtain evidence for possible mechanisms of the observed in vivo effects, the four glycoalkaloids (α-chaconine, α-solanine, solasonine and α-tomatine) mentioned above and the aglycones solanidine and tomatidine were also evaluated in in vitro assays for estrogenic activity. Only solanidine at 10 μM concentration exhibited an increase in the MCF-7 human breast cancer cell proliferation assay. Generally, the biological effects of solanidine differ from those of the parent potato glycoalkaloids. Possible mechanisms of these effects and the implication of the results for food safety and plant physiology are discussed.
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Keywords: Feeding studies; Hepatomegaly; Pregnant mice; Solanidine; Solasodine; Tomatidine

1. Introduction

In order to improve the nutritional value and safety of some plant foods consumed by humans, a need exists to delineate the biological potencies and mechanisms of action of structurally different potentially toxic glycoalkaloids and their steroidal aglycone metabolites. Glycoalkaloid-containing potatoes and potato products are widely consumed (Friedman and Dao, 1992). The daily per capita intake of potatoes in Sweden is 300 g (Slanina, 1990), in the United States it is about 170 g (Friedman et al., 1996), and in the United Kingdom, 140 g (Hopkins, 1995). The cited amount for the UK is estimated to contain 14 mg of glycoalkaloids. Although the glycoalkaloid concentration of most commercial potatoes is usually below a suggested safety guideline of 200 mg/kg of fresh potatoes, the concentration can increase substantially on exposure of potatoes to light or as a result of mechanical injury including peeling and slicing (Dao and Friedman, 1994). Glycoalkaloids are largely unaffected by food processing including baking, cooking and frying (Friedman and McDonald, 1999). Moreover, the potato glycoalkaloid α-chaconine is more toxic than α-solanine, certain ratios of the two glycoalkaloids may act synergistically, and their ratios may vary in different potato varieties. The estimated highest

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; ER, estrogen receptor; %LW/BWs,% liver weights to body weights; ODC, ornithine decarboxylase.

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safe level of total potato glycoalkaloids for human consumption is about 1 mg/kg body weight, the acute toxic dose is estimated to be at 1.75 mg/kg body weight, and a lethal dose may be 3–6 mg/kg body weight.

Glycoalkaloids appear to be more toxic to humans than to rodents (reviewed in Morris and Lee, 1984; Friedman and McDonald, 1997). The reported toxicity of glycoalkaloids a due to anticholinesterase effects on the central nervous system (Duan, 1995; Nigg et al., 1996) and disruption of cell membranes (Blankemeyer et al., 1992, 1997, 1998). Symptoms of glycoalkaloid toxicity experienced by humans include colic pain in the abdomen and stomach, gastroenteritis, diarrhea, vomiting, burning sensation about the lips and mouth, hot skin, fever, rapid pulse and headache. Another undesirable effect may be glycoalkaloid- and/or aglycone-induced craniofacial malformations in hamsters (Gaffield and Keeler, 1993, 1996) and multi-organ malformations in frog embryos (Friedman et al., 1991, 1992) at levels similar to those present in some potato varieties. Folic acid, glucose 6-phosphate and NADP protected frog embryos both against disruption of cell membranes and glycoalkaloid-induced teratogenicity (Rayburn et al., 1995a; Friedman et al., 1997). Glucose 6-phosphate and NADP also inhibited glycoalkaloid-induced lysis of human erythrocytes (Roddick and Leonard, 1999).

The steroidal aglycones (Fig. 1) are formed after removal of the carbohydrate side-chain from the glycoalkaloids. Solanidine is derived from the potato glycoalkaloids α-chaconine and α-solanine, tomatidine from the tomato glycoalkaloid α-tomatine, and solasodine from the eggplant glycolkaloids solamargine and solasonine. The aglycones may exist in the plant as degradation products of glycoalkaloids (Zitnak, 1961; Bushway et al., 1988). Generally, the exposure of humans and animals to the aglycones occurs when glycoalkaloids are hydrolyzed in vivo either non-enzymatically (e.g. acid hydrolysis in the stomach) or enzymatically (e.g bacterial glycosidases in the gastrointestinal tract). Concern over physiological effects of the aglycones is based on the fact that plasma levels of solanidine in humans parallel the amount of potatoes consumed (Claringbold et al., 1982; Matthew et al., 1983; Harvey et al., 1985a,b, 1986; Hellenas et al., 1992), their ability to affect steroid hormone physiology (Dixit et al., 1989), their induction of liver weight increases (hepatomegaly) in mice (Friedman et al., 1996), and their possible accumulation by newly introduced varieties of potatoes (Moehs et al., 1997). These considerations, as well as the sparsity of studies on the biological effects of orally fed glycoalkaloids and metabolites, suggest the need for nutritional and safety studies of both glycoalkaloids and the aglycones (Hopkins, 1995).

Glycoalkaloids and aglycones may also have beneficial effects. Glycoalkaloids are reported to inactivate the *Herpes simplex*, *Herpes zoster*, and *Herpex genitalis* viruses in humans (Chataing et al., 1997), to protect mice against infection by *Salmonella typhimurium* (Gubarev et al., 1998), to enhance the duration of action of anesthetics, which act by inhibiting acetylcholinesterase (McGehee et al., 2000), and to potentiate the immune response of vaccines in mice (Rajananthanan et al., 2000). Solasodine may protect against skin cancer (Cham, 1994) and tomatidine may benefit cancer chemotherapy by inhibiting multidrug resistance in human cancer cells (Lavie et al., 2001).

As part of an effort designed to improve food safety through identification and reduction of the content of the most toxic alkaloids in plant foods using safety evaluation in parallel with plant genetic studies, we previously evaluated the ability of dietary steroidal aglycones to induce hepatomegaly in non-pregnant mice at the 2.4 mmol/kg diet level (Friedman et al., 1996). The present study extends and complements the earlier investigation by comparing effects in non-pregnant to pregnant mice. In this study, we looked for differences in body weight and liver weight in non-pregnant and pregnant mice, differences in litter size and litter weight and fetal weight in pregnant mice following dietary exposure to 2.4 mmol/kg of solasodine, tomatidine or solanidine for 14 days. This dose is based approximately on the amount of solanidine that can be produced after consumption of the previously mentioned maximum safe level of potato glycoalkaloids. To obtain evidence for a possible mechanism for the observed in vivo effects we also evaluated the four glycoalkaloids and two aglycones in several estrogen receptor (ER) in vitro assays.

2. Materials and methods

2.1. Materials

Time-mated mice and Simonsen Custom Chow #7 were obtained from Simonsen Laboratories (Gilroy, CA, USA). The test compounds were obtained from Sigma (St. Louis, MO, USA). They tested as chromatographically pure by HPLC (Friedman and Levin, 1992).

2.2. Diets

Mice were fed pelleted or ground Simonsen Custom Chow #7 diet with or without test compound. Diets were stored in a cold room for 3 weeks. They were not tested for stability or purity. The % of test compound was based on equivalent mmol/kg diet. For solasodine, for example, 0.1% (1 g per kg diet) corresponds to [(1 g/416 (mol. wt))×1000 (mmol/m)] or 2.4 mmol/kg diet. To prepare the diet, all compounds were each triturated into a fine powder, weighed into one half of the total diet, and mixed for 10 min in a Twin Shell Dry Blender (Patterson Kelly Co., East Strousber, PA, USA). The remaining diet was then added and mixed for 10 min.

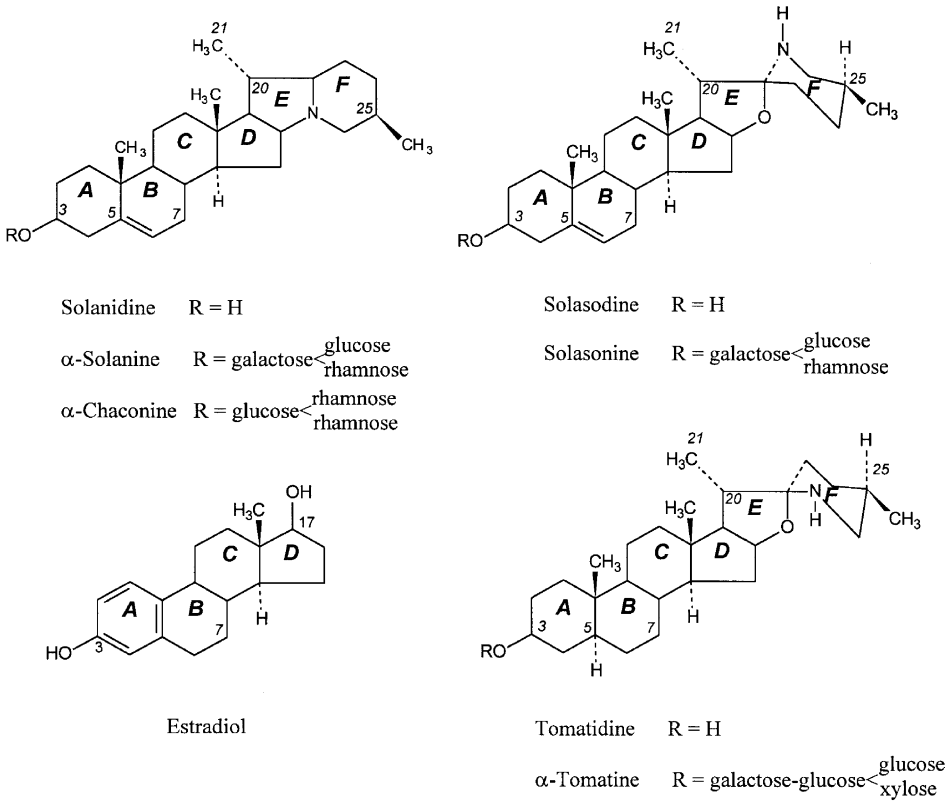

Fig. 1. Structures of aglycones, glycoalkaloids, and estradiol.

In a previous study (Friedman et al., 1996), dose–response data demonstrated that increased liver weights (hepatomegaly) were observed in adult female Swiss–Webster mice treated with up to 2.4 mmol/kg diets of solanidine, solasodine or tomatidine for 14 days. Significant percent increases in %LW/BW for these groups were: solasodine, 25.5%; solanidine, 21%. This concentration of alkaloids in the diet and the 14-day treatment period was therefore selected for comparison of effects in non-pregnant and pregnant mice.

2.3. Feeding studies

The study protocol was approved by our Animal Care and Use Committee, which follows the guidelines on the humane use of laboratory animals (NRC, 1996). For each experiment, 20 timed-pregnant Swiss Webster mice were obtained from Simonsen Laboratories (Gilroy, CA, USA) (1–2 days' gestation, 25–36 g). Sixteen 2-week experiments were run for a total of 320 animals received. The 20 mice were housed individually and randomized at the time of arrival. They were housed in plastic shoe-box cages with hardwood shavings for litter and were fed pelleted Simonsen Custom Chow #7. "Time-mated" mice were given a 48-h period after transport for adaptation to our animal facility. Thus, the 2-week experiments were started on day 4 of gestation.

At this time, mice were divided into two groups of 10 and fed Simonsen Custom Chow #7 or diet with test compound starting with 70 g/week in glass food-cups fitted with stainless-steel retainers. Each mouse was also weighed at this time. The mice were observed daily and litter was changed daily. An automatic timer regulated the 6 am–6 pm light/dark cycle in an animal room at 20 °C. Mice were given water ad lib. Food consumption is considered an estimate because food could be kicked out of the cup and/or contaminated with feces or urine. For unexplained reasons, the initial body weights in the tomatidine group were lower than for the other groups.

The 320 mice were used for eight experiments with solasodine diets and four each with tomatidine and solanidine diets. Data were pooled and reported in Tables 1–4 for each test diet and its matched control. At the time of necropsy (17–18 days' gestation, 2–3 days before parturition), the mice were divided into pregnant and non-pregnant groups. A non-pregnant mouse was defined as one that showed no signs of placenta or uterine horn development at the time of necropsy. Dead fetuses were observed to be pale in color, limp and 'watery'. Aborted fetuses (observed only with group treated with solanidine) expelled from the dam were found in the litter and were not in the process of resorption (Table 1). At necropsy, the mice were weighed, livers were dissected, and live fetuses were removed from the uterine horn. Livers and live fetuses were blotted with

Table 1
Acceptance of pregnant mice used in this study

Pregnant group	Litter size[a]	Accepted range[a]	Rejected	Dead
Control	12±3[b]	9–15	14[c]	10
Solasodine	11±4	7–15	11[d]	9
Control	13±3	10–16	4[e]	10
Tomatidine	12±4	8–16	3[f]	2
Control	11±4	7–15	5[g]	4
Solanidine	11±4	7–15	10[h]	16

[a] Live plus dead fetuses.
[b] Average±standard deviation.
[c] One moribund; five low litter weight; eight high litter weight.
[d] Seven low litter weight; four high litter weight.
[e] Two low litter weight; two high litter weight.
[f] Three low litter weight.
[g] Five low litter weight.
[h] One moribund; two low litter weight; two high litter weight; five abortions.

Data were reported for 95 non-pregnant and 174 pregnant animals (Tables 2–4). The acceptance of pregnant animals was based on litter size of live plus dead fetuses. We determined average litter size and standard deviations and rejected animals with low or high litter sizes outside the standard deviation (Table 1). The rejection of low litter sizes was based on dams with only one to three fetuses and with no evidence, such as dead fetuses, that litter size had been greater at time of gestation. The rejection of high litter sizes (16–19 fetuses) was based on the lower fetal weights. We thus normalized the population with respect to litter size and subjected the data pools to statistics. Pooled data for the solanidine test group was significantly lower for litter vs its matched control (Table 4). Five animals from the solanidine test group were rejected due to abortions. Three mice were moribund at the start of the experiment

Table 2
Effects of dietary solasodine, tomatidine and solanidine on body weight, liver weight and food intake in non-pregnant adult female mice treated with 2.4 mmol/kg diet for 14 days[a]

Group	Sample size (*n*)	Body wt initial (g)	Body wt final (g)	Body wt difference (g)	Food intake (g/d)	Food intake (g/day/kg/mouse)	Liver wt (g)	Liver wt/body wt (%)
Control	24	29.9±1.9	30.2±1.9	0.3±0.9	6.0±0.9	200±27	1.61±0.20	5.33±0.49
Solasodine	24	31.2±2.8	30.3±2.8	−0.9±1.6[b]	5.9±0.6	196±23	1.89±0.29[c]	6.22±0.64[c]
Control	11	32.7±2.4	32.3±2.3	−0.4±1.2	5.7±0.9	177±24	1.72±0.17	5.30±0.28
Tomatidine	14	29.6±2.5[b]	29.1±2.7[b]	−0.5±1.3	5.7±0.8	195±27	1.64±0.20	5.62±0.37[c]
Control	12	30.2±2.3	29.9±2.4	−0.3±0.8	5.9±0.8	196±23	1.50±0.17	5.00±0.27
Solanidine	10	31.1±2.6	28.4±2.3	−2.7±0.9[b]	6.1±0.9	215±44	1.78±0.19[c]	6.27±0.66[c]

[a] Listed values are means±S.D.
[b] Means which were significantly less than group control value. Statistically significant from control ($P<0.05$).
[c] Means which were significantly greater than group control value. Statistically significant from control ($P<0.05$).

Table 3
Effects of dietary solasodine, tomatidine and solanidine on body weight, liver weight and food intake in pregnant mice treated with 2.4 mmol/kg diet for 14 days[a]

Group	Sample size (*n*)	Body wt final (g)	Body wt initial (g)	Body wt difference (g)	Food intake (g/d)	Food intake (g/d/kg/mouse)	Liver wt (g)	Liver wt/body wt (%)
Control	38	52.1±4.2	30.5±2.0	21.5±3.3	6.8±0.7	164±20	2.71±0.21	5.20±0.29
Solasodine	45	48.1±4.0[b]	30.4±2.4	17.7±3.1[b]	6.2±0.9[b]	157±21	3.32±0.39[c]	6.92±0.78[c]
Control	25	57.7±4.5	33.1±2.8	24.6±3.3	7.1±0.8	157±18	2.99±0.31	5.20±0.49
Tomatidine	23	52.2±5.0[b]	30.6±1.7[b]	21.7±4.4[b]	6.9±0.6	167±19	2.94±0.29	5.64±0.48[c]
Control	23	54.3±5.7	31.0±3.5	23.3±4.4	6.6±0.6	156±17	2.85±0.26	5.26±0.26
Solanidine	19	44.4±4.4[b]	29.5±2.2	14.9±3.4[b]	6.1±0.5[b]	167±17[c]	2.45±0.24[b]	5.54±0.56[c]

[a] Listed values are means±S.D.
[b] Means which were significantly less than group control value. Statistically different from control ($P<0.05$).
[c] Means which were greater than group control value. Statistically different from control ($P<0.05$).

a paper towel and weighed. Parameters used for non-pregnant mice were: differences in body weight (final weight minus initial body weight, g); liver weight upon necropsy (g); and liver weight as a percent of body weight (%LW/BW). Additional parameters used for pregnant mice were: litter size (live fetuses); litter weight (live fetuses) (g); average fetus weight (g); and litter weight as a percent of body weight (%LTW/BW).

for reasons unknown—two in the pregnant in one in the non-pregnant groups (Table 1). The fertility of the 320 animals was 70%.

2.4. Estrogen activity assays

Estrogen activity assays were adapted from published procedures (Cover et al., 1999; Riby et al., 2000) to

Table 4
Effects of dietary solasodine, tomatidine and solanidine on dam body weight, litter size and fetus weight in pregnant female mice treated with 2.4 mmol/kg diet for 14 days[a]

Group	Sample size (*n*)	Body wt final (g)	Litter size (group average)	Litter wt (g)	Litter wt/body wt (%)	Fetus wt (average) (g)
Control	38	52.1±4.2	11.9±1.6	11.9±2.1	22.8±3.1	1.00±0.09
Solasodine	45	48.1±4.0[b]	11.4±2.2	10.0±2.1[b]	20.8±3.1[b]	0.89±0.09[b]
Control	25	57.7±4.5	13.0±1.7	13.0±2.1	22.4±2.5	0.99±0.09
Tomatidine	23	52.2±5.0[b]	12.4±2.6	13.1±3.7	24.8±5.0	1.05±0.16
Control	23	54.3±5.7	12.1±1.9	11.9±3.0	21.6±3.8	0.97±0.13
Solanidine	20	44.4±4.4[b]	10.2±2.6[b]	8.7±2.0[b]	19.4±3.4[b]	0.86±0.15[b]

[a] Listed values are means±S.D.
[b] Means which were significantly less than group control value. Statistically significant from control ($P<0.05$).

assess possible estrogenic activities of four glycoalkaloids (α-chaconine, α-solanine, tomatine and solasonine) and two aglycones (solanidine and tomatidine). The following assays were used: human MCF-7 breast cancer cell proliferation; induction of transcriptional activity in estrogen-receptor (ER) responsive reporters; and induction of ER binding by gel shift analysis.

The human breast adenocarcinoma cell line MCF-7, obtained from American Type Culture (ATTC, MD, USA), was grown as adherent monolayers in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.0 g/l glucose and 3.7 g/l sodium bicarbonate in a humidified incubator at 37 °C and 5% CO_2. Cells were passaged at approximately 80% confluence. Cultures of cells were used in subsequent experiments for fewer than 25 passages.

Before the beginning of the treatments, cells were depleted of estrogen for 7–10 days in medium composed of DMEM base without phenol red, with 4 g/l glucose, 3.7 g/l sodium bicarbonate and 5% calf serum twice stripped in dextran-coated charcoal and micro-filtered, supplemented with non-essential amino acids, 2 mM glutamine and 10 ng/ml insulin. The medium was changed every other day during the depletion period. Treatments were administered by addition of 1 μl of 1000× solution in dimethyl sulfoxide (DMSO) per ml medium. Once the treatment started, the medium was changed daily to counter possible loss of readily metabolized compounds. Cells were harvested by trypsinization and counted in a Coulter (Miami, FL, USA) particle counter.

The following experimental procedure was used with all other compounds. Estrogen-depleted MCF-7 cells were plated at a density of 10^5 cells per well in six-well plates and treated with the test compound in DMSO at the concentrations indicated, in the presence or absence of estradiol, E2 (1 nM). Duplicate aliquots of cells from individual wells were counted after 7 days. Data from three identical wells are averaged and calculated as the mean and S.D. for four separate experiments.

2.5. Statistical analysis

For statistical analyses, the test data for each compound were matched with the corresponding control diets. A simple two-sample *t*-test for each combination of test and control diet was used (Steel and Torrie, 1980). The standard deviations listed with means and sample sizes are based on non-pooled variance estimates. The error bars on the plots of % difference of test from the control diets represent 95% confidence intervals on means, and are based on non-pooled variance estimates.

3. Results and discussion

Tables 2–4 compare treatment means with their corresponding control means, including results of tests of significance. Figs. 2 and 3 give a visual overview of the results in terms of% difference of the treatment means and of corresponding control values along with 95% confidence intervals. These results show that %LW/BWs in non-pregnant mice fed solanidine, solasodine and tomatidine were greater than the control values (Table 2). The current study differs from Friedman et al. (1996) in that mice were fed Simonsen Custom Diet #7—a specialized diet for pregnant animals—and mice were plugged for pregnancy and found not to be pregnant at the time of autopsy.

It is instructive to compare the liver/body weight ratios reported previously with the results of the present study (values are controls, treated). For female mice of the previous study: (a) solanidine, 5.40±0.34, 6.42±0.32; (b) solasodine −5.36±0.43, 6.80±0.56; (c) tomatidine, −5.13±0.35, 5.37±0.44. The corresponding values for non-pregnant mice of the present study (Table 2) are (a) solanidine, 5.00±0.27, 6.27±0.66; (b) solasodine, −5.33±0.49, 6.22±0.64; (c) tomatidine, −5.30±0.28, 5.62±0.37. The corresponding values for pregnant mice (Table 3) are (a) solanidine, 5.26±0.26, 6.54±0.56; (b) solasodine, −5.20±0.29, 6.92±0.78; (c) tomatidine, −5.20±0.49, 5.64±0.48. The data indicate

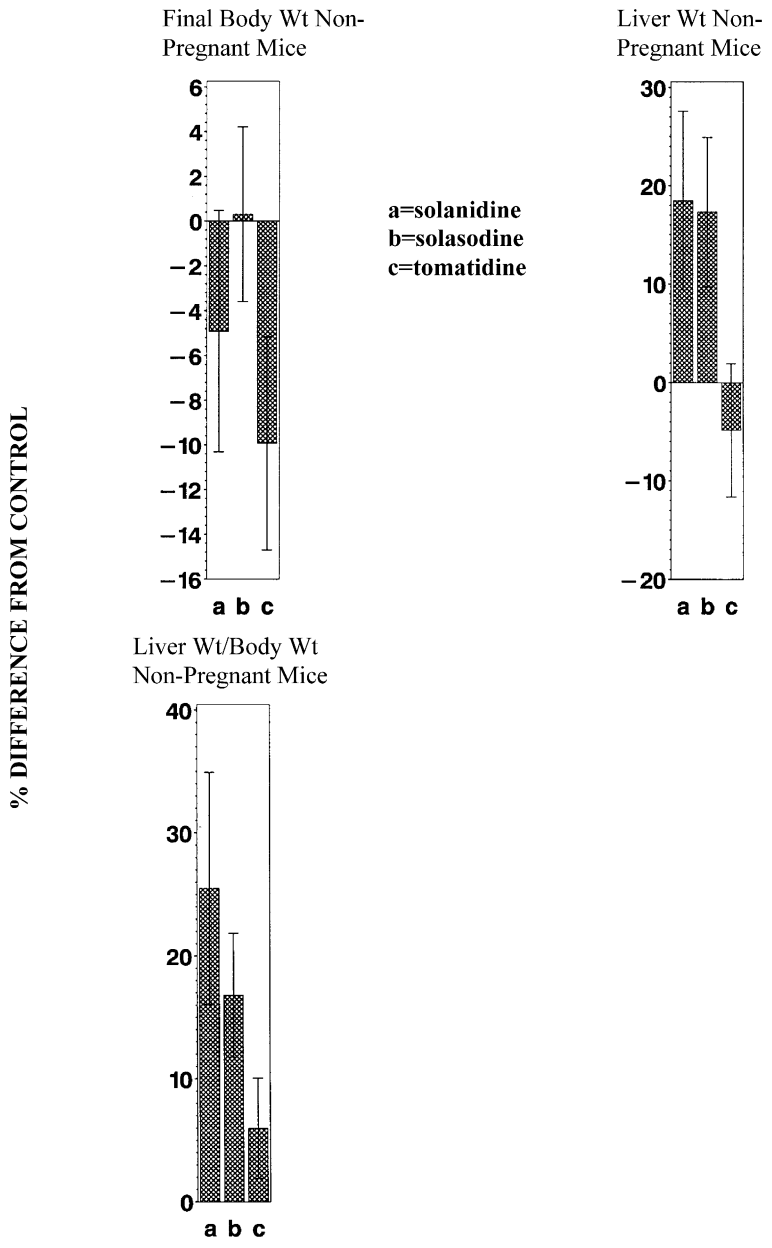

Fig. 2. Body and liver weights (% difference from controls) in non-pregnant Swiss–Webster mice fed for 14 days a diet containing solasodine, tomatidine or solanidine (2.4 mmol/kg diet). Error bars represent 95% confidence intervals on means based on non-pooled variance estimates.

that aglycone-induced hepatomegaly is similar for the non-pregnant mice of both studies and for the pregnant mice of this study. The %LW/BWs for the three aglycones from the two studies are not statistically different. The hepatomegaly induced by the three compounds is comparable in both studies.

In the current study,%LW/BWs in pregnant mice were also greater than in controls (Table 3). The livers grew in direct proportion to the body weight gains in the pregnant mouse. Thus, if hepatomegaly were to occur, it would add weight to a growing liver. We did observe, however, that solanidine-induced increase in %LW/BW was considerably less in pregnant mice than in non-pregnant mice. Possibly (a) the steroid hormones of the pregnant mouse are in competition with solanidine induction of hepatomegaly, (b) there is a counteractive hepatotoxicity which concomitantly lowers liver weight, and/or (c) there is less absorption in the pregnant than in the non-pregnant mice.

The data in Tables 2–4 and Figs. 2 and 3 also show that the body weight gains in the dams were significantly lower in mice treated with the three alkaloids, litter weight as a percent body weight (%LTW/BW) was lower than the control values for solasodine, and the average weight of the fetuses was lower than control values for solanidine and solasodine.

In a previous study on adult female mice, we observed that tomatidine has only one-third the potency of sola-

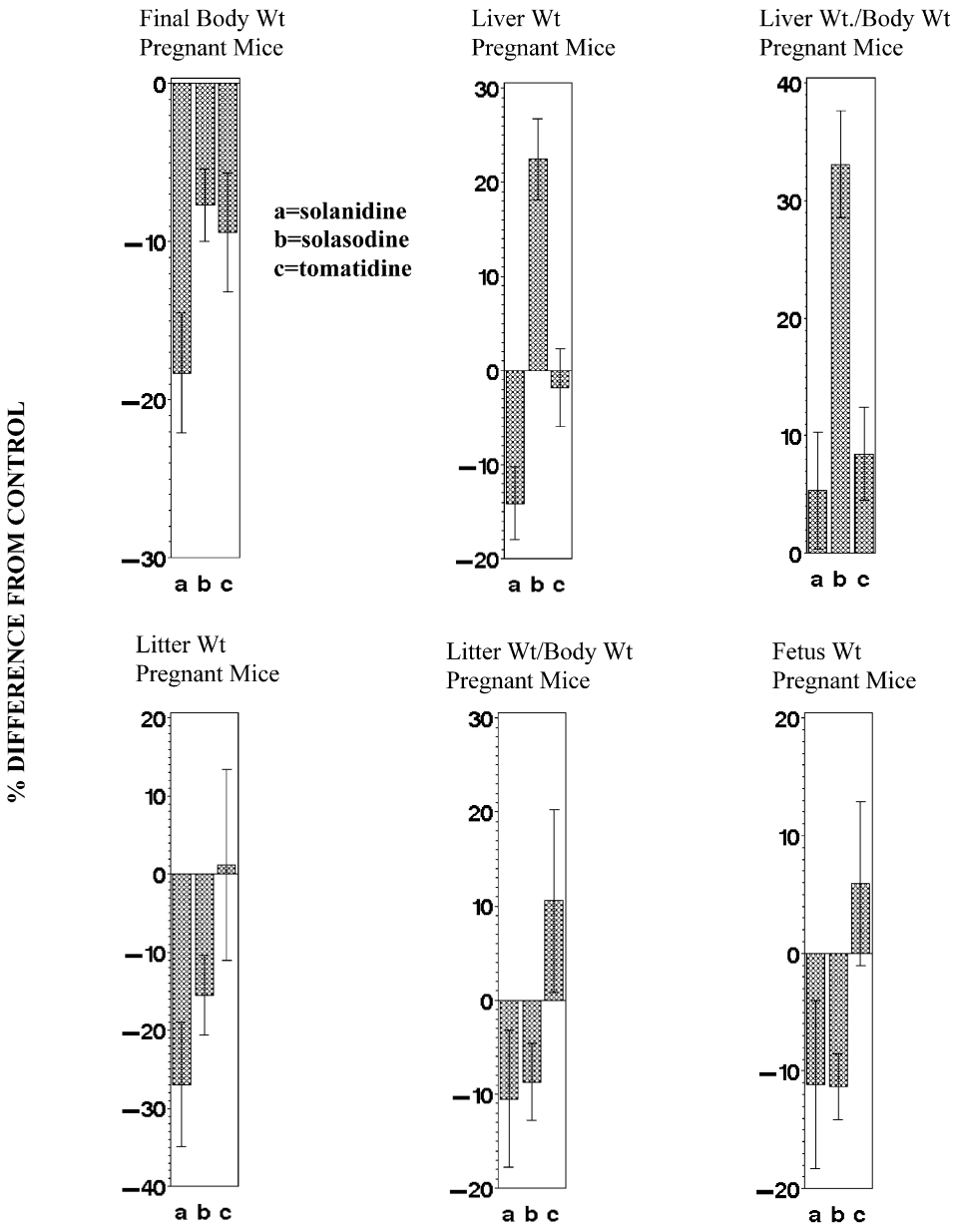

Fig. 3. Liver, litter and fetus weights (% difference from controls) in pregnant Swiss–Webster mice fed for 14 days a diet containing solasodine, tomatidine or solanidine (2.4 mmol/kg diet). Error bars represent 95% confidence intervals on means based on non-pooled variance estimates.

sodine in inducing hepatomegaly. This study confirms that tomatidine is the least toxic of the three compounds evaluated, roughly one-third as effective as solanidine in increasing %LW/BW in both non-pregnant mice (Fig. 2) and pregnant mice (Fig. 3). These data also show that for tomatidine, body weight gains in the dam and litter weights were comparable to controls (Table 4 and Fig. 3).

We were most interested in solanidine because it is formed in vivo via either acid or enzymatic hydrolysis from the widely consumed potato glycoalkaloids α-solanine or α-chaconine, and it is absorbed and/or persist in tissues after hydrolysis. The current study shows that dietary administration of 2.4 mmol/kg solanidine to pregnant mice results in significantly lower litter size (10.2 ± 2.6 vs 12.1 ± 1.9) and lower fetus weights (0.86 ± 0.15 vs 0.97 ± 0.13) (Table 4). Additional studies are warranted to assess the relevance of these results to humans.

To place our findings into a wider perspective, we briefly summarize other reported biological effects of solanidine. It is the major metabolite in rats following oral consumption of the potato glycoalkaloid α-chaconine (Norred et al., 1976). It is present in the plasma (up to 92.5 nmol/l) and saliva (up to 20% of serum levels) of humans consuming potatoes—plasma levels are proportional to the amount of potatoes consumed, suggesting that glycosidases can hydrolytically remove the

carbohydrate side-chain from the glycoalkaloids in vivo (Matthew et al., 1983; Harvey et al., 1985a,b, 1986; Hellenas et al., 1992). It has been detected in human post-mortem liver, suggesting that it is stored in the liver for prolonged periods (Claringbold et al., 1980, 1982). It does not induce micronuclei formation in erythrocytes of weanling or fetal mice (Friedman and Henika, 1992). It induces formation of terata (cranial malformations) in hamsters (Gaffield and Keeler, 1996) and reversible hepatomegaly in adult female mice (Friedman et al., 1996). Unlike the glycoalkaloids, solanidine was not toxic to frog embryos (Friedman et al., 1991; Blankemeyer et al., 1992), did not induce ornithine decarboxylase (ODC) in rat liver (Caldwell et al., 1991), showed low activity as an inhibitor of human acetylcholinesterase (Duan, 1995), and has estrogen-like properties (present study). It is also noteworthy that the non-toxicity of α-tomatine compared to other glycoalkaloids is due to its ability to form an insoluble complex with cholesterol in the digestive tract of hamsters which is then eliminated in the feces (Friedman et al., 2000a,b).

These observations and the results of the present study on solanidine-induced changes in liver weights, fetal mortality, and loss of fetuses suggest that the biological effects of solanidine often differ from those of the parent potato glycoalkaloids α-chaconine and α-solanine from which it is derived. However, we do not know whether consumption by humans of potatoes containing the highest recommended concentrations of glycoalkaloids mentioned earlier will produce sufficient solanidine in vivo to elicit any of the biological effects observed in mice.

3.1. Mechanistic aspects

Possible mechanisms that govern biological activities of aglycones are not well understood. Because solanidine was the most toxic compound, it was of interest to find out whether induction of estrogen may be involved in its mechanism of action at the cellular level. The results show that of all the compounds tested, only solanidine was found to be active in the breast cancer cell proliferation assay (Fig. 4). In this assay, solanidine (a) enhanced the activity of estradiol in the estrogen-depleted cells (bar graphs); and (b) at a concentration of 10 μM, significantly increased cell numbers after 7 days in the estrogen-depleted cells without added estradiol (dose–response plot). The other aglycone (tomatidine) as well as the four glycoalkaloids tested (α-chaconine, α-solanine, solasonine and α-tomatine) were inactive in the cell proliferation assay.

In additional assays, aimed at finding possible mechanistic aspects, there was no significant effect on gene transcriptional activation by any of the compounds tested in either estrogen-depleted conditions and or the presence of estradiol. The results from these assays suggest that solanidine's small but statistically significant positive effect on cell proliferation is independent of the ER signaling pathways. It is possible that solanidine operates through membrane-bound estrogen receptors that stimulate signal transduction pathways (Nemere and Farach-Carson, 1998).

It is also instructive to examine the structural features of solanidine that may be responsible for the estrogenic activity. The 3-OH group of solanidine (Fig. 1) must be involved in estrogen receptor binding since the activity is lost on glycosylation to the glycoalkaloids α-chaconine and α-solanine. The steroidal part of the molecule must also be involved since the inactive tomatidine also has an OH group.

In an earlier study, mice were fed freeze-dried potato berries (containing 221 and 159 mg/kg of fresh weight of the glycoalkaloids α-chaconine and α-solanine, respectively) at 1, 5, 10, 20 and 40% of the diet, as well as 10% casein diets supplemented with 50–1600 mg of the aglycone solasodine (Friedman, 1992). At day 14, all surviving animals were necropsied, organs weighed, and blood chemistries analyzed. Selected tissues from mice fed the control, solasodine and 20% potato berry diets were examined histologically. All mice fed the 40% potato berry diets died. Effects noted with the solasodine diets included elevated serum alkaline phosphatase, glutamic–pyruvic transaminase (GPT) and glutamic-oxaloacetic transaminase (GOT); elevated liver weight as a percent of body weight; decreased body weight gain; and increased incidence of liver cholangiohepatitis and gastric gland dilation/degeneration. Solasodine- and solanidine-induced hepatomegaly is reversible when adult female mice are taken off the alkaloid-supplemented diet (Friedman et al., 1996). This indicates that hepatomegaly may be a benign adaptive response.

It is also relevant that the steroid hormone dehydroepiandrosterone (DHEA) is reported to induce hepatomegaly in rats (Bellei et al., 1992) and mice (Friedman et al., 1996). Hepatomegaly induced by the hormone in rat livers was associated with a significant increase in the size and number of hepatocyte peroxisomes and of peroxisomal marker enzymes catalase and fatty acyl CoA oxidase. It remains to be shown whether the solanidine-induced hepatomegaly is also accompanied by changes in liver peroxisomes.

The biological effects are probably not due to genotoxicity at the DNA level since glycoalkaloids and aglycones were inactive in both the in vitro Ames mutagenicity and in the in vivo adult and foetal red blood cell micronucleus chromosome assays (Friedman and Henika, 1992).

Finally, although rumen microorganisms catalyze the transformation of potato glycoalkaloids to solanidine and its dihydro derivative, 5β-solanidan-3β-ol (King and McQueen, 1981), solanidine was not found in milk from cows fed a glycoalkaloid-rich potato waste

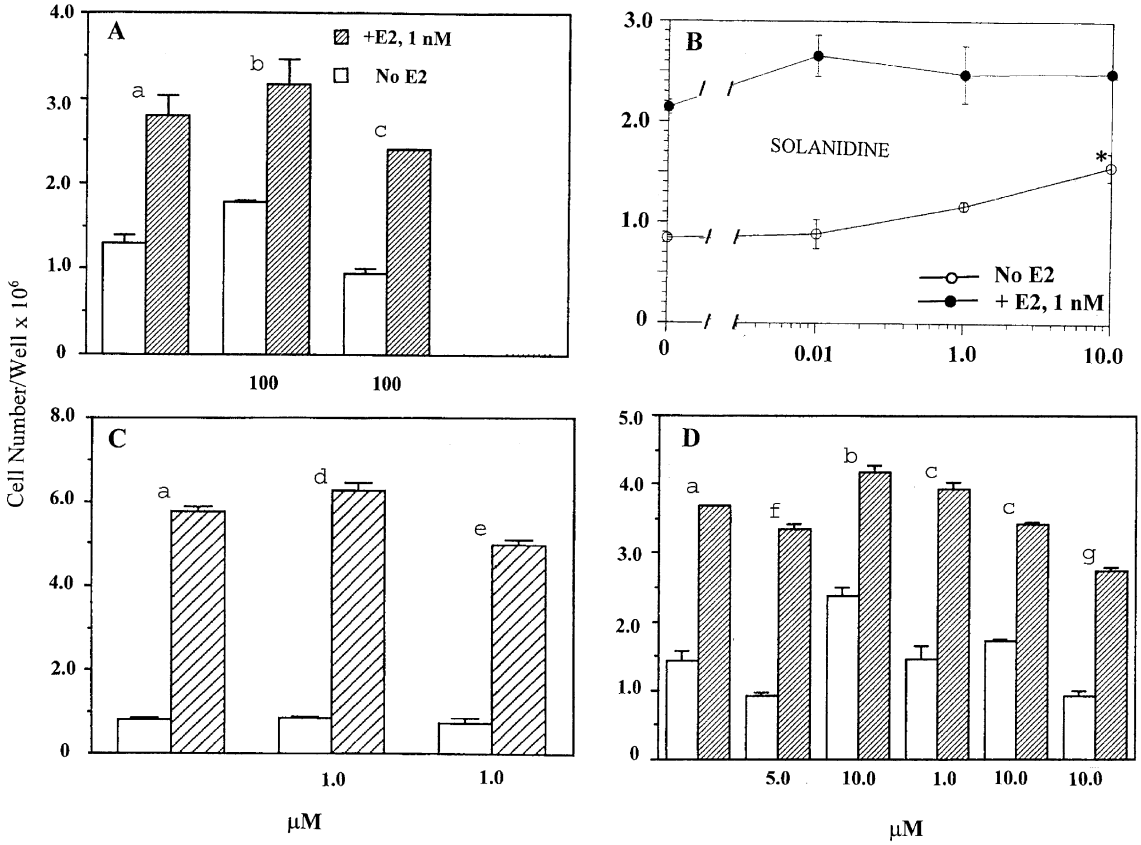

Fig. 4. Results from separate experiments on the effect of aglycones and glycoalkaloids on proliferation of MCF-7 breast cancer cells. (A): a, DMSO control; b, solanidine; c, tomatidine. Bars represent mean values. Error bars represent SD deviations from the mean ($n=4$). Solanidine but not tomatidine enhanced the number of estrogen-depleted cells with added estradiol (E2). (B): Dose–response of solanidine on proliferation of breast cancer cells. Estrogen-depleted MCF-7 cells were plated at a density of 10^5 cells per well in six-well plates and treated with solanidine at the indicated concentrations, in presence (closed circles) or absence (open circles) of estradiol, E2 (1 nM). Duplicate aliquots of cells form individual wells were counted after 7 days. Results are shown as the mean and SD for four separate experiments. *Significantly different ($P<0.05$) from DMSO control. (C): a, DMSO control; d, α-chaconine; e, α-tomatine. Both glycoalkaloids were inactive. (D): a, DMSO control; b, solanidine; c, tomatidine; f, α-solanine; g, solasonine. Only solanidine was active in this assay.

product known as tater meal (Bushway et al., 1984). Solanidine is therefore not transported to the milk of cows. It remains to be shown whether human milk contains diet-derived glycoalkaloids or metabolites.

3.2. Research needs

Additional studies are needed to define (a) whether the observed effects of solanidine on litter size and fetus weights will also occur in other species, especially primates; (b) the pharmacokinetics (absorption, transport, residence times in tissues, elimination) of the three aglycones solanidine, solasodine and tomatidine; (c) their effects on liver structure and function; (d) the significance of glycoalkaloid-induced formation of ODC in rat livers (Caldwell et al., 1991); (e) possible synergistic effects, as reported by Rayburn et al. (1995b) for potato glycoalkaloids α-chaconine and α-solanine; (f) the possible significance for human health of concurrent consumption of phytoestrogen-containing soybeans (Friedman and Brandon, 2001), cruciferous vegetables (Riby et al., 2000) and potato-derived solanidine (present study); and (g) whether solanidine can accumulate in transgenic potatoes in which the production of the enzyme solanidine glucosyltransferase that catalyzes the glucosylation of solanidine is suppressed through antisense RNA methodology (Stapleton et al., 1992; Moehs et al., 1997).

Finally, our observation that the absence of a 5,6-double bond in the B-ring of tomatidine results in a much less toxic molecule in both pregnant and non-pregnant mice as compared to the structurally similar solasodine (which contains such a double bond; Fig. 1) confirms related findings by Gaffield and Keeler (1993, 1996) on the influence of the structure of glycoalkaloids and aglycones on teratogenicities in hamsters. They reported the following relative oral teratogenicities in hamsters: jervine, 100; α-chaconine, 43; α-solanine, 32; solanidine, 32; α-tomatine, 1; tomatidine, 0. It may therefore benefit food safety to create, through plant breeding and/or plant molecular biology methods, potatoes containing the tomato glycoalkaloid

α-tomatine which does not appear to be toxic when consumed orally in moderate amounts (Friedman et al., 2000a,b). Our studies (Kozukue et al., 1999; Friedman, in press) on the inheritance of tomatine in potatoes of somatic hybrids suggest that this may be a feasible approach.

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