NUTRITION AND CANCER, *49*(1), 94–99

Inhibition of the Rat Breast Cytosolic Bioactivation of Ethanol to Acetaldehyde by Some Plant Polyphenols and Folic Acid

Maria Eugenia Maciel, Gerardo Daniel Castro, and José Alberto Castro

Abstract: *There is a well-established association between alcohol consumption and breast cancer risk. About 4% of the breast cancers in developed countries are estimated to be attributable to drinking alcohol. The mechanism of tumor promotion by alcohol remains unknown. Recent studies from our laboratory and others showed the ability of mammary tissue to bioactivate ethanol to mutagenic/carcinogenic acetaldehyde and free radicals. Xanthine oxidoreductase (XOR) is an enzyme involved in those biotransformation processes. In the present study, we provide evidence of the ability of different natural polyphenols and of folic acid derivatives to inhibit the biotransformation of alcohol to acetaldehyde by rat breast cytosolic XOR. Folic acid and dihydrofolic acid, at sconcentrations of 10 μM, inhibited 100% and 84%, respectively, of the cytosolic acetaldehyde formation. Thirty-five polyphenols were tested in these initial experiments: ellagic acid, myricetin, quercetin, luteolin, and apigenin inhibited 79–95% at 10 μM concentrations. The remaining polyphenols were either less potent or noninhibitory of acetaldehyde formation at similar concentrations in these screening tests. Results are relevant to the known preventive effects of folic acid against alcohol-induced breast cancer and to their potential preventive actions if added to foods or alcoholic beverages.*

Introduction

There is abundant evidence that even moderate levels of drinking alcohol (ETOH) increase risk of breast cancer (1,2). This association shows a clear dose-response relationship. In a combined analysis of data from 53 studies around the world, it was reported that the relative risk for breast cancer increased 7% for each additional 10 g of alcohol consumed daily and that about 4% of the breast cancers in developed countries are attributable to drinking alcohol (2). The World Health Organization estimated that about 3% of breast cancer cases worldwide, or 26,800 women, were attributable to alcohol consumption in 1990 (1).

Despite the relevance of the problem, the mechanism for the ethanol-increased risk of breast cancer remains unknown. Different studies have linked the increased risk of breast cancer with the estrogenic effect of the consumption of alcoholic beverages and the matter was recently reviewed (3–5). However, recent studies from our laboratory and others provided evidence that the carcinogenic effects of alcohol consumption in breast may not necessarily be indirect via increased levels of estrogen. In fact, our laboratory recently demonstrated that rat mammary gland cytosolic xanthine oxidoreductase (XOR) is able to bioactivate ETOH to acetaldehyde and free radicals (6). Further, the breast microsomal fraction also exhibited the presence of enzymatic NADPH dependent and NADPH nondependent pathways requiring oxygen for the biotransformation of ETOH to acetaldehyde (7). More recently, Triano et al. (8) reported that the cytosolic fraction from human mammary tissue contains a class I alcohol dehydrogenase (ADh) that can oxidize ETOH when concentrations of ETOH are in the range of 0.05 to 4.0 mM, but which is inhibited by concentrations of ETOH above 10 mM. The significance of the aforementioned studies is that they are pointing to the possibility that in situ production of acetaldehyde and/or free radicals could be causative because both metabolites have mutagenic, carcinogenic, or cancer-promoting potential (9–12). The relevance of the in situ direct effects of ETOH in the breast is also emphasized by studies of other laboratories, which showed that ETOH promotes the growth rate of human breast cancer epithelial cells in vitro by modulating putative growth-promoting signaling pathways (13–15).

The previously mentioned considerations led us to consider the possibility of inhibiting those ETOH bioactivation pathways with plant polyphenols that could be present in or added to alcoholic beverages or food.

In an initial effort in that direction, we selected a pathway of ETOH bioactivation for study mediated by cytosolic XOR, which results in formation of acetaldehyde from ethanol (6).

There are literature reviews on XOR inhibition by plant polyphenols (16–22) and a limited but interesting study on inhibition of milk XOR by folic acid (23). This literature prompted us to conduct studies on some of those chemicals against the rat breast cytosolic XOR-mediated biotransformation of ETOH to acetaldehyde.

All authors are affiliated with Centro de Investigaciones Toxicológicas (CEITOX)–CITEFA/CONICET, Buenos Aires, Argentina.

Materials and Methods

Chemicals

Absolute ETOH (analytical grade) was from Sintorgan (Argentina). Acetaldehyde was from Fluka (Switzerland). The compounds tested for their effects on the metabolism of ETOH were of the best quality available: nordihydroguaiaretic acid; quercetin dihydrate, (±)-naringenin, naringin, (+)-catechin, (-)-catechin, (+)-epicatechin, (-)- epigallocatechin gallate, ellagic acid, resveratrol, caffeic acid and its phenethyl ester, curcumin, propolis extract (from propolis collected in the state of Pennsylvania), hesperetin, folic acid, pelargonidine chloride, silibinin, ferulic acid, and morin were from Sigma Co. (St. Louis, MO). Baicalein and silymarin were from Aldrich Co. (Milwaukee, WI). Daidzein, enterodiol, and enterolactone were from Fluka.

Animals and Treatments

Non-inbred female Sprague Dawley rats (18 weeks, 220–260 g) were used. The rates were postlactation young mothers (2 weeks after weaning of their pups). The animals were fasted for 12–14 h before sacrifice ,but water was available ad libitum. They were killed by decapitation, and their mammary tissue was rapidly excised and processed. Purified cytosolic fractions were obtained from whole mammary tissue homogenates by subcellular fractionation procedures via ultracentrifugation at 2–4°C. These preparations were essentially free from cross contamination with other cellular fractions (e.g., nuclear, mitochondrial, or microsomal; 7,24).

Ethanol Biotransformation to Acetaldehyde in the Cytosolic Fraction

Incubation mixtures containing purified cytosol (2.32 ± 0.29 mg protein per ml) in STKM buffer (0.25 M sucrose/50 mM Tris-HCl, pH 7.5/2.5 mM KCl/5 mM $MgCl_2$), 0.25 mM hypoxanthine, 0.3 mM NAD^+, and 0.14 M ethanol (3 ml final volume) were conducted for 1 h at 37°C under air atmosphere. Incubations were performed in aluminum-sealed neoprene-septum stoppered glass vials (15 ml). The reaction was terminated by placing on ice. After adding 1 ml of saturated NaCl solution, samples were kept at 37°C for 10 min, and an aliquot (100 µl) of the headspace was analyzed by GC-FID. Chromatographic conditions were as follows: column, GS-Q, 25 m × 0.53 mm i.d. (J&W Scientific, CA); temperature, 110°C isothermal; injection port temperature, 150°C; FID, 200°C (25,26).

Inhibition of Xanthine Oxidoreductase Activity in Mammary Tissue by Plant Polyphenols and Folic Acid

Xanthine oxidoreductase activity was measured in mammary tissue by analysis of the uric acid formed, according to the methodology described by Terada et al. (27). Briefly, uric acid formation from xanthine was assayed under two experimental conditions that reveal the presence of xanthine oxidase (XO) or XOR (XOR = XO + XDh) activities, as follows:

$$\text{Xanthine} + 2\,O_2 + H_2O \rightarrow \text{uric acid} + 2\,O_2^{-\cdot} + 2\,H^+ \quad \text{(xanthine oxidase)}$$

$$\text{Xanthine} + O_2 + H_2O \rightarrow \text{uric acid} + H_2O_2 \quad \text{(xanthine oxidase)}$$

Superoxide dismutase (SOD) + catalase are necessary in the incubation media to prevent oxidative inactivation of the enzyme by its own products, $O_2^{-\cdot}$ and H_2O_2.

$$\text{Xanthine} + NAD^+ + H_2O \rightarrow \text{uric acid} + NADH + H^+ \quad \text{(xanthine dehydrogenase)}$$

Lactate dehydrogenase (LDH) and pyruvate are required to prevent inhibition of XDh by NADH. Results were expressed as percentage of inhibition compared with the value corresponding to the XOR activity.

Statistics

The significance of the differences between mean values was assessed by analysis of variance test and Tukey posttests (28). Calculations were performed using GraphPad software (29).

Results

Effect of Plant Polyphenols and Folic Acid on the Biotransformation of ETOH to Acetaldehyde by Breast Cytosolic Fraction

Acetaldehyde levels in incubation mixtures containing the cytosolic fraction of rat mammary tissue are summarized in Table 1. The XOR-mediated ETOH activation pathway was strongly inhibited by ellagic acid (95%) at concentration as low as 10 µM. This effect is of the same order as equimolar concentrations of known inhibitors of XOR such as allopurinol (94%), oxypurinol (99%), folic acid (99%), and dihydrofolic acid (84%). At the same concentration, other polyphenols were also able to significantly decrease the biotransformation of ETOH to acetaldehyde, the more potent being quercetin (79%), myricetin (86%), kaempferol (70%), apigenin (83%), luteolin (85%), and hesperetin (60%). At a lower extent, inhibitory effects were also observed at the same concentrations with morin (38%), rutin (24%), baicalein (51%), daidzein (30%), genistein (33%), (-)-catechin (31%), (+)-epicatechin (33%), (-)-epigallocatechin gallate (33%), curcumin (25%), ferulic acid (29%), silibinin (27%), enterodiol (46%), enterolactone (32%), and pelargonidine (32%). Other polyphenols showed little or no inhibitory ability, for example, (+)-catechin, naringenin, caffeic acid, resveratrol, or nordihydroguaiaretic acid.

Table 1. Effect of Plant Polyphenols on the Biotransformation of ETOH to Acetaldehyde in the Mammary Gland Cytosolic Fraction

Experimental[a]	Acetaldehyde (nmol)/Protein (mg)		
	Hypoxanthine + NAD^+	-Hypoxanthine – NAD^+	% Inhibition[b]
Air	2.75 ± 0.26	0.12 ± 0.01	—
10 µM allopurinol	0.19 ± 0.01	0.03 ± 0.01	94
10 µM oxypurinol	0.03 ± 0.01	0.24 ± 0.01[c]	99
10 µM folic acid	0.03 ± 0.01	0.21 ± 0.01[c]	99
10 µM dihydrofolic acid	0.44 ± 0.01	0.68 ± 0.01[c]	84
Flavonols			
10 µM quercetin	0.70 ± 0.01	0.16 ± 0.01[c]	79
10 µM morin	1.77 ± 0.18	0.14 ± 0.01	38
50 µM morin	1.68 ± 0.12	0.32 ± 0.02[c]	48
10 µM myricetin	0.54 ± 0.03	0.18 ± 0.01[c]	86
10 µM kaempferol	0.96 ± 0.03	0.16 ± 0.01[c]	70
10 µM rutin	2.23 ± 0.07[d]	0.22 ± 0.01[c]	24
Flavones			
10 µM baicalein	1.48 ± 0.03	0.20 ± 0.01[c]	51
10 µM apigenin	0.67 ± 0.05	0.22 ± 0.01[c]	83
10 µM luteolin	0.65 ± 0.03	0.25 ± 0.01[c]	85
Isoflavones			
10 µM daidzein	2.03 ± 0.03	0.18 ± 0.01[c]	30
10 µM genistein	1.87 ± 0.07	0.12 ± 0.01	33
Flavanols			
10 µM (+)-catechin	2.66 ± 0.02	0.05 ± 0.01	1
50 µM (+)-catechin	1.98 ± 0.02	0.05 ± 0.01	26
10 µM (-)-catechin	1.96 ± 0.04	0.14 ± 0.01	31
10 µM (+)-epicatechin	1.88 ± 0.13	0.12 ± 0.01	33
10 µM (-)-epigallocatechin gallate	2.04 ± 0.07	0.28 ± 0.01[c]	33
Flavanones			
10 µM naringenin	2.38 ± 0.02[e]	0.09 ± 0.01	13
50 µM naringenin	2.02 ± 0.06	0.07 ± 0.01	26
50 µM naringin	2.70 ± 0.08	0.04 ± 0.01	0
10 µM hesperetin	1.19 ± 0.03	0.13 ± 0.01	60
Phenolic acids and derivatives			
50 µM caffeic acid	2.69 ± 0.08	0.10 ± 0.01	2
100 µM caffeic acid phenethyl ester	2.09 ± 0.03[d]	0.28 ± 0.01[c]	31
10 µM curcumin	2.20 ± 0.08[d]	0.23 ± 0.01[c]	25
10 µM ferulic acid	2.10 ± 0.03[d]	0.23 ± 0.01[c]	29
Propolis extract 5 µg/ml	2.29 ± 0.03[d]	0.20 ± 0.01[c]	21
Stilbenes			
10 µM resveratrol	2.89 ± 0.04	0.05 ± 0.01	0
50 µM resveratrol	2.87 ± 0.09	0.24 ± 0.01[c]	0
Lignans			
Silymarin 25 µg/ml	2.30 ± 0.01[d]	0.22 ± 0.01[c]	21
Silymarin 50 µg/ml	1.84 ± 0.03	0.57 ± 0.01[c]	52
10 µM silibinin	2.08 ± 0.12[d]	0.15 ± 0.01[c]	27
10 µM enterodiol	1.56 ± 0.04	0.14 ± 0.01	46
10 µM enterolactone	1.94 ± 0.01	0.16 ± 0.01[c]	32
Anthocyanidins			
10 µM pelargonidin	1.90 ± 0.15	0.11 ± 0.01	32
Other Polyphenolics			
10 µM ellagic acid	0.13 ± 0.01	0.17 ± 0.01[c]	95
10 µM nordihydroguaiaretic acid	2.75 ± 0.04	0.18 ± 0.01[c]	2
50 µM nordihydroguaiaretic acid	3.05 ± 0.02	0.18 ± 0.01[c]	0

a: Incubation mixtures containing cytosol (2.32 ± 0.29 mg of cytosolic protein/ml), 0.14 M ethanol, and, when indicated, 0.25 mM hypoxanthine and 0.3 mM NAD^+ in STKM buffer, were conducted for 1 h at 37°C. Acetaldehyde was measured in the head space of each sample after adding 1 ml NaCl saturated solution. See **Materials and Methods** section for details. Each result is the mean of three separate lots of pooled mammary tissue samples.

b: Percent inhibition was calculated with respect to the air group as the control. Each data of the blank group (-hypoxanthine – NAD^+) was subtracted from the corresponding experimental group (+hypoxanthine + NAD^+).

c: When incubations were performed in the absence of hypoxanthine and NAD^+, this compound was shown to increase the production of acetaldehyde compared with the respective control. This behavior would be attributable to interactive effects of polyphenol, allowing the expression of competitive pathways for ethanol oxidation or inhibition of acetaldehyde consumption under the experimental condition.

d: $P < 0.01$, compared with air + hypoxanthine + NAD^+.

e: $P < 0.05$, compared with air + hypoxanthine + NAD^+.

Table 2. Inhibition of Xanthine Oxidoreductase Activity in Mammary Gland Cytosolic Fraction by Plant Polyphenols and Folic Acid[a]

Experimental[b]	Uric acid (nmol/mg protein)	% Inhibition
XO[c]	117.8 ± 0.5	—
XOR = XO+XDh[d]	212.9 ± 1.1	0
10 μM allopurinol	32.8 ± 0.9	85
10 μM folic acid	26.5 ± 0.4	88
10 μM dihydrofolic acid	30.7 ± 0.6	86
10 μM ellagic acid	51.1 ± 0.2	76
10 μM myricetin	67.2 ± 0.1	68
10 μM quercetin	95.7 ± 1.5	55
10 μM luteolin	92.5 ± 1.0	57
10 μM apigenin	95.8 ± 1.8	55

a: Abbreviations are as follows: XO, xanthine oxidase; XOR, xanthine oxidoreductase; XDh, xanthine dehydrogenase.

b: XOR activity was measured in mammary tissue by analysis of the uric acid formed by high-performance liquid chromatography (HPLC)-UV, according to the methodology described by Terada et al. (27). Results were expressed as percentage of inhibition compared with the value corresponding to the XOR activity. The effect of allopurinol, folic acid, and the polyphenols was tested using the XOR reaction conditions.

c: XO means: buffer + sample + SOD + catalase + xanthine.

d: XOR = XO + XDh means: buffer + sample + SOD + catalase + xanthine + NAD^+ + LDH + pyruvate.

Some of the compounds that inhibited the production of acetaldehyde from ETOH also proved to be potent inhibitors of XOR activity in mammary tissue cytosolic fractions (Table 2).

Discussion

In agreement with previous studies from our laboratory, rat breast cytosolic fractions exhibited purine-supported, allopurinol-inhibitable bioactivation of ETOH to acetaldehyde (6). In the absence of hypoxanthine and NAD^+, there was also an additional pathway of ETOH biotransformation to acetaldehyde, which accounted for up to 0.57 nmol/mg of acetaldehyde with the tested compounds (e.g., sylimarin; Table 1). This pathway is also strongly inhibited by allopurinol and is supported to some extent with many of the other XOR inhibitory polyphenols and folic acid derivatives (Table 1). This behavior might suggest that breast cytosolic XOR could catalyze the metabolism of alcohol to acetaldehyde, even in the absence of purine and NAD^+. It is well known that XOR has a broad substrate specificity that includes alcohols, aldehydes, nitroderivatives, and other chemicals (24,27,30). The ability of both ETOH and the cosubstrates to interact with XOR at different sites of the enzyme might explain the efficient purine + NAD^+ enhancing effect of alcohol oxidation by XOR. The XOR-mediated biotransformation of alcohol in mammary cells might occur in vivo. In fact, rat mammary tissue lacks detectable alcohol dehydrogenase (6,31). Its human counterpart, a class I ADh, was only detectable when alcohol concentrations were in the range from 0.05 to 4 mM, but that was inhibited by alcohol concentrations above 10 mM (8). In many countries, when mild impairment is observed and car driving is forbidden, the ETOH level in blood is about 0.5 g per liter (32), which is already 11 mM.

CYP2E1-mediated alcohol bioactivation pathways were not detectable in rat mammary tissue (7) but were detectable in human breast tissue (33). However, expression levels of cytochrome P450 in mammary tissue are up to 500 times lower in the breast than in the liver (34). That casts doubt on their potential role in activation of ETOH in situ. In contrast, mammary tissue is the richest source of XOR activity in the entire body (35). This XOR-mediated pathway of ETOH metabolism to a toxic/mutagenic/carcinogenic metabolite, as is acetaldehyde (9–12), was strongly inhibited by other potent inhibitors of XOR, such us folic acid and dihydrofolic acid (23). These results might be of particular interest in light of previous reports that higher folate consumption was associated with decreased breast cancer risk among women consuming alcohol regularly but not among nondrinkers in three cohort studies (36–39). Whether the preventive effect of folate on alcohol-promoted breast cancer is related to the inhibitory effects of folic acid on XOR-mediated cytosolic bioactivation of ETOH to metabolites such as acetaldehyde and free radicals (6) remains to be established. However, it is a provocative possibility. The preventive effects of folate were not observable when breast cancer risk was not associated with high alcohol intake, despite an increasing number of specific cancers having been linked to folate status (40). In those cases, several alternative explanations were put forward to explain the beneficial effects of folate (40). The XOR-dependent cytoplasmic route of ETOH bioactivation was shown here to be strongly inhibited by several phytochemicals of polyphenolic nature. Some of them (e.g., quercetin, myricetin, kaempferol, baicalein, apigenin, luteolin, hesperetin, silibinin, enterodiol, and ellagic acid) were found to be potent inhibitors of the XOR-mediated alcohol bioactivation pathway at concentrations as low as 10 μM (Table 1). The most potent inhibitors among all these polyphenols were the flavonols quercetin, myricetin, and kaempferol; the flavones, apigenin and luteolin; and the polyphenol, ellagic acid (Table 1). Their structures are depicted in Fig. 1.

The inhibition of XOR was confirmed for some of these compounds by checking their effect on the biotransformation of xanthine to uric acid by breast cytosol (Table 2). The XOR inhibitory properties of many plant polyphenols were previously established (16–22) and, consequently, their effectiveness to interfere with the XOR-mediated bioactivation of ETOH to acetaldehyde in breast cytosolic fractions is not unexpected. It is important to note, however, that the bioavailability of these compounds determines their in vivo ability to exert their benefitial effects (41). For most of these chemicals, peak plasma concentrations were in the low micromolar level (42–45). Further, for some polyphenols, biphasic and synergistic effects were reported (43,46). In those cases, inhibitory properties were observable at low concentrations, and stimulatory properties were observable at higher concentrations (46). In our case, no stimulation of

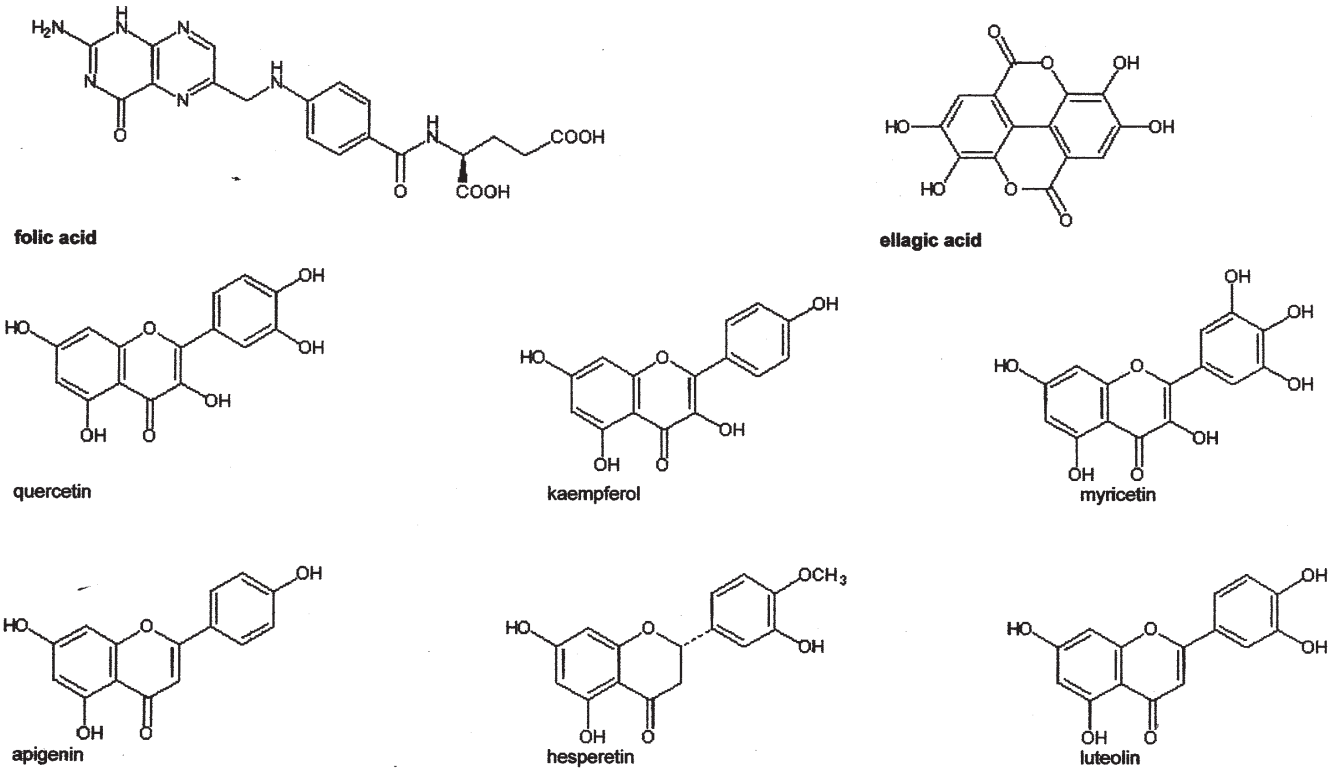

Figure 1. Structures of some of the compounds with the most powerful inhibitory ability on the biotransformation of ethanol to acetaldehyde in rat mammary tissue.

acetaldehyde formation from ETOH was observed with the polyphenols tested at the 10 μM level employed for this initial screening study. Further detailed studies are required to determine whether or not these biphasic or synergistic effects might occur before designing appropriate in vivo studies with ETOH-treated animals. To that end, it is also relevant to consider the effects of these polyphenols on the other pathways of rat breast bioactivation of ETOH to acetaldehyde. For example, the rat microsomal biotransformation of ETOH to acetaldehyde through a non-CYP2E1 pathway was inhibited by nordihydroguaiaretic acid but not by the other polyphenols tested (47).

In contrast to the case of folic acid, there are no reports available in the literature on the effect of diets rich in plant polyphenols on breast cancer risk among women consuming alcohol regularly. However, it is known that diets rich in vegetables and other plant products significantly reduce breast cancer risk (48,49). These diets are an important source of polyphenols (42,46,50) and of other cancer preventive agents (49). Because excess breast cancer risk related to alcohol consumption was observed, even in women consuming relatively modest amounts of alcohol (1,2), the possibility exists that diets containing sufficient plant polyphenols are protective in those cases. The present studies and available literature on the preventive effects of plant polyphenols on cancer risk (42,43,50) suggest the need to evaluate the potential preventive contribution of diets rich in polyphenols on breast cancer risk in women consuming varying amounts of alcohol.

Acknowledgments and Notes

This work was supported by grant FONCyT PICT/99 05–6045 and by the University of San Martin, Argentina. Address correspondence to J. A. Castro, CEITOX–CITEFA/CONICET, J.B. de La Salle 4397, B1603ALO Villa Martelli, Buenos Aires, Argentina. FAX: 54–11–4709–5911. E-mail: ceitox@dd.com.ar; postmast@ceitox.edu.ar.

Submitted 14 November 2003; accepted in final form 24 March 2004.

References

1. Stewart BW, Kleihues P: *World Cancer Report.* WHO, International Agency for Research on Cancer. Lyon, France: IARC Press, 2003.
2. Collaborative Group on Hormonal Factors in Breast Cancer: Alcohol, tobacco and breast cancer: collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *Br J Cancer* **87,** 1234–1245, 2002.
3. Sarkar DK, Liehr JG, Singletary KW: Role of estrogen in alcohol promotion of breast cancer and prolactinomas. *Alcohol Clin Exp Res* **25,** 230S–236S, 2001.
4. Singletary KW, Randall SR, Wen Y: Effect of ethanol on proliferation and estrogen receptor-α expression in human breast cancer cells. *Cancer Lett* **165,** 131–137, 2001.
5. Singletary KW, Gapstur SM: Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. *JAMA* **286,** 2143–2151, 2001.
6. Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA: Cytosolic xanthine oxidoreductase mediated bioactivation of ethanol

to acetaldehyde and free radicals in rat breast tissue. Its potential role in alcohol-promoted mammary cancer. *Toxicology* **160,** 11–18, 2001.
7. Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA: Rat breast microsomal biotransformation of ethanol to acetaldehyde but not to free radicals: its potential role in the association between alcohol drinking and breast tumor promotion. *Teratog Carcinog Mutag* **23**(Suppl 1), 61–70, 2003.
8. Triano EA, Slusher LB, Atkins TA, Beneski JT, Gestl SA, et al.: Class I alcohol dehydrogenase is highly expressed in normal human mammary eipithelium but not in invasive breast cancer: implications for breast carcinogenesis. *Cancer Res* **63,** 3092–3100, 2003.
9. Dellarco VL: A mutagenicity assessment of acetaldehyde. *Mutat Res* **195,** 1–20, 1988.
10. Lieber CS: Acetaldehyde and acetate. In *Medical and Nutritional Complications of Alcoholism. Mechanisms and Management*, Lieber CS (ed). New York: Plenum Medical, 1992, pp 37–54.
11. Woritersen RA, Appelman LM, Feron VJ, Van Der Heigden CA: Inhalation toxicity of acetaldehyde in rats. 3. Carcinogenicity study. *Toxicology* **41,** 213–232, 1984.
12. Woritersen RA, Appelman LM, Feron VJ, Zimmering S: Inhalation toxicity of acetaldehyde in rats II. Carcinogenicity study: interim results after 15 months. *Toxicology* **31,** 123–133, 1985.
13. Meng Q, Gai B, Goldberg ID, Rosen EM, Fan S: Stimulation of cell invasion and migration by alcohol in breast cancer cells. *Biochem Biophys Res Commun* **273,** 448–453, 2000.
14. Singletary KW, Frey RS, Yan W: Effect of ethanol on proliferation and estrogen receptor-α-expression in human breast cancer cells. *Cancer Lett* **165,** 131–137, 2001.
15. Izevbigie EB, Ekune SI, Jordan J, Howard CB: Ethanol modulates the growth of human breast cancer cells in vitro. *Exp Biol Med* **227,** 260–265, 2002.
16. Lin CM, Chen CS, Chen CT, Liang YC, Lin JK: Molecular modeling of flavonoids that inhibits xanthine oxidase. *Biochem Biophys Res Commun* **294,** 167–172, 2002.
17. Heim KE, Tagliaferro AR, Bobilya DJ: Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* **13,** 572–584, 2002.
18. Nagao A, Seki M, Kobayashi H: Inhibition of xanthine oxidase by flavonoids. *Biosci Biotechnol Biochem* **63,** 1787–1790, 1999.
19. Iio M, Ono Y, Kai S, Fukumoto M: Effect of flavonoids in xanthineoxidation as well as on cytochrome c reduction by milk xanthine oxidase. *J Nutr Sci Vitaminol* **32,** 635–647, 1986.
20. Vivot E, de Dios Muñoz J, del Carmen Cruanes M, Cruanes MJ, Tapia A, et al.: Inhibitory activity of xanthine-oxidase and superoxide scavenger properties of Inga verna subsp. affinis. Its morphological and micrographic characteristics. *J Etnopharmacol* **76,** 65–71, 2001.
21. Cos P, Ying M, ColommeJP, Hu K, Cimanga B, et al.: Structure activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. *J Nat Prod* **61,** 71–76, 1998.
22. Van Hoorn DEC, Nifveldt RJ, Van Leeuwen PAM, Hofman Z, Rabet LM, et al.: Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. *Eur J Pharmacol* **451,** 111–118, 2002.
23. Nishino T, Tsushima K: Interaction of milk xanthine oxidase with folic acid. Inhibition of milk xanthine oxidase by folic acid and separation of the enzyme into two fractions in sepharose 48/ folate gel. *J Biol Chem* **261,** 11242–11246, 1986.
24. Masana M, Toranzo EGD de, Castro JA: Reductive metabolism and activation of Benznidazole. *Biochem Pharmacol* **33,** 1041–1045, 1984.
25. Castro GD, Delgado de Layño AMA, Castro JA: Liver nuclear ethanol metabolizing systems (NEMS) producing acetaldehyde and 1-hydroxyethyl free radicals. *Toxicology* **129,** 137–144, 1998.
26. Diaz Gomez MI, Fanelli SL, Castro GD, Costantini MH, Castro JA: Liver nuclear ethanol metabolizing systems. Formation of metabolites that bind covalently to macromolecules and lipids. *Toxicology* **138**, 19–28, 1999.
27. Terada LS, Leff JA, Repine JE: Measurement of xanthine oxidase in biological tissues. *Methods Enzymol* **186**, 651–656, 1990.
28. Gad SC, Weil CS: Statistics for toxicologists. In *Principles and Methods of Toxicology*, Hayes AW, (ed). New York: Raven Press, 1992, pp 273–320.
29. Graph Pad Software: Graph Pad Instat. Instant Biostatistics. Graph Pad Software, Inc. San Diego, Ca, USA.
30. Rajagopalan KV: Xanthine oxidase and aldehyde oxidase. In *Enzymatic Basis of Detoxification, Volume I,* Jakoby WB (ed). New York: Academic Press, 1980, pp 295–310.
31. Guerri C, Sanchis R: Alcohol and acetaldehyde in rat's milk following ethanol administration. *Life Sci* **38,** 1543–1556, 1986.
32. Albert-Avila J, Schmidt EW, Nichols CG: Alcohols. In *Handbook of Medical Toxicology,* Viccellio P (ed). Boston: Little, Brown and Company, 1993, pp 616–621.
33. Kapucuoglu N, Coban T, Raunio H, Pelkonen O, Edwards RJ, et al.: Immunohistochemical demonstration of the expression of CYP2E1 in human breast tumour and non-tumour tissues. *Cancer Lett* **196,** 153–159, 2003.
34. Hellmold H, Rylander T, Magnusson M, Reihner E, Warner M, et al.: Characterization of cytochrome P450 enzymes in human breast tissue from reduction mammaplasties. *J Clin Endocrinol Metab* **83,** 886–895, 1998.
35. Frederiks WM, Bosch KS, Van der Munckhof RJM, Van Noorden CJFA: Quantitative histochemical study of xantine oxidase activity in rat liver using the cerium capture method in the presence of polyvinyl alcohol. *J Histochem Cytochem* **42,** 1091–1097, 1994.
36. Zhang S, Hunter DJ, Hankinson SE, Giovanucci FE, Rosner BA, et al.: A prospective study of folate intake and the risk of breast cancer. *JAMA* **281,** 1632–1637, 1999.
37. Rohan TE, Jain M, Miller AB: Alcohol consumption and risk of benign proliferative epithelial disorders of the breast: a case-cohort study. *Public Health Nutr* **1,** 139–145, 1998.
38. Sellers TA, Vierkant RA, Cerhan JR, Gapstur SM, Vachon CM, et al.: Interaction of dietary folate intake, alcohol, and risk of hormone receptor-defined breast cancer in a prospective study of postmenopausal women. *Cancer Epidemiol Biomarkers Prev* **11,** 1104–1107, 2002.
39. Sellers, TA, Kushi, LH, Cerhan, JR, Vierkant, RA, Gapstur SM, et al.: Dietary folate intake, alcohol and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology* **12,** 420–428, 2001.
40. Lucock M: Folic acid: nutritional biochemistry, molecular biology and role in disease processes. *Mol Gen Metabol* **71,** 121–138, 2000.
41. Kim S, Lee MJ, Hong J, Li C, Smith TJ, et al.: Plasma and tissue levels of tea catechins in rats and mice during chronic consumption of gree tea polyphenols. *Nutr Cancer* **37,** 41–48, 2000.
42. Yang CS, Maliakal P, Meng X: Inhibition of carcinogenesis by tea. *Annu Rev Pharmacol Toxicol* **42,** 25–54, 2002.
43. Moran TF, Klucas RV, Grayer RJ, Abian J, Becana M: Complexes of iron with phenolic compounds from soybean nodules and other legume tissues: prooxidant and antioxidant properties. *Free Radic Biol Med* **22,** 861–870, 1997.
44. Rasmussen SE, Miller Breinholt V: Non-nutritive bioactive food constituents of plants: bioavailability of flavonoids. *Int J Vitam Nutr Res* **73,** 101–111, 2003.
45. Adlercreutz H, Markkanen H, Watanabe S: Plasma concentrations of phytoestrogens in Japanese men and women consuming a traditional Japanese diet. *Am J Clin Nutr* **342,** 1209–1210, 1993.
46. Heim KE, Tagliaferro AR, Bobilya DJ: Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* **13,** 572–574, 2002.
47. Maciel ME, Castro GD, Castro JA: Effect of plant polyphenols on the rat breast microsomal and cytosolic bioactivation of ethanol (ETOH) to acetaldehyde. *Alcohol Clin Exp Res* **26,** 141A, 2002.
48. Riboli E, Norat T: Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr* **78,** 559S–569S, 2003.
49. Temple NJ, Gladwin KK: Fruit, vegetables and the prevention of cancer: research challenges. *Nutrition* **19,** 467–470, 2003.
50. Yang CS, Prabhu S, Landau J: Prevention of carcinogenesis by tea polyphenols. *Drug Metab Rev* **33,** 237–253, 2001.