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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

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We recently showed that mammary cytosolic xanthineoxidoreductase had the ability to bioactivate ethanol (EtOH) to acetaldehyde (AC) and free radicals. In the present study, we report that the microsomal fraction also biotransforms EtOH to AC. One pathway requires NADPH and the others do not. Both need oxygen. The NADPH-dependent pathway is not inhibited by $CO:O_2$ (80:20) or SKF 525A and that excludes the participation of cytochrome P450. It is inhibited by diethyldithiocarbamate (DDTC), sodium azide, and diphenyleneiodonium (DPI) but not by desferrioxamine, which suggests a possible role of a non-iron copper-requiring flavoenzyme. The process was partially inhibited by thiobenzamide (TBA), methylmercaptoimidazole (MMI), and nordihydroguaiaretic acid (NDG) but not by dapsone, aminotriazole, or indomethacin. These results suggest the potential participation of flavine monooxygenase and of lipooxygenase or of peroxidases/oxidases having similar characteristics but not of lactoperoxidase or cyclooxygenase. The pathway not requiring NADPH could also be partially inhibited by DDTC, NDG, azide, DPI, and TBA or MMI but not by the other chemicals. Little activity proceeds under nitrogen. Oxidases or peroxidases might be involved. No formation of 1-hydroxyethyl radicals was detected either in the presence or absence of NADPH. The nature of the EtOH bioactivating enzymes involved remains to be established. However, the fact remains that an activation of EtOH to AC was found in mammary tissue and could have a significant effect in some stages of the process of breast tumor promotion by EtOH. *Teratogenesis Carcinog. Mutagen. Suppl. 1:61–70, 2003.*

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INTRODUCTION

Breast cancer is the most common malignancy in women in the modern world [1, 2]. Epidemiologists have identified several risk factors for breast cancer. The incidence of breast cancer is related to genetic, physical, dietary, environmental, and hormonal factors [1–3]. The most studied environmental risk factors for breast cancer in humans are radiation exposure and alcohol ingestion [1, 4].

There is at present considerable evidence from epidemiological studies to support a positive association between alcohol intake and the risk of breast cancer [reviewed in references 3–5]. However, there is limited information regarding the possible mechanisms for this effect [3–5]. Several studies implicated endogenous hormones, particularly increases in estrogen levels, as the underlying biological determinants of alcohol-related breast cancer incidence [6]. However, it is thought that hormones fundamentally play a promotional role in breast carcinogenesis by stimulating mitotic division of already initiated cells [3]. Notwithstanding, the nature of the process is unknown. A role for chemical carcinogens in breast cancer is supported by the analysis of the mutational spectrum of the p53 gene [7, 8]. Recent studies from our laboratory, suggest one alternative possibility for explaining alcohol-related effects on breast cancer incidence. These studies provide evidence that breast cytosolic fractions are able to bioactivate ethanol to a mutagenic-carcinogenic chemical as is acetaldehyde and to hydroxyl and 1-hydroxyethyl (1HEt) free radicals, which might play a role in both the initiation and promotion of cancer [9].

In the present study, the occurrence of additional sources of metabolic activating pathways of ethanol to acetaldehyde but not to 1-hydroxyethyl free radicals is shown in the mammary tissue microsomal fraction.

MATERIALS AND METHODS

Chemicals

Absolute ethanol (analytical grade) was from Sintorgan (Argentina).

N-t-Butyl-α-phenylnitrone (PBN), N,O-bis(trimethylsilyl)trifluoracetamide (BSTFA), and the drugs tested for their effects on the metabolism of ethanol were from Sigma (St. Louis, MO): SKF 525A, thiobenzamide (TBA), N,N-diethyldithiocarbamic acid sodium salt (DDTC), 2-mercapto-1-methylimidazole (MMI), $NADP^+$, desferrioxamine mesylate (DFA), 3-amino-1,2,4-triazole (AT), diphenyleneiodonium chloride (DPI), nordihydroguaiaretic acid (NDG), dapsone, sodium azide, and indomethacin (IM). Nitrogen (ultra high purity) was from AGA (Argentina) and the carbon monoxide was from Matheson Co. Both gases were further deoxygenated by bubbling through a solution containing 0.05% 2-anthraquinone sulfonic acid sodium salt and 0.5% $Na_2S_2O_4$ in 0.1 M NaOH. The polyclonal antibody against rat liver microsomal P450 reductase was purchased from Gentest.

Animals and Treatments

Non-inbred female Sprague Dawley rats (220–260 g) were used. These were post-lactation young mothers (2 weeks after weaning of their pups). The animals were fasted for 12–14 h before being killed by decapitation and their mammary tissue was rapidly excised and processed. Water was available ad libitum. Microsomal fractions were obtained as previously described [10].

Metabolism of Ethanol to Acetaldehyde in Rat Breast Microsomes

Incubation mixtures contained mammary tissue (0.37 ± 0.08 mg prot/ml), NADPH generating system (0.45 mM $NADP^+$, 4 mM dl-isocitric acid trisodium salt, and 0.25 U of isocitric dehydrogenase) and 0.21 M ethanol in 50 mM KH_2PO_4, pH 7.4, in a final volume of 3 ml. Incubations were conducted for 1 h at 37°C under an air atmosphere. These 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 40°C for 10 min and an aliquot (100 µl) of the head space was analyzed by GC-FID [10–13].

Chromatographic conditions: column, Poraplot Q, 25 m × 0.53 mm i.d. (Chrompack, The Netherlands); temperature 140°C isothermal, injection port temperature: 150°C, FID: 200°C.

1-Hydroxyethyl Radicals Determination in In Vitro Biological Systems

The potential formation of 1HEt radicals was investigated via the prior formation of a spin adduct of the 1HEt radical and its detected ion by the GC/MS method described previously [11–13]. Selected ion monitoring (SIM) of mass spectrum of the adduct was employed to increase sensitivity. Procedures for sample preparation in the case of the Fenton or when using liver microsomes or nuclei are described elsewhere [11–13]. In experiments involving mammary tissue microsomal activation of ethanol, purified microsomes (0.4 to 1.2 mg protein per ml) were added to the NADPH generating system, 0.15 M $MgCl_2$, 18 mM PBN, and 0.21 M ethanol in 0.25 M sucrose-Tris-potassium-magnesium (STKM) buffer [11]. Incubation was performed under air atmosphere for 1 h at 37°C. In another experiment, incubation vials were purged with nitrogen and the reaction was run anaerobicaly [10]. After incubation, the volume (3 ml) was extracted with 500 µl toluene, centrifuged, and the organic layer evaporated under nitrogen. The residue was silylated with BSTFA:acetonitrile (1:1), 60°C, 15 min and analyzed by GC/MS-SIM. Chromatographic conditions were as follows: column, 5% phenylmethyl silicone, 12 m × 0.2 mm i.d., programmed from 100° to 300°C at a ramp of 10°C/min. Injection port was at 250°C and transfer line to MS, 300°C. Selected masses were 250 (M - $\cdot CHCH_3OTMS$) and 194 (m/z 250, C_4H_8). The dwell time was 50 ms for both masses selected.

Statistics

The significance of the difference between the two mean values was assessed by the Student's *t*-test [14].

RESULTS

Biotransformation of EtOH to AC in the Mammary Tissue Microsomal Fraction

Results depicted in Table I showed that the mammary tissue microsomal fraction was able to biotransform EtOH into AC in the presence (+NADPH) or absence of NADPH (−NADPH). Both processes were mostly enzymatic because about 83% of the +NADPH and about 91% of the −NADPH activities were destroyed by heating the breast microsomal fraction for 5 min at 100°C. The activity in the absence of NADPH was about 57% of that observed when NADPH was present. Oxygen from air was required for most of the +NADPH requiring process (~76% of it) and for 58% of the −NADPH (Table I).

The +NADPH requiring pathway was fully inhibited by metal chelators such as DDTC or sodium azide. The potent iron chelator DFA, however, did not inhibit the process. Further, in its presence the bioactivation of EtOH to AC was enhanced to 169%. The −NADPH pathway is strongly inhibited by DDTC and azide but remains unchanged by incubation with DFA. Neither the +NADPH or −NADPH metabolisms of EtOH to AC was inhibited by 1 mM SKF 52A; $CO:O_2$ (80:20); 30 μM indomethacin; 5 mM AT, or 1 mM dapsone (Table I). The polyclonal antibody against liver microsomal P450 reductase was not able to inhibit the biotransformation of ETOH to acetaldehyde.

TABLE I. Ethanol Biotransformation to Acetaldehyde by Mammary Tissue Microsomes

	Acetaldehyde (nmole)/protein (mg)	
Experimental[a]	+NADPH	−NADPH
Air	5.8±0.8	3.3±0.9
Heated (100°C, 5 min)	1.0±0.1	0.3±0.1
Nitrogen	1.4±0.1	1.4±0.1
$CO:O_2$ (80:20)	5.8±0.1	2.9±0.1
1 mM SKF 525A	6.0±0.1	3.2±0.2
Polyclonal antibody against P450 reductase	6.8±0.1	6.1±0.3[b]
1 mM DDTC	0.9±0.1	0.9±0.1
1 mM DFA	9.8±0.1	3.4±0.5
1 mM sodium azide	0.6±0.1	0.8±0.1
10 μM DPI/air	0.8±0.3	1.7±0.2
10 μM DPI/nitrogen	0.8±0.1	0.7±0.1
5 mM AT	5.4±0.3	3.0±0.1
1 mM dapsone*	5.3±0.1	3.4±0.1
30 μM indomethacin*	5.8±0.2	3.5±0.1
1 mM MMI**	4.6±0.2	2.2±0.1
1 mM TBA	2.7±0.1	2.1±0.2
10 μM NDG	2.6±0.1	2.1±0.2

[a]Incubation mixtures containing microsomal preparations (0.37±0.08 mg of microsomal protein/ml), NADPH generating system, and 0.21 M ethanol 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 for details. Each result is the mean of three separate samples.

[b]Corresponding to the antiserum, in the presence of NADPH.

*$P > 0.05$, when compared to "air+NADPH".

**$P < 0.05$, when compared to "air+NADPH".

Partial inhibitory effects of 1 mM MMI were observed for the +NADPH pathway (21% inhibition) and for the −NADPH metabolism of EtOH to AC (33% inhibition).

TBA (1 mM) inhibited 53% of the bioactivating ability of mammary tissue microsomes in the presence of NADPH and 36% in its absence (Table I).

The potent inhibitor of flavoproteins DPI inhibited the ability of mammary tissue microsomes to biotransform EtOH into AC either under air (86%) or under nitrogen (43%). In the absence of NADPH, DPI blocked 48% of the biotransformation process under air, and 50% in its absence.

The inhibitor of lactoperoxidase dapsone (1 mM), cyclooxygenase (30 μM indomethacin), and catalase (5 mM aminotriazole) did not inhibit either the +NADPH or the −NADPH biotransformation steps of EtOH by mammary tissue microsomes (Table I).

NDG 10 μM (an inhibitor of lipooxygenase) partially inhibited the +NADPH metabolism of EtOH to AC (55% inhibition) and 36% of the −NADPH process.

Attempt for Potential Detection of the Formation of 1HEt Radicals During EtOH Biotransformation to AC by Mammary Tissue Microsomes

In Figure 1a, the ability of the Fenton reagent [12, 13] to generate 1HEt from EtOH can be observed. In Figure 1b and c, the formation of 1HEt by the liver microsomal and nuclear fractions, in the presence of NADPH, can be observed.

No formation of 1HEt was detectable under the experimental conditions tested when mammary tissue microsomes were incubated in the presence of NAPDH, either under air or nitrogen atmosphere. Either no formation occurred or, if formed, they were below our detection level and below that observable when liver nuclear preparations were used (Fig. 1d).

DISCUSSION

The results reported here provided evidence for the existence of at least two general pathways of mammary tissue microsomal biotransformation to acetaldehyde. One is NADPH dependent and the other did not require NADPH. Both required the presence of oxygen from air and were of an enzymatic nature, since heating at 100°C for 5 min abolished most of the biotransformation process. In contrast to what it is known to occur in the case of liver microsomes [15], no formation of 1HEt or of hydroxyl radical was detected in our experiments.

The NADPH and air dependent pathways of mammary tissue ethanol metabolism to acetaldehyde did not involve the participation of any cytochrome P450 (P450) isoenzyme, as indicated by the lack of inhibitory effects of two general inhibitors of P450 mediated processes which were carbon monoxide/ air mixtures (80:20) or SKF 525A [16;17].

The potent inhibitory effects of DPI, a well-known inhibitor of flavoprotein-mediated metabolic processes [18–21], suggested that flavoenzymes were involved in the mammary tissue microsomal NADPH-dependent biotransformation of ethanol to acetaldehyde. A potential flavoenzyme candidate for consideration might be the flavin-dependepent monooxygenase system (FMO), as suggested by the inhibitory effects of two typical inhibitors of FMO-mediated biotransformations, which were

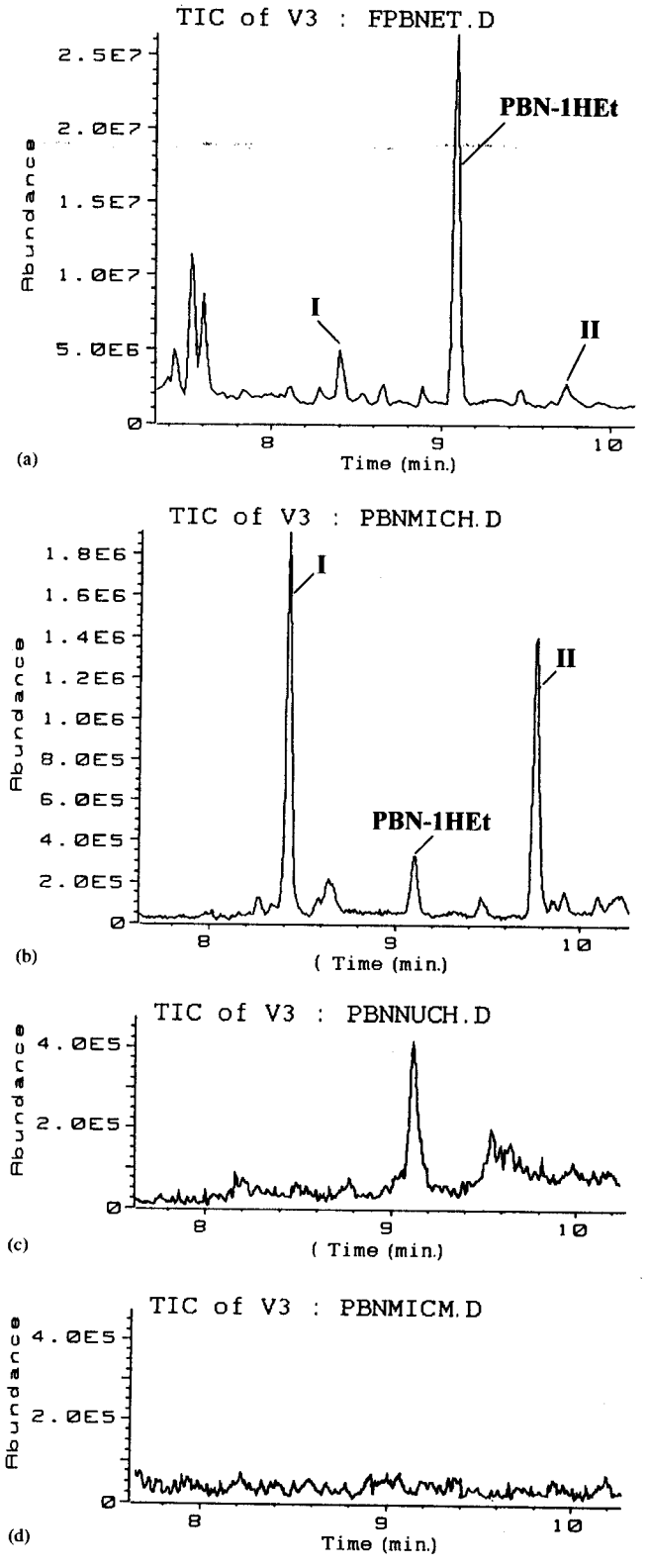
TIC of V3 : FPBNET.D
Abundance
2.5E7
2.0E7
1.5E7
1.0E7
5.0E6
0
PBN-1HEt
I
II
8
9
10
Time (min.)
(a)
TIC of V3 : PBNMICH.D
Abundance
1.8E6
1.6E6
1.4E6
1.2E6
1.0E6
8.0E5
6.0E5
4.0E5
2.0E5
0
I
II
PBN-1HEt
8
9
10
(Time (min.)
(b)
TIC of V3 : PBNNUCH.D
Abundance
4.0E5
2.0E5
0
8
9
10
(Time (min.)
(c)
TIC of V3 : PBNMICM.D
Abundance
4.0E5
2.0E5
0
8
9
10
Time (min.)
(d)

Figure 1.

MMI and TBA [22–25]. Other flavoenzymes might be either responsible or additionally be involved, since the DPI effect was far more potent than that of either one of the two FMO inhibitors. Those flavoenzymes, however, did not include P450 reductase, as could be anticipated from its known ability to activate ethanol to acetaldehyde aerobically and anaerobically [10]. In effect, the NADPH-dependent mammary tissue microsomal process was not inhibited by the polyclonal antibody against P450 reductase.

The participation of a metal-dependent process in this ethanol biotransformation pathway could be anticipated from the potent inhibitory effect of DDTC, an effective metal chelating agent [26]. The putative metal, however, was not iron because a very specific inhibitor of iron mediated processes such as desferrioxamine [27] does not inhibit the biotransformation of the NADPH dependent metabolism of ethanol in breast microsomes. The potent inhibitory effects of 1 mM sodium azide suggested that peroxidase-type of enzymes might be involved in the ethanol biotransformations. In effect, azide is a well-known inhibitor of peroxidases and oxidases [28;29]. The lack of inhibitory effects of dapsone, a specific inhibitor of lactoperoxidase [30–32], suggested that lactoperoxidase would not be involved in this ethanol metabolic process. Indomethancin, a specific inhibitor of cyclooxygenase [28, 33, 34] was not able to block the bioactivation of ethanol to acetaldehyde. That excluded the participation of this enzyme in this mammary tissue ethanol microsomal metabolism. Nordihydroguaiaretic acid (NDG), in contrast, inhibited about half of the mammary tissue microsomal alcohol metabolism. This might suggest that lipooxygenase could be one enzyme at least partially involved in the mammary tissue microsomal metabolism of ethanol. In effect, NDG is a specific inhibitor of this enzyme [28, 34, 35]. The nature of these metal- and flavoenzyme-mediated processes remains to be established.

The microsomal metabolism of ethanol not requiring NADPH, but having a need for oxygen, is far less clear to us at present than the one that was NADPH-dependent. Non-iron metals were also involved, as evidenced by the inhibitory effects of DDTC [26, 27] but not of DFA. Again, part of the process appears to require a flavoenzyme since DPI partially inhibits this process [19–21]. A partial inhibitory effect of two inhibitors of FMO, MMI, and TBA [22–25] was also observed. This was unexpected for an NADPH-independent pathway and this result might point to the existence of other enzymes not requiring NADPH but having an equivalent behavior concerning inhibitory effects of MMI and TBA. These two sulfur-containing compounds could have been acting on other oxidizing enzymes (e.g., oxidases or peroxidases or other) [28, 36–38]. These peroxidases might also

Fig. 1. **a:** Gas chromatogram obtained from a sample of the reaction mixture containing PBN and ethanol in Fenton system after trimethylsilylation. Peaks: I and II, derived from PBN-hydroxyl adduct; PBN-1HEt, trimethylsilylated spin adduct of 1-hydroxyethyl radical. See reference 13 for details. **b:** Selected ion current (SIM) profile obtained from the analysis of a sample of an incubation mixture containing liver microsomes, NADPH, ethanol, and PBN, after trimethylsilylation. Masses selected for SIM are 250 and 194. See reference 13 for details. **c:** SIM profile obtained from the analysis of a sample of an incubation mixture containing liver nuclear suspensions, NADPH, ethanol and PBN, after trimethylsilylation. Masses selected for SIM are 250 and 194. See reference 11 for details. **d:** SIM profile obtained from the analysis of a sample of an incubation mixture containing breast microsomes, NADPH, ethanol, and PBN (under air atmosphere), after trimethylsilylation. Masses selected for SIM are 250 and 194. See Materials and Methods for details.

have been responsible for the NADPH and oxygen pathway of ethanol biotransformation. If that was the case, NADPH and oxygen might have been needed, e.g., to generate the hydrogen peroxide necessary for them to operate [28]. They need to be identified in future studies.

The lack of formation of 1HEt in all of the mammary tissue microsomal biotransformations reported here suggest that the processing enzymes did not involve free radical intermediate forms. In the case of FMO, that is known to be the case. In effect, the FMO active form is a FAD hydroxyperoxide of the enzyme [22–25]. Alternatively, our detection system may not have sufficient sensitivity to detect them under the experimental conditions used or they were consumed by reacting with other molecules present in the microsomal samples. Regardless of the complete knowledge of the enzymology of these mammary tissue microsomal ethanol biotransformation processes, the fact remains that mammary tissue microsomes were able to biotransform ethanol to acetaldehyde, a very well-known mutagen and carcinogen [39–41]. These microsomal bioactivating pathways and the one previously reported by our laboratory in which the mammary tissue cytosolic fraction mediated by xanthine oxidoreductase occurred [9], might potentially provide additional rational links to the possible susceptibility of women consuming alcohol and to the risk of breast cancer. These mechanisms may operate separately and/or in conjunction with others postulated by other workers [41–45].

REFERENCES

1. Sasco AJ. Epidemiology of breast cancer: an environmental disease? APMIS 2001;109:321–332.
2. Parkin DM. The global burden of cancer. Seminars Cancer Biol 1998;8:219–235.
3. American Institute for Cancer Research: Food, nutrition and the prevention of cancer. A global perspective. Washington, DC: WCRF and AICR; 1997. p 252–287. .
4. Wolff MS, Weston A. Breast cancer risk and environment. Environ Health Perspect 1997;105 (Suppl 4):891–896.
5. Longnecker MP. Alcoholic beverage consumption in relation to risk of breast cancer: meta-analysis and review. Cancer Causes Control 1994;5:73–82.
6. Pike MC, Kraito MD, Henderson BE, Casagrande JT, Hoel DG. Hormonal risk factors, beast tissue age and the age-incidence of breast cancer. Nature 1983;303:767–770.
7. Biggs PJ, Warren W, Venitt S, Stratton MR. Does a genotoxic carcinogen contribute to human breast cancer? The value of mutational spectra in unraveling the etiology of cancer. Mutagenesis 1993;8: 275–283.
8. Eyfgord JE, Thoriacins S, Steinarsdottir M. p53 abnormalities and genomic instability in primary human breast carcinomas. Cancer Res 1995;55:646–651.
9. 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 2001;160:11–18.
10. Díaz Gómez MI, Castro GD, Delgado de Layño AMA, Costantini MH, Castro JA. Cytochrome P450 reductase-mediated anaerobic biotransformation of ethanol to 1-hydroxyethyl free radicals and acetaldehyde. Toxicology 2000;154:13–122.
11. Castro GD, Delgado de Layño AMA, Castro JA. Liver nuclear ethanol metabolizing system (NEMS) producing acetaldehyde and 1-hydroxyethyl free radicals. Toxicology 1998;129:137–144.
12. Castro GD, Delgado de Layño AMA, Castro JA. Hydroxyl and 1-hydroxyethyl free radical detection using spin traps followed by derivatization and gas chromatography-mass spectrometry. Redox Report 1997;3:343–347.
13. Castro JA, Castro GD. Hydroxyl and 1-hydroxyethyl radical detection by spin trapping and GC-MS. In: Amstrong D, editor. Oxidative stress and antioxidant protocols. Part I: techniques for free radical derived biomarkers. Methods in Molecular Biology series. Totowa, NJ: Humana Press; 2001. p 89–99.

14. Gad SC, Weil CS. Statistics for toxicologists. In: AW Hayes, editor. Principles and methods in toxicology. New York: Raven Press; 1982. p 273–320.
15. Lieber CS. The metabolism of alcohol and its implications for the pathogenesis of disease. In: Preedy VR, Watson RR, editors. Alcohol and the gastrointestinal tract. Boca Raton: CRC Press; 1996. p 19–39.
16. Guenguerich FP. Human cytochrome P450. In: Ortiz de Montellano PR, editor. Cytochrome P450: structure, mechanism and biochemistry, 2nd ed. New York: Plenum Press; 1995. p 473–535.
17. Omiecinski C, Remmel RP, Hosagrahara V. Concise review of the cytochrome P450s and their role in Toxicology. Toxicol Sci 1999;48:151–156.
18. Stuehr DJ, Fasehum OA, Kwon NS, Gross SS, Gonzalez JA, Levi R, Nathan CF. Inhibition of macrophage and endothelial cell nitric oxide synthase by diphenyleneiodonium and its analogs. Faseb J 1991;5:98–103.
19. Donssiere J, Gaillard J, Vigneis PV. The heme component of the neutrophil NADPH complex is a target for aryliodonium compounds. Biochemistry 1999;38:3694–3703.
20. O'Donnell VB, Tew DG, Jones OT, England PJ. Studies of the inhibition mechanisms of iodonium compounds with special reference to neutrophil NADPH oxidase. Biochem J 1993;290:41–49.
21. McGuire JJ, Anderson DJ, McDonald BJ, Narayanasami R, Bennett BM. Inhibition of NADPH cytochrome P450 reductase and glyceryl trinitrate biotransformation by diphenyleneiodonium sulfate. Biochem Pharmacol 1998;56:881–893.
22. Hodgson E, Cherrington NJ, Philpot RM, Rose RL. Biochemical aspects of flavin containing monooxygenase (FMOs). In: Arinç E, Schenkman JB, Hodgson E, editors. Molecular and applied aspects of oxidative drug metabolizing enzymes. New York: Plenum Press; 1991. p 55–70.
23. Ziegler DM. S-oxygenase, I. Chemistry and biochemistry. In: Damani LA, editor. Sulphur containing drugs and related organic compounds: chemistry, biochemistry and toxicology, Vol 2. Chichester: Ellis Horwood Publishers; 1989. p 53–66.
24. Poulsen LL, Ziegler DM. Multisubstrate flavin-containing monooxygenase: applications of mechanism to specificity. Chem Biol Interact 1995;96:57–73.
25. Elfarra AA. Potential role of the flavin-containing monooxygenase in the metabolism of endogenous compounds. Chem Biol Interact 1995;96:47–55.
26. Cheng KL, Veno K, Imamura T. CRC handbook of organic analytical reagents. Boca Raton: CRC Press Inc. 1982.
27. Halliwell B. Use of desferrioxamine as a probe for iron-dependent formation of hydroxyl radicals. Biochem Pharmacol 1985;34:229–233.
28. O'Brien PJ. Peroxidases. Chem Biol Interact 2000;129:113–139.
29. Kalyanaraman B, Janzen EG, Mason RF. Spin trapping of the azidyl radical in azide/ catalase/H_2O_2 and various azide/peroxidase/H_2O_2 peroxidazing systems. J Biol Chem 1985;260:4003–4006.
30. Doerge DR. Mechanism-based inhibition of lactoperoxidase by thiocarbamide goitrogens. Bichemistry 1986;25:4724–4728.
31. Shin K, Tamita M, Lonnerdal B. Identification of lactoperoxidase in mature human milk. J Nutr Biochem 2000;11:94–102.
32. Shin K, Hayawawa H, Lonnerdal B. PCR cloning and baculovirus expression of human lactoperoxidase and myeloperoxidase. Biochem Biophys Res Commun 2000;271:831–836.
33. Capasso A, Sorrentino L. Arachidonic acid and its metabolites are involved in the expression of morphine dependence in guinea-pig isolated ileum. Eur J Pharmacol 1997;330:199–204.
34. Salari H, Braquet P, Borgeat P. Comparative effects of indomethacin, acetylenic acids, 15-HETE, nordihydroguaiaretic acid and BW755C in the metabolism of arachidonic acid in human leukocytes and platelets. Prostaglandins Leukot Med 1984;13:53–60.
35. Natarajan R, Nadler J. Role of lipoxygenases in breast cancer. Funt Biosci 1998;3:E81–E88.
36. Lagorce JF, Moulard T, Rousseau A, Comby F, Buxeraud J, Raby C. Anti-inflammatory action of methimazole. Pharmacology 1997;55:173–178.
37. Petry TW, Eling TE. The mechanism for the inhibition of prostaglandin H synthase-catalyzed xenobiotic oxidation by methimazole. Reaction with free radical oxidation products. J Biol Chem 1987;262:14112–14118.
38. Bandyopadhyay V, Batthacharyya DK, Chatterjee R, Banerfee RK. Irreversible inactivation of lactoperoxidase by mercaptomethylimidazole through generation of a thiyil radical: its use as a probe to study the active site. Biochem J 1995;306:751–757.
39. Dellarco VL. A mutagenicity assessment of acetaldehyde. Mutat Res 1988;195:1–20.

40. Woritersen RA, Appelman IM, Feron VJ, Van der Heigden CH. Inhalation toxicity of acetaldehyde in rats. 3. Carcinogenicity study. Toxicology 1984;41:213–232.
41. Woritersen RA, Appelman IM, Feron VJ, Zimmering S. Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study. Interior results after 15 months. Toxicology 1985;31:123–133.
42. Singletary K. Ethanol and experimental breast cancer. A review. Alcohol Clin Exp Res 1997;21: 334–339.
43. Wright RM, McManaman JL, Repine JE. Alcohol-induced breast cancer: A proposed mechanism. Free Rad Biol Med 1999;26:348–354.
44. Ginsburg ES. Estrogen, alcohol and breast cancer. J Steroid Biochem Mol Biol 1999;69:299–306.
45. Chabra SK, Anderson LM, Perella C, Desai D, Amin S, Kyrtopoulos SA, Souliotis VL. Coexposure to ethanol with N-Nitrosodimethylamine or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone during lactation of rats: Marked increase in O^6-methylguanine-DNA-adducts in maternal mammary gland and in suckling lung and kidney. Toxicol Appl Pharmacol 2000;169:191–200.