Pergamon

Biochemical Pharmacology, Vol. 49, No. 11, pp. 1703–1707, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00083-6

EFFECT OF EUGENOL ON DRUG-METABOLIZING ENZYMES OF CARBON TETRACHLORIDE-INTOXICATED RAT LIVER

PARASAKTHY KUMARAVELU,* DEEPALAKSHMI P. DAKSHINAMOORTHY,* SHANTHI SUBRAMANIAM,* HALAGOWDER DEVARAJ† and NIRANJALI S. DEVARAJ*‡

Departments of *Biochemistry and †Zoology, University of Madras, Madras-600 025, India

(*Received* 21 *June* 1994; *accepted* 1 *November* 1994)

Abstract—The chemoprotection extended by eugenol against carbon tetrachloride (CCl_4) intoxication was established by studies on drug-metabolizing phase I and phase II enzymes. An overall decrease in drug-metabolizing enzymes, namely NADPH-cytochrome *c* reductase, NADH-cytochrome reductase, coumarin hydroxylase, 7-ethoxy coumarin-*O*-deethylase, UDP-glucuronyltransferase and glutathione-*S*-transferase, was observed with CCl_4 intoxication, with a subsequent decrease in cytochrome P450 and cytochrome b_5 content. CCl_4 caused a significant decrease in microsomal phospholipids and the marker enzymes glucose-6-phosphatase and 5′-nucleotidase, and an increase in thiobarbituric acid reactive substances (TBARS). Simultaneous administration of eugenol with CCl_4 inhibited the accumulation of TBARS and the decrease in the microsomal phospholipids and marker enzymes. Further, the chemical onslaught imposed by CCl_4 on the drug-metabolizing system was removed successfully by eugenol. Eugenol appears to act as an *in vivo* antioxidant and as a better inducer of phase II enzymes than phase I enzymes. It is therefore suggested that eugenol could be an interesting basic structure for drug design.

Key words: lipid peroxidation; eugenol; CCl_4; microsomes; phospholipids; antioxidant

Hepatic diseases are always associated with impaired drug metabolism, and this may lead to toxic complications of drug therapy. [1]. Experimental cirrhosis induced by chronic administration of carbon tetrachloride (CCl_4) to rats resulted in lowered levels of hepatic cytochrome P450, the principal component of MFO§ [2] of the phase I drug-metabolizing system. A decrease in UDP-GT, the predominant phase II drug-metabolizing enzyme, upon acute exposure to CCl_4 was also reported by Deliconstantinos *et al.* [3]. The rates of both hydroxylation and glucuronidation are diminished markedly in hepatitis and hepatocirrhosis [4].

The hepatoprotective action of various plant extracts has been known for several years [5, 6]. Eugenol (4-allyl-1-hydroxy-2-methoxybenzene), an allyl benzene, is an active principle of various plant extracts, namely *Ocimum*, clove and nutmeg. It is widely used as a food-flavouring agent, but thus far the medicinal application of pure eugenol has been limited to the field of dentistry [7, 8].

We have reported the hepatoprotective action of eugenol against CCl_4-induced hepatic damage [9]. Eugenol was reported to act as an *in vitro* antioxidant [10, 11]. However, one single mechanism, namely the antioxidant property alone, cannot account for the chemoprotection extended by eugenol. Its action on the drug-metabolizing system is suggested as another mode of protection. Eugenol was reported to induce phase II drug-metabolizing enzymes, viz. UDP-GT, GST [12, 13] and DT-diaphorase [14]. Hence, a biochemical approach was undertaken to determine the effect of eugenol on the drug-metabolizing system of the CCl_4-intoxicated rat model.

MATERIALS AND METHODS

Chemicals. Eugenol, TBA, 1,1,3,3-tetramethoxypropane, UDP-glucuronic acid, NADP, NADPH, glucose-6-phosphate, isocitrate dehydrogenase and *p*-nitrophenol were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). CCl_4 and olive oil were obtained from S.D. Fine-Chemical Limited (India). All other chemicals used were of analytical grade.

Animals. Male Wistar rats, weighing 100–120 g, were purchased from the Frederick Institute of Plant Protection and Toxicology, Padappai, India, and housed in a well-aerated room and acclimatized to the laboratory conditions for 1 week.

Experimental design. Animals were divided into four groups, and the dosage for eugenol [9] and CCl_4 [15] administration was fixed as reported earlier. Olive oil was used as a carrier, and both eugenol (10.7 mg/kg body weight/day) for 14 consecutive days) and CCl_4 (1.195 g/kg body weight three times a week for 2 weeks) were injected intraperitoneally.

‡ Corresponding author: Niranjali S. Devaraj, Ph.D., 9/2, III Main Road, Gandhi Nagar, Adyar, Madras - 600 020, India. Tel. 91-44-419596; FAX 91-44-415856.

§ Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GST, glutathione transferase; MDA, malondialdehyde; MFO, mixed-function oxidase; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; and UDP-GT, UDP-glucuronyltransferase.

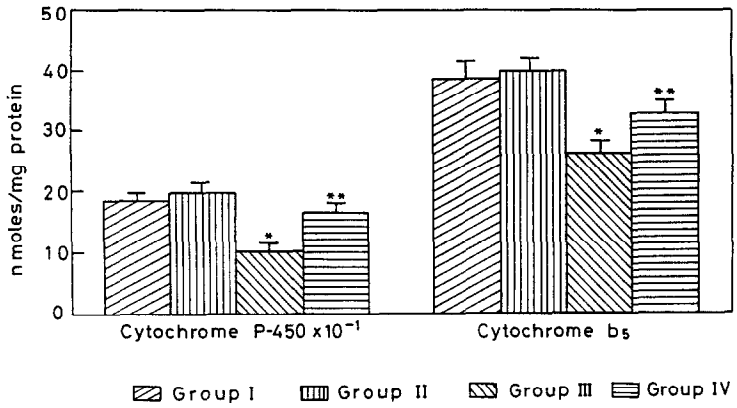

Fig. 1. Effect of eugenol on hepatic cytochrome P450 and cytochrome b_5 of CCl_4-intoxicated rats. Values are means ± SD, N = 6. Key: (*) $P < 0.001$ compared with Group I, and (**) $P < 0.001$ compared with Group III.

Group I: control animals (receiving only olive oil); Group II: eugenol control rats (treated with eugenol in olive oil); Group III: animals receiving CCl_4 in olive oil; and Group IV: animals treated simultaneously with CCl_4 and eugenol by two separate injections.

Microsomal preparation. The animals were fasted overnight and were killed by cervical dislocation, 24 hr after the last injection. The liver was thoroughly perfused *in situ* by way of the portal vein with phosphate-buffered saline (0.1 M, pH 7.2) and removed immediately. It was homogenized in 4 vol. of ice-cold medium, containing 1.15% KCl-0.02 M Tris–HCl buffer, pH 7.4. The hepatic microsomes were prepared by differential centrifugation [16]. The homogenate was centrifuged for 15 min at 9000 *g*. This supernatant was centrifuged at 105,000 *g* for 60 min to obtain the microsomes. The pellet was resuspended in 1.15% KCl-0.02 M Tris–HCl buffer. The supernatant represents the soluble cytosolic fraction. Activity of succinate dehydrogenase was assayed in the pellet to evaluate mitochondrial contamination, if any.

Analytical procedures. Microsomal and cytosolic protein were estimated by the method of Lowry *et al.* [17], using bovine serum albumin as the standard. TBARS were determined by the method of Ohkawa *et al.* [18]. Microsomal lipids were extracted by the method of Folch *et al.* [19]. Microsomal phospholipid [20, 21] and cholesterol [22] content were quantitated. The microsomal marker enzymes glucose-6-phosphatase [23] and 5′-nucleotidase [24] were assayed.

Microsomal cytochrome P450 and cytochrome b_5 contents were determined by the method of Omura and Sato [25]. Cytochrome P450-related enzymes, coumarin hydroxylase and 7-ethoxy coumarin-*O*-deethylase were assayed fluorimetrically as described by Aitio [26]. NADPH-cytochrome *c* reductase activity was assayed at 25° by measuring the rate of reduction of cytochrome *c* at 550 nm [27]. NADH-cytochrome reductase was assayed according to Strittmatter and Velick [28]. Microsomal UDP-GT activity was assayed by following the disappearance of *p*-nitrophenol upon glucuronidation [29, 30]. Cytosolic GST activity was determined using CDNB as a substrate as described by Habig *et al.* [31].

Statistical analysis. Data are presented as means ± SD. Statistical differences were calculated by one-way ANOVA, followed by Student's Newman–Keuls test.

RESULTS

Effects on phase I drug-metabolizing system. Figure 1 depicts the levels of cytochrome P450 and cytochrome b_5 content in the control and experimental animals. CCl_4 intoxication caused a reduction of both cytochrome P450 and b_5, but the simultaneous treatment with eugenol inhibited the decrease of these phase I components.

Other phase I enzymes, viz. NADH-cytochrome b_5 reductase, NADPH-cytochrome *c* reductase, 7-ethoxy coumarin-*O*-deethylase and coumarin hydroxylase were also found to be inhibited ($P < 0.001$) by CCl_4 administration when compared with controls (Table 1), indicating that there is a partial loss of phase I drug metabolism. Simultaneous administration of eugenol maintained the activities of phase I enzymes at near normal levels.

Indicators of microsomal damage and microsomal lipid alterations. The microsomal damage by CCl_4 intoxication and the protection extended by eugenol were studied based on TBARS production, microsomal marker enzymes glucose-6-phosphatase and 5′-nucleotidase, and microsomal lipids. Eugenol successfully protected against CCl_4-induced peroxidative damage by preventing both the accumulation of TBARS and damage to the microsomal lipids (Table 2); the membrane-bound enzyme activities were at near normal levels (Table 3).

Activities of phase II enzymes. The detoxification capacity of CCl_4-poisoned rats was diminished significantly, as evidenced by the decreased activities of UDP-GT and GST. Simultaneous treatment of eugenol with CCl_4 prevented almost completely the effects of CCl_4 on these phase II enzymes (Table 4).

Table 1. Effect of eugenol on phase I drug-metabolizing enzymes of CCl_4-intoxicated rats

	NADH-cytochrome b_5 reductase (U/mg protein)	NADPH-cytochrome *c* reductase (U/g protein)	7-Ethoxy coumarin-*O*-deethylase (U/mg protein)	Coumarin hydroxylase (U/mg protein)
Group I	0.597 ± 0.03	101.94 ± 6.28	597 ± 19.29	81.81 ± 6.62
Group II	0.514 ± 0.06	104.88 ± 5.53	589 ± 6.71	78.03 ± 4.30
Group III	0.243 ± 0.01*	61.43 ± 2.91*	170 ± 12.02*	43.54 ± 3.43*
Group IV	0.575 ± 0.02†	87.51 ± 3.33†	575 ± 10.24†	64.71 ± 4.04†

Values are means ± SD for six animals.
* $P < 0.001$, compared with Group I.
† $P < 0.001$, compared with Group III.

Table 2. Effect of eugenol on microsomal TBARS and lipids of CCl_4-intoxicated rats

	TBARS (nmol MDA/mg protein)	Microsomal lipids Cholesterol (C) (μg/mg protein)	Phospholipid (P) (μg/mg protein)	C/P
Group I	454.79 ± 30.07	36.53 ± 2.36	168.97 ± 5.45	0.217
Group II	467.92 ± 22.35	36.26 ± 2.73	174.24 ± 7.48	0.208
Group III	1043.23 ± 54.92*	34.36 ± 2.17†	141.43 ± 11.02*	0.244
Group IV	616.96 ± 15.23‡	34.86 ± 2.05NS§	162.84 ± 6.68‡	0.215

Values are means ± SD for six animals.
* $P < 0.001$, compared with Group I.
† $P < 0.05$, compared with Group I.
‡ $P < 0.001$, compared with Group III.
§ NS = not significant.

Table 3. Effect of eugenol on microsomal marker enzymes of CCl_4-intoxicated rats

	5′-Nucleotidase (U/mg protein)	Glucose-6-phosphatase (U/mg protein)
Group I	30.60 ± 2.32	3.73 ± 0.20
Group II	31.42 ± 2.27	3.46 ± 0.38
Group III	22.26 ± 1.55*	2.71 ± 0.17*
Group IV	30.96 ± 1.47†	3.72 ± 0.27†

Values are means ± SD for six animals.
* $P < 0.001$, compared with Group I.
† $P < 0.001$, compared with Group III.

A highly significant increase in the activities of UDP-GT and GST was observed in rats treated with only eugenol (group II) when compared with control rats (group I), indicating the ability of eugenol to induce the activities of UDP-GT and GST.

DISCUSSION

Our study shows the *in vivo* protective action of eugenol on rat liver microsomes intoxicated with CCl_4 by inducing the drug-metabolizing enzymes and by acting as an *in vivo* antioxidant. CCl_4 intoxication causes damage not only to cytochrome P450 [2], UDP-GT [3] and GST [15], as reported earlier, but also to other cytochrome P450-associated enzyme systems.

Table 4. Effect of eugenol on phase II drug-metabolizing enzymes of CCl_4-intoxicated rats

	UDP-GT (nmol/min/mg protein)	GST (nmol CDNB/min/mg protein)
Group I	25.09 ± 2.25	910.42 ± 39.25
Group II	29.60 ± 3.21*	1939.68 ± 101.25*
Group III	23.35 ± 2.10†	471.97 ± 22.39*
Group IV	28.09 ± 2.06‡	1025.78 ± 54.61‡

Values are means ± SD for six animals.
* $P < 0.001$, compared with Group I.
† $P < 0.01$, compared with Group I.
‡ $P < 0.001$, compared with Group III.

It has been hypothesized that CCl_4 undergoes reductive oxidation at cytochrome P450 of liver microsomes to produce reactive $CCl_3\cdot$ [32], which, in turn, transfers one electron either to molecular oxygen, forming superoxide anion, or to a microsomal lipid molecule, forming lipid radicals, or reacts with O_2 to give trichloromethylperoxy radical. These reactive species would interact readily with unsaturated membrane lipids to produce lipid peroxidation [33].

Effect on the phase I drug-metabolizing system. During the bio-transformation of CCl_4 in the microsomal MFO, the reducing equivalents of NADPH are transferred through cytochrome P450 reductase to cytochrome P450 via cytochrome b_5. In general, during this process a certain amount of superoxide ions is released within the system [34]. Will [35] had hypothesized that the system involved in lipid peroxidation at least partially resembles the drug-hydroxylating system, and approximate parallelism was observed between the formation of MDA and the loss of microsomal enzymes and cytochrome P450 [36].

The observed decrease in the cytochrome P450, cytochrome b_5, hydroxylating and oxidizing enzymes (Fig. 1 and Table 1) in CCl_4-intoxicated rats appears to be due either to damage to membrane lipids by free radicals or to a complex formation with lipid peroxides and $CCl_3\cdot$. Organo halogen compounds have been shown to undergo covalent binding to rabbit liver microsomal protein following their microsomal metabolism [37]. Murray and Farrell [38] have reported a reduction of the content of P450 undergoing spectral changes during hepatic cirrhosis induced by CCl_4.

A well-known antioxidant, GSH, chelating agents [36] and free radical trapping agents [39] have been reported to preserve cytochrome P450 by blocking lipid peroxidation. Similarly, simultaneous administration of eugenol maintained the cytochrome P450 and phase I components during CCl_4 intoxication, which supports its antioxidant nature.

Protection to microsomes. The decreased activities of the microsomal marker enzymes (Table 3) 5′-nucleotidase and glucose-6-phosphatase during CCl_4 poisoning confirm the microsomal damage. Though these two enzymes are active in two different metabolic pathways, their functional integrity depends on the chemical composition and physical status of the lipid environment where they are embedded [40]. The decrease in microsomal phospholipids due to an increase in phospholipase A_2 [41] and C [42] and increased lipid peroxidation (Table 2) in CCl_4-intoxicated rats could be the reason for the decreased enzyme activities.

Koster and Slee [43] have demonstrated the inactivation of glucose-6-phosphatase during increased lipid peroxidation. They further emphasized that glucose-6-phosphatase is located in an environment containing more polyunsaturated fatty acid. Since this location lies closely associated with NADPH-cytochrome P450 reductase, there is a reasonable possibility that highly reactive radicals formed at cytochrome P450 diffuse quickly from their site of formation [44] and inactivate glucose-6-phosphatase.

Eugenol extends protection against both microsomal damage and accumulation of toxic lipid peroxidation products. This is evident from the unaltered activities of membrane-bound enzymes and the reversed C/P ratio and the decreased lipid peroxides (Table 2) in the group IV rats. This suggested that eugenol could act as an *in vivo* antioxidant at the microsomal level and thereby diminish the peroxidative action of the free radicals on the membrane.

Effects on phase II enzymes. In our experiments, the observed striking decrease in the activities of UDP-GT and GST suggested a total inhibition of drug metabolism during CCl_4 intoxication. Again, there is considerable evidence indicating that UDP-GT is constrained to phospholipids of the microsomal membrane [45]. Hence, the observed decrease may be due to the peroxidative damage to the microsomal lipids. The significant increase in UDP-GT and GST in those animals treated only with eugenol (group II) shows the overall ability of eugenol to induce the phase II drug-metabolizing system. It appears that eugenol is a more effective inducer of phase II than phase I enzymes. The increased activity of GST with eugenol may be due to increased expression of phenolic antioxidant responsive element, which results in the induction of GST subunit 1 gene without a concomitant induction of cytochrome CYP1A1 [46].

The simultaneous treatment of eugenol with CCl_4 is found to curtail the toxic effects of CCl_4 (group IV). This may be: (i) by acting as an *in vivo* antioxidant and thereby inhibiting the initiation and the promotion of lipid peroxidation and its subsequent toxic effects on microsomes, or (ii) by an accelerated scavenging of toxic free-radicals and their products by conjugation with GSH or glucuronic acid with the help of increased GST and UDP-GT, respectively.

In conclusion, it is suggested that eugenol could be considered as a basis for drug design and/or as a co-drug for any therapeutic agent that may cause hepatic damage as a side-effect either by lipid peroxidation or by interacting with drug-metabolizing enzymes.

REFERENCES

1. Hoyumpa JAM, Greene HL, Dewey DL and Schenker S, Fatty liver: Biochemical and clinical considerations. *Am J Dig Dis* **20**: 1142–1170, 1975.
2. Marshall WJ and McLean AEM, Effect of cirrhosis of the liver on microsomal detoxications and cytochrome P-450. *Br J Exp Pathol* **30**: 578–583, 1969.
3. Deliconstantinos G, Mykoniatis M and Papadimitriou D, Carbon tetrachloride modulates the rat hepatic microsomal UDP-glucuronyl transferase activity and membrane fluidity. *Experientia* **42**: 181–183, 1986.
4. Kazuhisa R, Drug metabolism in hepatic insuffiency in hepatitis and hepatocirrhosis. *Nippon Rinsho Taisha Gakkai Kiroku* **12**: 44–48, 1975.
5. Ansari RA, Tripathi SC, Patnaik GK and Dhawan BN, Antihepato toxic properties of picroliv: An active fraction from rhizomes of *Picrorhizo kurrooa*. *J Ethanopharmacol* **34**: 61–68, 1991.
6. Leng Peschlow E and Strenge-Hesse A, Die Mariendistel (*Silybum marianum*) und Silymarin als

lebertherapeutikum. *Z Phytotherapie* **12**: 162–174, 1991.
7. US Food and Drug Administration, *Scientific Literature: Review of Eugenol and Related Substances in Flavor Usage*, pp. 1–134. US Food and Drug Administration, Rockville, MD, 1978.
8. IARC Working Group on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Eugenol. *IARC Monogr Eval Carcinog Risk Chem Hum* **36**: 75–97, 1985.
9. Parasakthy K, Deepalakshmi PD, Shanthy S and Niranjali SD, The hepatoprotective action of eugenol. *Med Sci Res* **21**: 611–612, 1993.
10. Nagababu E and Lakshmaiah N, Inhibitory effect of eugenol on non-enzymatic lipid peroxidation in rat liver mitochondria. *Biochem Pharmacol* **43**: 2393–2400, 1992.
11. Pulla Reddy ACh and Lokesh BR, Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol Cell Biochem* **111**: 117–124, 1992.
12. Yokota H, Hashimoto H, Motoya M and Yuasa A, Enhancement of UDP-glucuronyltransferase, UDP-glucose dehydrogenase and glutathione *S*-transferase activities in rat liver by dietary administration of eugenol. *Biochem Pharmacol* **37**: 799–802, 1988.
13. Zheng GQ, Kenney PM and Lam LKT, Sesquiterpenes from clove (*Eugenia caryophyllata*) as potential anticarcinogenic agents. *J Nat Prod* **55**: 999–1003, 1992.
14. Rompelberg CJM, Verhagen H and Van Bladeren PJ, Effects of the naturally occurring alkenylbenzenes eugenol and *trans*-anethole on drug-metabolising enzymes in the rat liver. *Food Chem Toxicol* **31**: 637–645, 1993.
15. Fumio F, Sachiko N, Masae K, Kumiko T and Takashi K, Carbon tetrachloride induced alteration of glutathione-*S*-transferase in rat liver cytosol and plasma. *J Clin Biochem Nutr* **6**: 175–185, 1989.
16. Boyd MR and Burka LT, *In vivo* studies on the relationship between target organ alkylation and pulmonary toxicity of a chemically reactive metabolite of 4-ipomeanol. *J Pharmacol Exp Ther* **207**: 687–697, 1978.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
18. Ohkawa H, Ohishi AN and Yagi K, Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**: 351–358, 1979.
19. Folch J, Lees M and Stanley GH, A simple method for the isolation and purification of total lipids from animal tissue. *J Biol Chem* **226**: 497–509, 1957.
20. Fiske CM and Subbarrow Y, The colorimetric determination of phosphorus. *J Biol Chem* **66**: 375–400, 1925.
21. Bartlett GR, Phosphorus assay in column chromatograpy. *J Biol Chem* **234**: 466–468, 1959.
22. Parekh AC and Jung DH, Cholesterol determination with ferric acetate–uranium acetate and sulfuric acid–ferrous sulfate reagents. *Anal Chem* **42**: 1423–1427, 1970.
23. Koide H and Oda T, Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. *Clin Chim Acta* **4**: 554–561, 1959.
24. Luly P, Barnabei O and Tria E, Hormonal control *in vitro* of plasma membrane bound (Na^+–K^+)-ATPase of rat liver. *Biochim Biophys Acta* **282**: 447–452, 1972.
25. Omura T and Sato R, The carbon monoxide-binding pigment of liver microsomes. *J Biol Chem* **239**: 2370–2378, 1964.
26. Aitio A, A simple and sensitive assay of 7-ethoxycoumarin deethylation. *Anal Biochem* **85**: 488–491, 1978.
27. Phillips AH and Langdon RG, Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: Isolation, characterization and kinetic studies. *J Biol Chem* **237**: 2652–2660, 1962.
28. Strittmatter P and Velick SF, A microsomal cytochrome reductase specific for diphosphopyridine nucleotide. *J Biol Chem* **221**: 277–286, 1956.
29. Isselbacher KJ, Chrabas MF and Quinn RC, The solubilization and partial purification of a glucuronyl transferase from rabbit liver microsomes. *J Biol Chem* **237**: 3033–3036, 1962.
30. Hollman S and Touster O, Alteration in tissue levels of UDP-glucose dehydrogenase, UDP-glucuronic acid pyrophosphatase and glucuronyl transferase induced by substances influencing the production of ascorbic acid. *Biochim Biophys Acta* **62**: 338–352, 1962.
31. Habig WH, Pabst UJ and Jakoby WB, Glutathione-*S*-transferase. *J Biol Chem* **249**: 7130–7139, 1974.
32. Slater TF, Necrogenic action of carbon tetrachloride in the rat: A speculative mechanism based on activation. *Nature* **209**: 36–40, 1966.
33. Halliwell B and Gutteridge JMC, Free radicals and toxicology. *Free Radicals in Biology and Medicine*, pp. 335–340. Clarendon Press, Oxford, 1989.
34. Halliwell B and Gutteridge JMC, Protection against oxidants in biological systems. The superoxide theory of oxygen toxicity. *Free Radicals in Biology and Medicine*, pp. 144–147. Clarendon Press, Oxford, 1989.
35. Will ED, Lipid peroxide formation in microsomes. *Biochem J* **113**: 333–341, 1969.
36. Reiner O, Athanassopoulos S, Hellmer KH, Murray RE and Uehleke H, Formation of chloroform from carbon tetrachloride in liver microsomes, lipid peroxidation and destruction of cytochrome P-450. *Arch Toxicol* **29**: 219–233, 1972.
37. Uehleke H, Werner T, Greim H and Krämer M, Metabolic activation of haloalkanes and tests *in vitro* for mutagenicity. *Xenobiotica* **7**: 393–400, 1977.
38. Murray M and Farrell GC, Different effects of carbon tetrachloride toxicity and cirrhosis on substrate binding to rat hepatic microsomal cytochrome P-450. *Biochem Pharmacol* **33**: 687–689, 1984.
39. Levin W, Lu AYH, Jacobson M and Kuntzman R, Lipid peroxidation and the degradation of cytochrome P-450 heme. *Arch Biochem Biophys* **158**: 842–852, 1973.
40. Brenner RR, Effect of unsaturated acids on membrane structure and enzymatic kinetics. *Prog Lipid Res* **23**: 69–96, 1984.
41. Ungemach FR, Plasma membrane damage of hepatocytes following lipid peroxidation: Involvement of phospholipase A_2. In: *Free Radicals in Liver Injury* (Eds. Poli G, Cheeseman KH, Dianzani MU and Slater TF), pp. 127–138. IRL Press, Oxford, 1985.
42. Coleman JB, Condie LW and Lamb RG, The role of CCl_4 bio-transformation in the activation of hepatocyte phospholipase C *in vivo* and *in vitro*. *Toxicol Appl Pharmacol* **95**: 208–219, 1988.
43. Koster JF and Slee RG, Lipid peroxidation of rat liver microsomes. *Biochim Biophys Acta* **620**: 489–499, 1980.
44. Mimnaugh EG, Trush MA and Gram TE, Stimulation by adriamycin of rat heart and liver microsomal NADPH-dependent lipid peroxidation. *Biochem Pharmacol* **30**: 2797–2804, 1981.
45. Erickson RH, Zakim D and Vessey DA, Preparation and properties of a phospholipid-free form of microsomal UDP-glucuronyltransferase. *Biochemistry* **17**: 3706–3711, 1978.
46. Rushmore ThH and Pickett CB, Regulation of glutathione *S*-transferase Ya subunit gene expression by planar aromatic compounds. In: *Glutathione S-Transferase and Drug Resistance* (Eds. Hayer JD, Pickett CB and Mantle TJ), pp. 157–164. Taylor & Francis, London, 1990.