Induction of prooxidant state by the food flavor cinnamaldehyde in rat liver

Muthusamy Raveendran, Jacob Thanislass, Govindan Uma Maheswari, and Halagowder Devaraj

Unit of Biochemistry, Department of Zoology, University of Madras, Madras, India

Cinnamaldehyde is an unsaturated aromatic aldehyde that is widely used as a food flavoring agent. The effect of cinnamaldehyde at the dose of 73.5 mg/kg body weight/day for 30 days, on male Wistar albino rats (80–90 g) was studied for an insight into the cellular antioxidant defense system. The antioxidant defense system in the liver of cinnamaldehyde-treated rats was different, as evidenced by the lower levels of glutathione and ascorbic acid. The primary antioxidant enzymes, superoxide dismutase and glutathione peroxidase activities were higher, whereas the catalase activity was diminished significantly. There was no appreciable difference in the activities of glutathione-S-transferase and glutathione reductase. The activity of glucose-6-phosphate dehydrogenase was significantly lower. Significantly higher levels of thiobarbituric acid reactive substances indicate oxidative damage. Thus, cinnamaldehyde treatment leads to the prooxidant state, which may be conducive for the carcinogenic process.

Keywords: liver; cinnamaldehyde; antioxidants; prooxidant state; carcinogenesis

Introduction

It is well known that chemical carcinogenesis is a multistage process. Recently, substantial evidence has suggested that free radicals play an important role in the complex course of multistep carcinogenesis.[1] Certain xenobiotics induce the formation of active oxygen species in the course of their metabolism, leading to the prooxidant state.[2] Some xenobiotics are known to alter the activity of antioxidants and thereby decrease their ability to destroy the active oxygen species, leading to the progression of tumors.

Cinnamaldehyde is an unsaturated aromatic aldehyde, used since the 1940's as an imitation cherry flavoring in foods.[3] The food flavor is used at concentrations ranging from 7.7 ppm (ice creams) to 700 ppm (sweets).[4] The World Health Organization (WHO) has established a temporary acceptable daily intake of 0.7 mg/kg body weight in 1984. The mutagenic nature of cinnamaldehyde has been shown in several prokaryotic and eukaryotic systems, including hamster fibroblast.[5] Cinnamaldehyde is also cytotoxic in vitro, its most likely mode of action being the suppression of protein synthesis.[6] Devaraj et al.[7] have shown that an increase in the hepatic cytochrome P450 level of cinnamaldehyde-treated rats leads to liver nodule formation. Incidentally, phenobarbital, a complete hepatocarcinogen, increases the level of cytochrome P450[8] and at the same time alters the antioxidant defense system.[9] Thus, suspicions of the carcinogenic nature of cinnamaldehyde are increased. To substantiate this, an attempt has been made to study the antioxidant defense system in cinnamaldehyde-treated rats.

Materials and methods

Male Wistar albino rats were purchased from the Fredrick Institute of Plant Protection and Toxicology, Padappai, Madras, India. They were divided into two groups. Each group consists of 10 rats, weighing from 80 to 90 g. One group served as control and the other as experimental. The experimental rats were treated orally with food grade cinnamaldehyde purchased from Basil & Company (Madras, India) at a dose of 73.5 mg/kg body weight per day for 30 days. This dose had been fixed to reach the LD_{50} value in 30 days. The LD_{50} value for cinnamaldehyde is 2.22 g/kg body weight.[10]

Supported by grant No F.3-156/90.SR II. from University Grants Commission, New Delhi, India.
Address reprint requests to Dr. H. Devaraj at the Unit of Biochemistry, Department of Zoology, University of Madras, Guindy Campus, Madras-600 025, India.
Received August 12, 1992; accepted August 20, 1992.

© 1993 Butterworth–Heinemann

At the end of the experimental period, the animals were fasted overnight and killed by cervical dislocation. The liver was quickly dissected out into ice-cold saline and connective tissues trimmed. Then 10% (wt/vol) liver homogenate was prepared in Tris-HCl buffer, 0.01 M, pH 7.0. by using a high speed Teflon homogenizer.

The following parameters were measured in liver homogenates: reduced glutathione (GSH) was determined by the method of Moron et al.[11] based on the reaction with 2,2′-dithiobis (2-nitrobenzoic acid) to produce a compound with maximum absorption at 412 nm; ascorbic acid was measured by the method of Omaye et al.[12]

The activity of catalase was measured as the amount of H_2O_2 consumed per minute per mg protein by the method of Sinha.[13] Glutathione peroxidase activity was assayed by the method of Rotruck et al.[14] The activity is expressed as μg GSH utilized per minute per mg protein. The superoxide dismutase activity was measured as the degree of inhibition of autoxidation of epinephrine at an alkaline pH by the method of Misra and Fridovich.[15]

Glutathione reductase activity was assayed by the method of Staal et al.[16] The activity of glutathione reductase was expressed as μg of NAD^+ formed per minute per mg protein. The rate of formation of NADPH with the help of added tissue homogenate was taken as the activity of glucose-6-phosphate dehydrogenase.[17] Glutathione-S-transferase activity was measured with 1-Chloro-2,4-dinitrobenzene.[18] Thiobarbituric acid reactive substances were assayed by the method of Utley et al.[19] Protein was estimated by the method of Lowry et al.[20]

Values were statistically analysed using the student t test and compared with controls.

Results

Table 1 shows the levels of enzymatic and non-enzymatic antioxidants in the liver of cinnamaldehyde-treated rats in comparison with those of the age-matched group of untreated rats. The levels of ascorbic acid ($P < 0.05$) and reduced glutathione ($P < 0.001$) were significantly lower. The activity of catalase ($P < 0.001$) was less than that of the untreated group. Superoxide dismutase ($P < 0.001$) and glutathione peroxidase ($P < 0.001$) activities were higher in the treated animals. Among the secondary antioxidant enzymes, the glutathione reductase activity was not altered significantly, but the activity of glucose-6-phosphate dehydrogenase ($P < 0.05$) was less compared with that of untreated rats. The thiobarbituric acid reactive substances ($P < 0.05$) were higher in the cinnamaldehyde-treated rats.

Discussion

Cinnamaldehyde, suspected to be a carcinogen, was found to have an effect on the antioxidant status of rat liver. Reduced glutathione is involved in the detoxification of xenobiotics by forming conjugates and by acting as a free radicals scavenger.[21] Boyland and Chasseaud[22] have shown the reduction in the level of GSH in cinnamaldehyde-treated rats. Similarly, our study also showed a diminished level of liver GSH. This may be due to two reasons: (1) GSH being involved in the formation of conjugates with cinnamaldehyde-derived byproducts, which are catalyzed by glutathione-S-transferase; (2) GSH acting as an intracellular electron donor for the activity of glutathione peroxidase.

In our study the glutathione peroxidase activity was higher in the treated rats. At the same time, there was no significant alteration in the glutathione-S-transferase activity. Therefore, we can attribute the observed reduction in the level of GSH to the increased activity of glutathione peroxidase.

Ascorbic acid is another non-enzymatic antioxidant. During the free radical scavenging action of ascorbic acid, semidehydro ascorbate is formed. GSH is required for the reduction of semidehydro ascorbate back to ascorbate.[23,24] When there is a reduction in the level of GSH, this conversion of semidehydro ascorbate is affected. Thus, the ascorbate level is lowered.

Superoxide dismutase, catalase, and glutathione peroxidase are the primary antioxidant enzymes. The activity of superoxide dismutase was found to be higher. This may be due to the increased production of reactive oxygen species. Prolonged treatment of animals with xenobiotics leads to the alteration in cytochrome P450 resulting in autoxidation and leading to lipid peroxidation.[25] Our previous study has shown the induction

Table 1 Effect of cinnamaldehyde on the enzymatic and non-enzymatic antioxidant defense system in the rat liver

Particulars	Control	Experimental
Ascorbic acid (μg/mg protein)	2.98 ± 1.28	2.12 ± 0.45*
Reduced glutathione (μg/mg protein)	16.06 ± 1.10	12.09 ± 2.30†
Catalase (μmoles of H_2O_2 consumed/min/mg protein)	207.16 ± 21.27	127.43 ± 18.44†
Glutathione peroxidase (μg of GSH consumed/min/mg protein)	70.86 ± 6.29	109.61 ± 17.99†
Superoxide dismutase (unit/min/mg protein)	1.48 ± 0.35	2.58 ± 0.42†
Glutathione reductase (μg of NAD^+ formed/min/mg protein)	54.22 ± 12.07	55.92 ± 8.82
Glucose-6-phosphate dehydrogenase (unit/mg protein)	1.89 ± 0.84	0.84 ± 0.03*
Glutathione-S-transferase (nmole of CDNB conjugated/min/mg protein)	908.54 ± 86.83	987.34 ± 65.69
TBA reactive substances (nmole/mg protein)	0.29 ± 0.03	0.37 ± 0.06*

CDNB, 1-Chloro-2,4-dinitrobenzene.
Values are mean ± SD (n = 8–10).
*$P < 0.05$.
†$P < 0.001$.

of cytochrome P450 levels in the liver microsomal fraction by prolonged treatment with cinnamaldehyde.[7] Due to enhancement in the activity of superoxide dismutase, the formation of H_2O_2 might be greater. To clear the excess H_2O_2, which is toxic to the cells, the glutathione peroxidase activity was increased. H_2O_2 can also be detoxified by catalase.[26] But the activity of catalase was lowered in the cinnamaldehyde-treated rats. NADPH serves to prevent and partially reverse the well-recognized inactivation of catalase by its toxic substrate, H_2O_2.[27] NADPH is supplied by glucose-6-phosphate dehydrogenase (G6PDH) of the hexose mono phosphate shunt.[28] In the cinnamaldehyde-treated rats, the activity of G6PDH was reduced, hence, there was less production of NADPH. This may be the reason for the inactivation of catalase.

Glutathione reductase, a secondary antioxidant enzyme, catalyzes the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) with the help of NADPH, which is supplied by glucose-6-phosphate dehydrogenase.[29] In the cinnamaldehyde-treated rats, there was no appreciable change in the activity of glutathione reductase. Thus, the conversion of GSSG to GSH is slow compared with that of GSH to GSSG by glutathione peroxidase. This may also be the reason for the observed lower level of reduced glutathione in the liver of experimental animals.

An increase in the reactive oxygen species may have resulted from the observed variations in the antioxidant defense system of cinnamaldehyde-treated rats. A growing body of data provides compelling evidence that the reactive oxygen species produced by radiation and some chemicals, including tumor promoters, play a role in the process of carcinogenesis in vivo and in vitro. This may indicate its potential for carcinogenicity mediated through reduction of antioxidant potential of liver cells.

Acknowledgments

The authors express deep felt gratitude to Dr. S. Niranjali, Lecturer, Department of Biochemistry, University of Madras for critically reviewing the manuscript.

References

1 Fisher, S.M., Floyd, R.A., and Copeland, E.S. (1988). Workshop report from the division of research grants, National Institute of Health. Oxy radicals in carcinogenesis-a. chemical pathology study section workshop. *Cancer Res.* **48,** 3882–3887

2 Mason, R. (1982). In *Free Radicals in Biology,* Vol. 5, (W. Pryor, ed.), p. 161, Academic Press, New York, NY USA

3 Opdyke, D.L.J. (1975). Cinnamyl anthranilate. *Fd. Cosmet. Toxicol.* **13,** 751–752

4 Mantovani, A., Stagi, A.V., Macri, C., Ricciardi, C., Piccioni, A., and Badellino, E. (1989). Pre-natal (Segment II) toxicity study of cinnamaldehyde in the Sprague-Dawley rat. *Fd. Cosmet. Toxicol.* **27,** 781–786

5 Ishidate, M. Jr., Sofuni, T., Yoshikawa, K., Hayashi, M., Nohmi, T., Sawada M., and Matsuoka, A. (1984). Primary mutagenicity screening of food additives currently used in Japan. *Fd. Chem. Toxic.* **22,** 623–636

6 Moon, K.H. and Pack, M.Y. (1983). Cytotoxicity of cinnamic aldehyde on leukemia L1210 cells. *Drug. Chem. Toxicol.* **6,** 521

7 Devaraj, H., Niranjali, S., and Raveendran, M. (1992). Effect of Food flavor cinnamaldehyde on liver microsomal cytochrome p-450 in rats. *Bull. Environ. Contam. Toxicol.* **49,** 306–311

8 Pitot, H. and Sirica, A. (1980). Quantitative evaluation of the promotion by 2,3,7,8 tetrachloro dibenzo-p-dioxin of hepato carcinogenesis from diethyl nitrosamine. *Biochem. Biophys. Acta* **605,** 191–215

9 Furukawa, K., Numoto, S., Furuya, K., Furukawa, N.T., and Williams, G.M. (1985). Effects of hepato carcinogen nafenopin, a peroxisome proliferator on the activities of rat liver glutathione-requiring enzymes and catalase in comparison to the action of Phenobarbital. *Cancer Res.* **45,** 5011–5019

10 Jenner, P.M., Hagan, E.C., Taylor, J.M., Cook, E.L., and Fitzhugh, O.G. (1964). Food flavorings and compounds of related structure. I. Acute oral toxicity. *Fd. Cosmet. Toxicol.* **2,** 327–343

11 Moron, M.S., Defierre, J.W., and Mannervik, K.B. (1979). Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. *Biochem. Biophys. Acta* **582,** 67–78

12 Omaye, S.T., Turnball, J.D., and Sauberlich, H.E. (1971). Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Meth. Enzymol.* **62,** 1–11

13 Sinha, A.K. (1972). Colorimetric assay of catalase. *Anal. Biochem.* **11,** 469–474

14 Rotruck, J.T., Pope, A.L., and Ganther, H.E. (1973). Selenium; Biochemical role as a component of glutathione peroxidase purification and assay. *Science* **179,** 588–590

15 Misra, H.P. and Fridovich, I. (1972). The role of superoxide anion in the antioxidation of epinephrine and a sample assay for SOD. *J. Biol. Chem.* **247,** 3170–3175

16 Staal, G.E.J., Visser, J. and Veeger, C. (1969). Purification and properties of glutathione reductase of human erythrocytes *Biochem. Biophys. Acta* **185,** 39–48

17 Zinkham, W.H. (1958). Defect of glutathione metabolism in erythrocytes from patients with a naphthalene induced hemolytic anemia. *Bull. Johns Hopkins Hosp.* **102,** 169

18 Habig, W.H., Pabst, U.J., and Jakoby, W.B. (1974). Glutathione-S-transferase. *J. Biol. Chem.* **249,** 7130–7139

19 Utley, G.H., Bernheim, F., and Hochstein, P. (1967). Effect of Sulfhydryl reagents on peroxidation in microsomes. *Biochem. Biophys. Acta* **118,** 29–32

20 Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **193,** 265–276

21 Comporti, M. (1985). Lipid peroxidation and cellular damage in liver injury. *Lab. Invest.* **53,** 599–623

22 Boyland, E. and Chasseaud, L.F. (1970). The effect of some carbonyl compounds on rat liver glutathione levels. *Biochem. Pharmacol.* **19,** 1526–1528

23 Tappel, A.L. (1962). Vitamin E as the biological lipid antioxidant. *Vitamin. Horm.* **20,** 493–510

24 Grimble, R.F. and Huges, R.E. (1967). Adehydroascorbic acid reductase factor in guinea pig tissues. *Experentia* **23,** 362

25 Parke, D.V. and Gray, T.J.B. (1978). In *Primary liver Tumors* (H. Remmer, H.M. Bolt, P. Bannasch, and H. Popper, eds) p 335, MTP press, Lancaster, UK

26 Chance, B., Sies, H., and Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* **59,** 527

27 Nicholls, P. and Schonbaum, G.R. (1963). In *The Enzymes,* 2nd Ed., Vol. 8, (P.D. Boyer, H. Lardy, and K. Myrback, eds.) p. 147–225, Academic Press, New York, NY USA

28 Dickens, F. and Glock, G.E. (1951). Direct oxidation of glucose-6-phosphate, 6-phosphogluconate and pentose-5-phosphate by enzymes of animal origin. *Biochem. J.* **50,** 81–95

29 Mann, P.J.G. (1931). The reduction of glutathione by a liver system. *Biochem. J.* **26,** 785–790