Bull. Environ. Contam. Toxicol. (1989) 42:301–306

Bulletin of Environmental Contamination and Toxicology

Studies on γ-Glutamyl Transpeptidase in Rodents Exposed to Benomyl

Yogeshwer Shukla, Mary Antony, and N. K. Mehrotra

Laboratory of Environmental Carcinogenesis, Industrial Toxicology Research Centre, Post Box 80, M.G. Marg, Lucknow-226 001, India

Benomyl is a protective and eradicant carbamate fungicide with systemic activity. It is found to be effective against a wide range of fungi affecting field crops, fruits, nuts, etc. It is also used against the mites, primarily as an ovicide (The pesticide manual 1983). In the preliminary studies, no effect level (NEL) was found to be more than 250 mg per kg body weight in rats, when no histological change was observed after two years exposure with benomyl (The pesticide manual 1983). Benomyl has been considered as one of the high priority chemicals required to be tested for its tumorigenic potential by the working group of International Agency for Research on Cancer, Lyon (IARC) a subsidiary of world health organisation (WHO) (IARC Working Group Report 1984). In view of the above and its increasing pattern of demand in India as a fungicide, benomyl technical grade (minimum purity 92%) was tested at low (1000 ppm) and high (4000 ppm) dose levels. After feeding of this fungicide for 15 days, the status of the enzyme γ-glutamyl transpeptidase (GGT, E.C.2.3.3.2) which catalyzes the transfer of glutamyl groups of peptides to other peptides and amino acids and has been proposed as a marker for assessment of tumorigenic activity of test substances (Kalengayi et al 1975, Young et al 1978) has been estimated.

MATERIALS AND METHODS

Benomyl (technical grade) was obtained from Bharat Pulverizing Mills, Bombay, India. γ-glutamyl-p-nitroanilide hydrochloride, p-nitro aniline and Tris buffer were obtained from Sigma Chemical Co., St. Louis,

Send reprint request to Dr. Yogeshwer Shukla at the above address.

USA. Glycylglycine was obtained from BDH, England and all other chemicals of analytical grade were procured from BDH, India and Sisco Research Laboratories, India.

Adult female albino rats (150-200 gm body weight) were randomly divided into three groups of eight animals each. The control group rats were provided synthetic pellet diet and water ad libitum. The other two groups were fed benomyl mixed with the crushed synthetic pellet diet in low (1000 ppm) and high (4000 ppm) doses respectively. Both, the pesticide mixed diet and water were provided ad libitum. Adult female swiss albino mice (20-25 gm body weight) were also taken and randomly divided into 3 groups each having 8 animals. The control mice were fed synthetic pellet diet and water and those in the experimental groups were kept on low dose (1000 ppm) and high dose (4000 ppm) of benomyl mixed in the diet respectively. As in case of rats, here also the diet and water were supplied ad libitum for the total experimental period which lasted for 15 days.

At the end of the study period, all the animals were killed by stunning and their blood was collected through cardiac puncture into clean and dry centrifuge tubes. The liver was immediately taken out and weighed on mettler balance (PE 160). Freshly collected blood was allowed to clot and stand in refrigerator at 8 - 10° C for 8 hr and clear serum was obtained after centrifugation at 2000 rpm for 15 min. The liver was homogenized (10% w/v) in 0.05 M tris buffer pH 7.0 with a Potter Elvehijem type homogenizer fitted with a teflon Pestle.

GGT activity was assayed in liver homogenates using the modified method of Roomi and Goldberg (1981). The enzyme activity in sera was estimated according to the method of Naftalin et al (1969). Protein content of the samples was estimated by the method of Lowry et al (1951) using Bovine serum albumin as the reference standard. The data were statistically analysed by the test described by Fischer (1950).

RESULTS AND DISCUSSION

Throughout the experimental period none of the animals from control or experimental groups showed any sign/s of overt toxicity or increased rate of mortality.

The data summarized in table 1 shows the effect of benomyl on absolute and relative liver weight of both rats and mice at low and high doses. A significant

Table 1. Effect of benomyl on the liver weights

	Absolute wt. (g)	Relative wt. (g/100 g)
Rat		
Control	6.29 ± 0.41	4.03 ± 0.33
Benomyl (1000 ppm)	7.69 ± 0.26*	4.59 ± 0.30
Benomyl (4000 ppm)	9.63 ± 0.61**	5.68 ± 0.43**
Mice		
Control	1.02 ± 0.21	3.92 ± 0.27
Benomyl (1000 ppm)	1.83 ± 0.29	6.81 ± 0.45**
Benomyl (4000 ppm)	1.93 ± 0.27	7.37 ± 0.72**

All the values represent the mean ± SE of 8 animals.
*P < 0.05; **P < 0.01, when compared with controls.

Table 2. Effect of benomyl on the activity of GGT© in liver and serum

	Liver	Serum
Rat		
Control	5.23 ± 0.81	56.39 ± 2.1
Benomyl (1000 ppm)	12.17 ± 1.37*	71.43 ± 2.9*
Benomyl (4000 ppm)	36.04 ± 3.72**	83.26 ± 3.2**
Mice		
Control	6.48 ± 0.63	63.81 ± 3.2
Benomyl (1000 ppm)	12.53 ± 1.36**	79.63 ± 2.8*
Benomyl (4000 ppm)	32.80 ± 2.92**	94.47 ± 3.4**

All the values represent the mean ± SE of 8 animals.
© nmoles p-nitroaniline liberated/min/mg protein.

increase in absolute liver weight was observed in both rats and mice after exposure with benomyl at low and high doses. However, when the data was expressed in relation to the body weight of animals, a significant increase in the liver weight was also observed in rats and mice at both the doses. This increase in absolute and relative liver weights was found to be dose dependent (table 1).

The effect of benomyl on the activity of liver and serum GGT in rats and mice is presented in table 2. Results show that the benomyl exposure to significantly induced the activity of GGT in both liver and serum of the exposed animals. This increase in the activity of GGT shows a dose dependent increase in both rats and mice.

In this study a significant liver enlargement after benomyl exposure through oral route was observed which indicates the increase in functional load of the organ (Conney 1967).

GGT, which catalyzes the transfer of γ-glutamyl group of compounds containing this group to a wide variety of amino acid acceptors (Meister 1973), is localized in the focal areas of hepatocytes (Luke et al 1975). GGT is widely used as a marker enzyme which is found to be raised in the preneoplastic lesions of the liver during chemical carcinogenesis (Perinio et al 1983). Abnormally high levels of GGT were observed in tumors of a variety of tissues including hepatocellular carcinomas (Boelsterli 1979, Hanigan and Pitot 1985), and malignant squamous cell carcinoma of the skin (Rosalki 1975), in experimental animals. Human hepatocellular carcinomas also showed increased levels of GGT (Gerber and Thung 1980, Tsuji et al 1980). Eight of the ten human hepatocellular carcinomas studied by Gerber and Thung (1980) were GGT-positive. In this study GGT was reported to be released from the cell membrane and the same could be detected in the serum. High serum activity has been also found in cases of cholestasis, excessive alcohol intake and acute viral hepatitis (Boelsterli 1979).

In the present investigation, the increase in serum and liver GGT levels is indicative of a toxic or preneoplastic response of the liver to benomyl, and could probably occur due to cellular lesions or as an adaptive response.

Acknowledgment. The authors are thankful to Prof. P.K. Ray, Director, Industrial Toxicology Research Centre, Lucknow, India, for his keen interest in the work.

REFERENCES

Boelsterili U (1979). γ-glutamyl transpeptidase (GGT) an early marker for hepatocarcinogens in rats. Trend Pharm Sci 1:47-49.

Conney AH (1967). Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 10:317-366.

Fischer RA (1950) Statistical methods for research workers, 11th edn. Oliver & Boyd, London.

Gerber MA, Thung SN (1980) Enzyme patterns in human hepatocellular carcinoma. Am J Pathol 98:395-400

Hanigan MH, Pitot HC (1985). Gamma glutamyl transpeptidase-its role in hepatocarcinogenesis. Carcinogenesis 6:165-172.

IARC working Group Report (1984) Chemicals and Exposures to complex mixture Recommended for Evaluation in IARC Monographs and Chemicals and Complex Mixtures Recommended for long term Carcinogenicity Testing. Technical Report No. 84/002 IARC, Lyon France.

Kalengayi MMK, Ronchi G, Desmet VJ (1975). Histochemistry of γ-glutamyl transpeptidase in rat liver during aflatoxin B_1 induced carcinogenesis. J Natl Cancer Instt 55:579-588.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951). Protein measurement with Folin-phenol reagent. J Biol Chem 193:265-275.

Lake BG, Gangoli SD, Grasso P, Llyod AG (1975). Studies on the hepatic effect of orally administered DEHP in the rat. Toxicol Appl Pharmacol 32:355-367.

Meister A, Tate SS, Ross LL (1976). Membrane bound gamma glutamyl transpeptidase: In Martonosi A (ed) The enzyme of Biological Membranes, Plenum Press, New York, pp 315-347.

Naftalin L, Sexton M, Whitaker T, Tracey D (1969). A routine procedure for estimating serum γ-glutamyl transpeptidase activity. Clin Chem Acta 26:293-296.

Peraino C, Richards WL, Stevens FJ (1983). Multistage hepatocarcinogenesis. Environ Health Persp 56:1-43.

Roomi MW, Goldberg DM (1981). Comparison of γ-GGTP induction by phenobarbitol in rat, guinea pig and rabbit. Biochem Pharmacol 30:1563-1571.

Rosalki SB (1975). γ-glutamyl transpeptidase. In advances in clinical chemistry, vol 17 eds. Bodansky O, Latner AL, Academic Press, New York, pp 53-107.

The Pesticide Manual (1983). Worthing CS (ed) published by British Crop Protection Council, pp 720.

Tsuji A, Matsuda Y, Katunuma N (1980). Characterisation of human serum gamma glutamyl transpeptidase. Clin Chem Acta 104:361-366.

Uchida T, Miyata H, Shikata T (1981). Human hepatocellular carcinoma and putative precancerous disorders. Arch Pathol Lab Med 105:180-186.

Young LM, Richards WL, Bonzelet WL, Boutwell RK (1978). Localization and significance of γ-glutamyl transpeptidase in normal and neoplastic mouse skin. Cancer Res 38:3697-3701.

Received May 17, 1988; accepted June 13, 1988.