Dioxane-Induced Changes in Mouse Liver Microsomal Mixed Function Oxidase System

by SITARAM S. PAWAR and AVINASH M. MUNGIKAR

Division of Biochemistry
Department of Chemistry
Marathwada University
Aurangabad (Maharashtra)
India

In recent years considerable attention has been focused on the industrial organic solvents as potential health hazards because of their presence in the environment. TERAYAMA (1967) demonstrated that carcinogenic azodye dimethylaminoazobenzene is metabolized in rat liver microsomes by several enzymatic systems. Recently, ARGUS et al (1965, 1973) reported dioxane induced liver carcinogenesis in rats and mice.

Data regarding the effect of dioxane on hepatic drug-metabolism and lipid peroxidation is not available. Therefore the present studies were designed to report such observations.

MATERIALS and METHODS

Female Hindustan Antibiotics strain mice weighing 30-50 g were used for all experiments. Mice were housed two to a cage, in an air conditioned room and fed standard laboratory diet and water ad libitum. The mice were classified into thr following groups. 1. Control group, 2. Dioxane treated group, 3. Phenobarbital treated group, 4. Phenobarbital and dioxane treated group.

Dioxane was administered orally (2 g/kg) in the morning for two successive days. Phenobarbital sodium was injected intraperitoneally at a dose of 50 mg/kg of body wt. for three days. The phenobarbital treated animals from group 4 were further treated with dioxane (2 g/kg) for two days. Control animals were given eqivalent volumes of distilled water.

The animals were sacrificed 24 hrs. after the last injection by decapitation and their livers were excised, weighed, minced with scissors and homogenized in ice cold 50 mM Tris-HCl buffer containing 1.15 % Kcl, using teflon pestle glass homogenizer. The microsomes were isolated by the $Ca^{2+}Mg^{2+}$sedimentation procedure of BAKER et al (1973). The microsomal protein was determined by the biuret method (GORNALL et al 1949) using crystalline bovine serum albumin as the standard protein.

Drug enzyme activities were carried out as reported earlier (PATEL and PAWAR 1974) using 1 mg/ml microsomal protein. The formaldehyde formed during N-demethylation was estimated by the method of NASH (1953). Acetanilide hydroxylation was estimated by measuring the formation of p-hydroxyacetanilide (WEISBURGER and GOODALL 1968).

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 6 © 1976 by Springer-Verlag New York Inc.

NADPH linked lipid peroxidation was assayed as described by ERNSTER and NORDENBRAND (1967). Ascorbate induced lipid-peroxidation was carried out in the same medium but nicotinamide was omitted and NADPH was replaced by 1 mM ascorbate. The malonaldehyde formation was measured by the thiobarbituric acid reaction (BERNHEIM et al 1948). Microsomal lipids were extracted according to the procedure of FOLCH et al (1957). The ultraviolet spectra were determined from 340 to 220 mμ with the lipid concentration of 1 mg/ml (DI LUZIO 1972).

Cytochrome b_5 and cytochrome P-450 contents of the liver microsomes were measured according to OMURA and SATO (1964). NADPH cytochrome c reductase activity was determined as previously described (PATEL and PAWAR 1974). Total heme content was determined by the pyridine hemochromogen procedure and calculated using 32.5 $cm^{-1} mM^{-1}$ as the extinction coefficient.

RESULTS

TABLE 1

Effect of dioxane and phenobarbital on the activity of drug-metabolizing enzymes in mice*

Group	% Liver wt body wt. (g)	Liver microsomal protein (mg/gm)	Aminopyrine N-demethylase**	Acetanilide hydroxylase***
Control	4.08±0.24	18.12±2.60	6.25±1.25	0.335±0.015
Dioxane treated	4.44±0.30[a]	32.62±0.10[c]	8.75±1.25[c]	0.270±0.015[b]
Phenobarbital treated	4.87±0.32[b]	33.68±0.20[c]	6.62±0.13[a]	1.98±0.06
Phenobarbital + dioxane treated	4.63±0.31[a]	24.21±1.10[b]	6.50±0.25[b]	2.31±0.39

* Results are mean ± SE (6 mice in each group).
** Activity is expressed as nmoles formaldehyde formed/min/mg protein.
*** Activity is expressed as μmoles p-hydroxyacetanilide formed/min/mg protein.
a = $P<0.05$, b = $P<0.01$, c = $P<0.001$

Dioxane treatment did not cause any change in relative liver weights. However, a significant increase in liver microsomal protein content was noticed. This was associated with concommitant increase in aminopyrine N-demethylase and acetanilide hydroxylase activities (Table 1).

Table 2 shows the effect of dioxane treatment on *in vitro* NADPH linked and ascorbate induced lipid peroxidation. Dioxane treatment lowered both the NADPH linked and ascorbate induced lipid peroxidation. NADPH linked lipid peroxidation was further decreased when dioxane was administered to phenobarbital pretreated mice.

TABLE 2

Effect of treatment of dioxane on hepatic microsomal lipid-peroxidation with* and without pretreatment of phenobarbital in adult female mice

Group	NADPH linked lipid peroxidation**	Ascorbate induced lipid peroxidation**
Control	8.00±0.16	7.64±0.04
Dioxane treated	6.68±0.20[b]	7.08±0.04[a]
Phenobarbital treated	6.80±0.16[a]	6.35±0.25[b]
Phenobarbital + dioxane treated	6.32±0.12[a]	-

* Results are mean ± SE (6 mice in each group).
** Activity is expressed as nmoles malonaldehyde formed/min/mg protein. a = $P<0.05$, b = $P<0.01$

Levels of microsomal electron transport components during dioxane treatment are summarized in Table 3.

TABLE 3

Hepatic microsomal electron transport components during dioxane treatment and the effect of phenobarbital pretreatment in adult female mice*

Group	Cytochrome b_5 (nmoles/mg)	Total heme (nmoles/mg)	NADPH cytochrome c reductase nmoles/min/mg	Cytochrome P-450 (nmoles/mg)
Control	0.14	0.76	12	0.16
Dioxane treated	0.16[a]	0.87[a]	18[c]	0.33[c]
Phenobarbital treated	0.23[c]	1.38[c]	36[c]	0.88[c]
Phenobarbital + dioxane treated	0.25[c]	0.88[c]	24[c]	0.49[c]

* Values are the average of three determinations of pooled livers of 3 mice. a = $P<0.05$, c = $P<0.001$

Levels of hepatic microsomal cytochrome b_5, total heme, cytochrome c reductase and cytochrome P-450 were elevated due to the administration of dioxane. Phenobarbital pretreatment prior to the administration of dioxane resulted in significantly higher levels of electron transport components as compared to dioxane treated mice, although their levels were lower than in the mice treated with phenobarbital alone.

The ultraviolet spectrum of total liver microsomal lipids of dioxane treated animals revealed a significant reduction in diene conjugation band (Fig. 1).

Fig. 2 shows the effect of dioxane treatment on pyridine binding spectra. A decrease in the magnitude of pyridine binding spectra was observed in dioxane treated animals.

Figure 1: UV spectrum of liver microsomal lipids in adult female mice.
—— Control animals —·— Dioxane treated animals

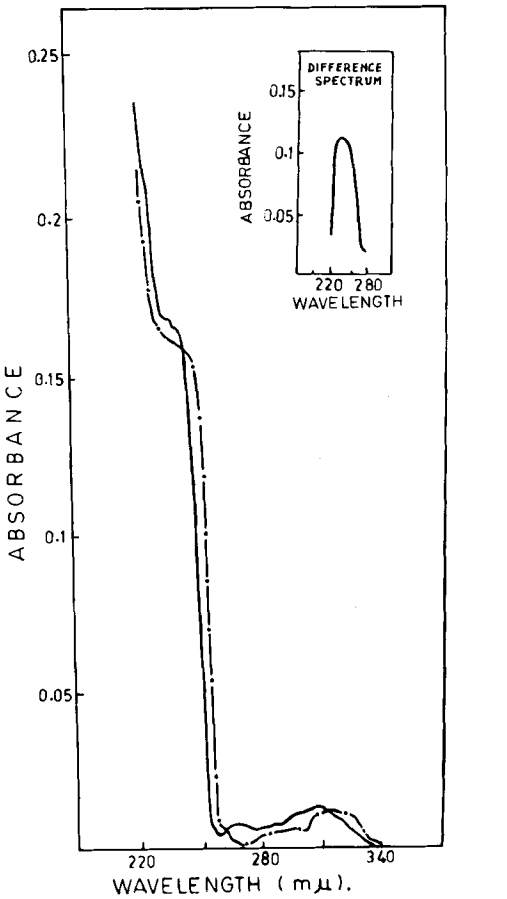
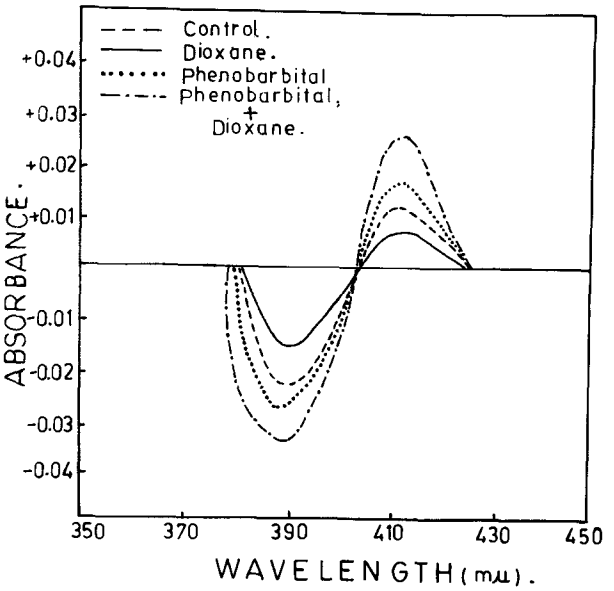


Figure 2: Effect of dioxane treatment on pyridine binding spectra with and without pretreatment of phenobarbital in adult female mice.



DISCUSSION

Present studies indicate the effect of dioxane treatment on mouse liver microsomal mixed function oxidase system. DECLOITRE et al (1975) studied the effect of phenobarbital and 3-methylcholanthrene administration on detoxication and activation of the carcinogenic azodye 4-dimethylaminoazobenzene in rat liver microsomes. Recently HOOK et al (1975) reported that following 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment , both cytochrome P-450 and benzpyrene hydroxylase were significantly stimulated in hepatic microsomes from male and female rats. In the present studies, the enhancement of drug-

metabolizing enzymes was associated with a significant increase in microsomal protein content, levels of cytochrome b_5 and cytochrome P-450. The increase in the drug metabolizing enzyme activities due to dioxane treatment is possibly due to several factors such as synthesis of more active enzymes, elevated levels of microsomal electron transport components (cytochrome P-450) and increased protein synthesis. The low levels of lipid peroxidation during dioxane treatment might be possibly due to the metabolites of dioxane acting as antioxidants in liver microsomes. The inhibition of lipid peroxide formation in mice pretreated with phenobarbital prior to dioxane administration could be due to the competition between drug hydroxylation and lipid peroxidation for a common flavoprotein NADPH cytochrome c reductase (ORRENIUS, 1964). After the administration of dioxane the lipid peroxidation showed a further inhibition probably due to the entry of this drug.

ACKNOWLEDGEMENTS

AMM is a Junior Research Fellow of University Grants Commission, New Delhi. SSP is grateful to Prof. Ronald W. Estabrook, Professor and Chairman, Department of Biochemistry University of Texas, Health Science Center, Dallas, Texas, USA for his generous help and encouragement. The authors wish to thank Dr. J.M.Patel (presently at National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, USA) for his help during the early stages of the work.

REFERENCES

ARGUS, M.F., ARCOS, J.C. and HOCH-LIGETI, C., J.Natl.Cancer Inst. 35,949(1965).
ARGUS, M.F., SOHAL, R.S., BRAYANT, G.M., HOCH-LIGETI, C. and ARCOS, J.C., Eur.J.Cancer 9,237(1973).
BAKER, R.C., COONS, L.B. and HODGSON, E., Chem.-Biol.Interactions 6,307(1973).
BERNHEIM, F., BERNHEIM, M.L.C. and WILBUR, L.M., J.Biol.Chem. 174,257(1948).
DECLOITRE, F., MARTIN, M. and CHAUVEAU, J., Chem.-Biol.Interactions 10,229(1975).
DI LUZIO, N.R., J.Agr.Food.Chem. 20,486(1972).
ERNSTER, L. and NORDENBRAND, K., Methods Enzymol. 10,574(1967).
FOLCH, J., LEES, M. and SLOANESTANLEY, G.H., J.Biol.Chem. 226,497(1957).
GORNALL, A.G., BARDAWILL, C.J. and DAVID, M.M., J.Biol.Chem. 177,751(1949).
HOOK, G.E.R., HASEMAN, J.K. and LUCIER, G.W., Chem.-Biol.Interactions 10,199(1975).
NASH, T., Biochem.J. 55,416(1953).
OMURA, T. and SATO, R., J.Biol.Chem. 239,2370(1964).
ORRENIUS, S., DALLNER, G. and ERNSTER, L., Biochem.Biophys,Res. Commun. 14,329(1964).
PATEL, J.M. and PAWAR, S.S., Biochem.Pharmacol.23,1467(1974).
TERAYAMA,H., Methods in Cancer Research(BUSCH, H.,Ed.) Academic press, New York pp.399(1967).
WEISBURGER, J.H. and GOODALL, C.M., Life Sci. 7,263(1968).