ABSENCE OF ETHANOL METABOLISM IN 'ACATALATIC' HEPATIC MICROSOMES THAT OXIDIZE DRUGS

Kostas P. Vatsis and Martin P. Schulman

Department of Pharmacology
University of Illinois College of Medicine
Chicago, Illinois 60612

Received March 26, 1973

Summary: When liver slices of Cs^a and Cs^b mice were incubated in vitro, they had similar catalase activities and equal rates of ethanol metabolism. While incubated liver homogenates and microsomes from Cs^a mice oxidized ethanol and retained catalase activity, preparations from Cs^b mice did not oxidize ethanol and lost all catalase activity. Addition of beef liver catalase restored ethanol oxidation by Cs^b microsomes. The oxidations of aniline and aminopyrine proceeded at the same rate in Cs^a and Cs^b microsomes and were inhibited by ethanol. It is evident that (a) the microsomal drug-metabolizing pathway is not involved in ethanol oxidation, and (b) the postulation of a unique microsomal ethanol-oxidizing system ("MEOS") that is independent of microsomal catalase is unwarranted.

The existence of a unique microsomal ethanol-oxidizing system that is independent of catalase activity has been the subject of lively debate. By using a combination of catalase inhibitors in vivo and in vitro, Lieber et al. reported a complete dissociation of microsomal ethanol oxidation by catalase and a H_2O_2-generating system from a NADPH-linked microsomal ethanol-oxidizing system, which has been termed "MEOS" (1). The credibility of "MEOS" has been challenged by several laboratories that have offered evidence that rates of microsomal H_2O_2 generation from NADPH via NADPH oxidase and the amounts of microsomal catalase can satisfactorily account for the oxidation of ethanol by hepatic microsomes (2, 3, 4).

Additionally, "MEOS" is believed to have features in common with the microsomal multifunction oxidase system that is responsible for drug oxidations. This conclusion was based on the findings that "MEOS" required oxygen and was partially inhibited by CO, properties commonly attributed to microsomal drug-

detoxifying enzymes, and that the microsomal oxidation of drugs was inhibited by ethanol (1, 5).

Since the use of catalase inhibitors has hampered the resolution of the conflict of whether microsomal ethanol oxidation is accomplished by "MEOS" and/or catalase, we have undertaken studies of ethanol metabolism in a strain of acatalasemic mice (Cs^b) whose hepatic microsomes can be rendered acatalatic without the addition of inhibitors. In these mutant mice, tissue catalases undergo rapid and irreversible inactivation in vitro at 37°C and pH 7.4 as opposed to those from the genetic control strain (Cs^a) which remain stable under these conditions (6, 7). In addition, we have examined ethanol oxidation in liver slices of acatalasemic mice, in which catalase activity was not altered by the normal incubation conditions. Drug oxidations and the interaction of ethanol with the drug-metabolizing system have also been examined in 'acatalatic' and control microsomes.

Experimental Procedure

Pooled liver slices (400 mg) of adult male and female mice (10 months old) were incubated for 30 min at 37°C in 4.0 ml of oxygenated Krebs-Ringer-phosphate buffer, pH 7.4, and 1.0 ml ethanol (final concentration 88 mM) in stoppered 25 ml Erlenmyer flasks. Flasks were chilled to 0°C and the supernatant fluid removed for ethanol assay by gas chromatography (8).

Liver homogenates (1.7 ml of 33% homogenate in Krebs-Ringer-phosphate, pH 7.4) were incubated in stoppered 25 ml flasks for 1 hr at 37°C with 0.5 ml ethanol (88 mM final concentration) and additional buffer in 2.5 ml final volume. After chilling, the contents were spun at 39,000 x g for 20 min, and ethanol determined in the supernatant fluid. Washed microsomes (equivalent to 560 mg liver) were incubated in Krebs-Ringer-phosphate, pH 7.4 with 88 mM ethanol and 1.35 mM NADPH (final volume 2.5 ml) for 1 hr at 37°C in air and analyzed for ethanol.

Table 1. Hepatic Ethanol Oxidation in Control (Cs^a) and Acatalasemic (Cs^b) Mice

	Cs^a mice	Cs^b mice
Slices		
Ethanol oxidation*	131	155
Catalase activity**	212	189
Homogenates		
Ethanol oxidation	26	0
Catalase activity	245***	0***
Microsomes†		
Ethanol oxidation	31	0
Ethanol oxidation with added catalase‡	40	36

*µmoles ethanol oxidized/gm liver/hr.

**mmoles H_2O_2 decomposed/gm liver/hr.

***Values for catalase activity before incubation were 245 and 218 mmoles H_2O_2 decomposed/gm liver/hr for Cs^a and Cs^b mice, respectively.

†The activity of microsomal catalase before incubation was 25.6 and 17.7 mmoles H_2O_2 decomposed/gm liver/hr for Cs^a and Cs^b mice, respectively. Microsomes from Cs^a mice lost no catalase activity after 1 hr incubation at 37° C; those of Cs^b mice lost 87%, 96% and 100% of catalase activity after 5, 15 and 60 min incubation, respectively.

‡1 mg crystalline beef liver catalase, 14,000 U/mg.

Microsomes for drug metabolism studies were isolated by centrifugation at 105,000 x g and were washed once in KCl-Tris buffer (8 parts of 1.15% KCl and 2 parts of 0.25 M Tris-HCl, pH 7.5) according to a modified method of Deckert and Remmer (9). The final microsomal pellet was resuspended in KCl-Tris buffer, pH 7.5, and adjusted so that each ml contained 6-8 mg protein. Incubation mixtures (1.1 ml total volume) contained 0.3 ml of microsomal suspension, 0.6 ml NADPH-generating system (10 mM $MgCl_2$, 2 mM NADPH and 15 mM Na isocitrate), 5 µl (200 mU) isocitrate dehydrogenase and 0.1 ml buffer or ethanol (8 mM or 100 mM final concentration for vessels containing aniline or aminopyrine, respectively). After 5 min of preincubation at 37° C, the reaction was initiated by adding 0.1 ml of substrate (aniline was freshly distilled). After 10 min incubation, 1.0 ml 15% trichloracetic acid was added to

aniline vessels; 1.0 ml 7.5% trichloracetic acid was added to vessels containing aminopyrine after 3 min of incubation. Aniline hydroxylation was determined by a modification of the method of Deckert and Remmer (9) while aminopyrine demethylation was measured as released formaldehyde (10).

Microsomal H_2O_2 production was measured as described by Thurman et al. (3). Alcohol dehydrogenase activity was measured in the 105,000 x g supernatant fraction according to the method of Bonnichsen and Brink (11). Catalase, microsomal NADPH oxidase, and NADPH-cytochrome c reductase activities were assayed by previously described methods (12, 13, 14).

Results

Liver slices of Cs^a and Cs^b mice metabolized ethanol at the same rate. Catalase activities of incubated liver slices were similar but somewhat lower than values obtained from liver samples that were analyzed without prior incubation. Disruption of hepatic cells of Cs^b mice and subsequent incubation at 37°C resulted in the loss of catalase and ethanol-oxidizing activities. For the control Cs^a mice, neither catalase activity nor ethanol oxidation was destroyed by incubation of liver homogenates (Table 1). While whole liver homogenates and washed hepatic microsomes of Cs^a mice metabolized ethanol, those derived from Cs^b mice did not exhibit any ethanol oxidation. Addition of beef liver catalase restored ethanol oxidation of Cs^b microsomes to values observed with hepatic microsomes from control Cs^a mice. Substitution of NADPH by a H_2O_2-generating system (1.5 mM hypoxanthine and 20 mU/ml xanthine oxidase) without added catalase did not result in ethanol oxidation by microsomes of Cs^b livers. Aside from the loss of endogenous catalase from the livers of Cs^b mice, no other enzymic changes were observed. The activities of alcohol dehydrogenase, NADPH-cytochrome c reductase and NADPH oxidase were the same for Cs^a and Cs^b livers and were not altered by one hour of preincubation

Table 2. Drug Metabolism in Hepatic Microsomes of Cs^a and Cs^b Mice

Drug Concentration	Cs^a microsomes		Cs^b microsomes	
	No ethanol	With ethanol	No ethanol	With ethanol
	μmoles product formed/gm liver/hr			
0.01 mM aniline	1.2	0.6	1.4	0.6
16 mM aniline	2.2	2.2	2.5	2.5
0.1 mM aminopyrine	3.1	1.7	3.2	1.3
16 mM aminopyrine	20.2	20.2	23.2	23.2

at 37° C; H_2O_2 production from added NADPH (35 μmoles/gm liver/hr) was also similar for microsomal preparations from Cs^a and Cs^b livers.

The results in Table 2 demonstrate that drug oxidation occurred with equal vigor in hepatic microsomes of both Cs^a and Cs^b mice despite the absence of ethanol oxidation by Cs^b microsomes. Furthermore, the drug-metabolizing capacity of either Cs^a or Cs^b livers was not at all altered by preincubating microsomes for one hour at 37°C before drug addition. Ethanol inhibited the oxidation of 0.01 mM aniline and 0.1 mM aminopyrine to the same degree by microsomal preparations of either type of liver; no inhibition of drug oxidation by ethanol was observed at the higher drug concentration studied. The inhibition of drug oxidation by ethanol confirms previous findings (5, 15).

Discussion

The possibility that catalase may account for "MEOS" activity has been rejected by Lieber's group (16). The recent report that "MEOS" activity can be separated from ADH and catalase (17) strengthens the argument for a distinct "MEOS", but unfortunately others have not been able to demonstrate ethanol oxidation in microsomal preparations that are devoid of catalase activity (18). In addition, the ability of microsomes to catalyze the conversion of

ethanol to acetaldehyde was lost under conditions which led to the solubilization and reconstitution of enzyme systems which retained drug-hydroxylating activity (19).

In the present study, microsomal NADPH-linked and H_2O_2-linked ethanol oxidation was observed in Cs^b microsomes only when exogenous catalase was added. The conclusion is inescapable that microsomal catalase was responsible for "MEOS" activity. The finding that drug oxidation was impaired in the presence of ethanol may very well represent a characteristic interaction between ethanol and the microsomal drug-metabolizing system; however, in view of the absence of ethanol metabolism in Cs^b microsomes, this interaction must be entirely unrelated to microsomal ethanol metabolism. Consistent with this formulation, Imai and Sato (20) consider the spectral changes of hepatic microsomal proteins induced by ethanol to represent an action of ethanol on a lipophilic phase of the microsomal membrane adjacent to the hemoprotein, rather than a substrate-like binding to the hemoprotein itself.

It is evident from these studies with 'acatalatic' microsomes that the drug-metabolizing pathway is not involved in the oxidation of ethanol. The postulation of a unique "MEOS" system that is independent of microsomal catalase and NADPH oxidase activities and which interacts with microsomal drug-metabolizing systems "through competition for an at least partially common detoxifying system in the liver" (16) is unwarranted.

Acknowledgements

This work was supported by U.S. Public Health Service Research Grant 2R01 MH 04559. The authors wish to express their sincere thanks to Dr. Robert N. Feinstein for kindly supplying the Cs^a and Cs^b mice to start our colony, and to Dr. Fred Deckert for his assistance in preparing a modification of the drug metabolism assays.

References

1. Lieber, C.S.and DeCarli, L. M. J. Biol. Chem., 245, 2505 (1970).
2. Isselbacher, K. J. and Carter, E. A. Biochem. Biophys. Res. Commun., 39, 530 (1970).
3. Thurman, R. G., Ley, H. G. and Scholz, R. Eur. J. Biochem., 25, 420 (1972).
4. Lin, G., Kalant, H. and Khanna, J. M. Biochem. Pharmacol., 21, 3305 (1972).
5. Rubin, E., Gang, H., Misra, P. S. and Lieber, C. S. Amer. J. Med., 49, 801 (1970).
6. Feinstein, R. N., Braun, J. T. and Howard, J. B. Arch. Biochem. Biophys., 120, 165 (1967).
7. Aebi, H., Suter, H. and Feinstein, R. N. Biochem. Genetics, 2, 245 (1968).
8. Roach, M. K. and Creaven, P. J. Clin. Chim. Acta, 21, 275 (1968).
9. Deckert, F. W. and Remmer, H. K. Chem-Biol. Interactions, 5, 255 (1972).
10. Nash, T. Biochem. J., 55, 416 (1953).
11. Bonnichsen, R. K. and Brink, N. G. In Methods in Enzymology, Vol. 1, p. 495. Colowick, S. P. and Kaplan, N. O. (Eds.) Academic Press, New York, 1955.
12. Baudhuin, P., Beaufay, H., Rahman-Li, Y., Sellinger, O. Z., Wattiaux, R., Jacques, P. and DeDuve, C. Biochem. J., 92, 179 (1964).
13. Gillette, J. R., Brodie, B. B. and LaDu, B. N. J. Pharm. Expl. Therap., 119, 532 (1957).
14. Sottocasa, G. L., Kuylenstierna, B., Ernster, L. and Bergstrand, A. J. Cell Biol., 32, 415 (1967).
15. Rubin, E. and Lieber, C. S. Science, 162, 690 (1968).
16. Lieber, C. S. New England J. Med., 288, 356 (1973).
17. Teschke, R., Hasumura, Y., Joly, J. G., Ishii, H. and Lieber, C. S. Biochem. Biophys. Res. Commun., 49, 1187 (1972).
18. Thurman, R. G. and Scholz, R. Drug Metabol. and Disp. 1 (1973).
19. Coon, M. J. Personal communication.
20. Imai, Y. and Sato, R. J. Biochem. (Tokyo), 62, 239 (1967).