Vol. 111, No. 1, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1983 Pages 219-223

ETHANOL AS AN INDUCER OF UDP-GLUCURONYLTRANSFERASE: A COMPARISON WITH PHENOBARBITAL AND 3-METHYLCHOLANTHRENE INDUCTION IN RABBIT HEPATIC MICROSOMES

Garold S. Yost and Brent L. Finley

College of Pharmacy

Washington State University

Pullman, WA 99164-6510

Received January 14, 1983

SUMMARY: In this report we have examined the ability of ethanol to induce UDP-glucuronyltransferase activity in male rabbits using p-nitrophenol as substrate. The rabbit was found to be an excellent species for studies of ethanol induction since almost 3-fold increases in activity were observed relative to controls. Ethanol induction of p-nitrophenol UDP-glucuronyltransferase activity was even greater than induction by 3-methylcholanthrene, the prototypic inducer of this type of activity. Thus, the rabbit shows promise for studies of UDP-glucuronyltransferase isozymes that are induced by chronic ethanol consumption.

Chronic ethanol consumption induces a unique, isolable set of hepatic cytochrome P-450 monoxygenase isozymes in rabbits (1). Since inducers of P-450 such as phenobarbital (PB) and 3-methylcholanthrene (3-MC) are also inducers (2) of UDP-glucuronyltransferase (UDP-GT)(EC 2.4.1.17), ethanol could be an effective inducer of UDP-GT isozymes in the rabbit. Ethanol induces p-nitrophenol UDP-GT activity in man (3) and rat (4), and the induction of UDP-GT activity (phenol red as substrate) by ethanol in cultured chick embryo hepatocytes was recently reported (5). In spite of these reports, however, some doubts remain as to the general phenomenon of induction of UDP-GT by ethanol (2,6) since bilirubin UDP-GT activity was induced by ethanol only in Wistar rats (7) and not in Sprague-Dawley rats (3) or man (3). It seems plausible, however, that the alcohol-induced UDP-GT isozymes are species and/or substrate selective as are the PB and 3-MC induced isozymes (8).

0006-291X/83/040219-05$01.50/0

Human alcoholism significantly alters drug metabolic processes and therefore therapeutic drug regimens may require alterations (9,10). Although the increased rates of drug metabolism observed in alcoholics are generally ascribed to the induction of the cytochrome P-450 enzymes (1,9,10), significant effects would be observed with drugs that are subject to first-pass glucuronidation if UDP-GT enzymes were likewise induced by ethanol (3-5). A recent report (11) described significant increases in metabolism of propranolol but not sotalol in humans after ethanol consumption. Propranolol is primarily eliminated as its glucuronide while sotalol is mostly excreted unchanged, thus, UDP-GT induction by ethanol may be a clinically important phenomenon.

The purpose of this study was to evaluate ethanol induction, using the rabbit as a model species, and to compare the p-nitrophenol UDP-GT activities of the ethanol induced animals to control and to PB and 3-MC induced animals. In addition, partial characterization of the UDP-GT isozymes from differently induced rabbits was accomplished by evaluating the p-nitrophenol activities of unactivated and Lubrol-activated microsomes from each type of induction treatment.

METHODS

Uridine 5'-diphosphoglucuronic acid ammonium salt (UDPGA), Lubrol PX, p-nitrophenol (spectrophotometer grade), and 3-methylcholanthrene (practical grade) were purchased from Sigma (St. Louis, MO). TRIS buffer, $MgCl_2 \cdot 6\ H_2O$, NaOH, acetic acid, and ethanol (95%) were all analytical grade chemicals and were purchased from Baker chemicals (Phillipsburg, N.J.). Sodium phenobarbital was purchased from Merck (Rahway, N.J.). All chemicals and reagents were used without further purification.

Male New Zealand white rabbits fed with normal rabbit chow and weighing 2-3 Kg were induced with PB (0.1% in drinking water for 1 week), 3-MC (40 mg/Kg in 4 ml corn oil i.p. - sacrifice after 3 days), or ethanol (10% in drinking water for 2 weeks). Hepatic microsomes were prepared from induced and control rabbits by the method of Coon et al (12) and stored at -70°C after dilution to approximately 10 mg protein/ml solution. When needed, the microsomes were thawed, centrifuged at 100,000 g for 1 hr, and the pellet resuspended in the original volume of Tris acetate (50 mM, pH 7.4) without glycerol or EDTA. Protein concentrations were determined with the Bio-Rad (Richmond, CA) Protein Assay Kit. Blood alcohol concentrations were determined from rabbit ear vein blood samples taken at various times during the two weeks of ethanol induction. Blood ethanol concentrations were measured with the Sigma (St. Louis, MO) ethyl alcohol diagnostic kit.

Incubations of the microsomes from the differently induced rabbits and from controls were performed essentially as in the method of Dutton (13). A typical incubation of 5.00 ml total volume in 50 mM Tris acetate (pH 7.4)

contained 1.2 mg/ml Lubrol PX, 10 mM $MgCl_2$, 1.0 mM p-nitrophenol, 2.0 mM UDPGA, and 1.0 mg/ml microsomal protein. Appropriate changes were made when unactivated microsomal incubations were performed, i.e. deletion of $MgCl_2$ and/or Lubrol PX. Incubations were initiated at 37°C in a shaker bath in the dark by substrate addition. Aliquots of 0.5 ml were withdrawn at 2 min intervals and the enzymatic reaction was quenched by transferring the aliqouts to tubes containing 1.5 ml ethanol. 1.0 M NaOH (0.25 ml) was then added to each tube to raise the pH and the precipitated protein was pelleted by centrifugation. The absorbance at 400 nm of the supernatant was then determined for each time point. All manipulations were carried out in glassware wrapped in aluminum foil. An extinction coefficient of 18,300 $M^{-1}cm^{-1}$ was used to calculate the rate of disappearance of p-nitrophenol, and thus the rate of conjugation.

RESULTS

Ethanol induction of rabbit hepatic UDP-GT enzymes was accomplished by using a 10% solution of ethanol in the drinking water for a two week period which gave rise to a constant blood alcohol content of 0.02%. Water consumption by the rabbits remained constant after inclusion of ethanol in the diet.

The catalytic capabilities of the ethanol induced UDP-GT were measured (Table 1) using p-nitrophenol as substrate. The rates of p-nitrophenol glucuronidation were measured in control microsomes as well as ethanol, PB, and 3-MC induced microsomes. The rates of p-nitrophenol glucuronidation

Table 1

Effects of Induction and Activation on Glucuronyltransferase Activities[1]

	Microsomes	Microsomes + $MgCl_2$	Microsomes + $MgCl_2$ + Lubrol
Control	3.1 ± 0.9	6.3 ± 0.1	69.0 ± 7.9
PB	4.3 ± 0.5	10.4 ± 1.4	75.1 ± 6.0
3-MC	6.5 ± 1.6	20.6 ± 5.6	92.3 ± 11.9
Ethanol	9.0 ± 1.1	25.5 ± 3.1	158.4 ± 9.2

[1] Activities (p-nitrophenol as substrate) are expressed as nmol/min·mg microsomal protein ± S.D., and are the mean values of at least three animals.

were evaluated for each type of inducer with unactivated, Mg^{+2}-activated, and Lubrol-activated (Mg^{+2} included) microsomes (Table 1). Phenobarbital showed no significant induction of p-nitrophenol UDP-GT activity, which was anticipated since PB is an inducer of UDP-GT specific for GT_2 type (8) substrates like morphine. A recent study, however, has reported a 60% increase in hepatic microsomal p-nitrophenol UDP-GT activity in Fauve de Bourgogne rabbits given 35 mg/kg doses of PB for 4 days (14). Also as anticipated, 3-MC induced the GT_1 type (8) activity (p-nitrophenol) approximately 2-fold. Significant induction by ethanol was observed in the rabbit. Induction of approximately 3-fold in the alcohol treated animals provided convincing evidence that ethanol is an inducer of UDP-GT.

DISCUSSION

The results demonstrate that ethanol effectively induces p-nitrophenol UDP-GT activity in the rabbit. The ability of ethanol to stimulate UDP-GT activities seems to be significantly substrate and species related (2,6). We are therefore currently evaluating the induction process using other UDP-GT substrates.

A 10% aqueous solution of ethanol was used to induce the rabbits, but this procedure increases the total caloric intake and possibly leads to some changes in UDP-GT enzymes. It is unlikely however, that the almost 3-fold increases that we observed could totally be the result of nutritional factors. For example, cytochrome P-450 induction in rats by ethanol has been recently attributed primarily to ethanol and not to nutritional requirements (15). To reduce nutritional effects, the induction process will be evaluated using liquid isocaloric diets (16) for control and ethanol (36% of the total calories) induced rabbits.

We have found no published accounts of 3-MC induction of rabbit hepatic UDP-GT activities using p-nitrophenol or other substrates. This paucity of literature is unfortunate since we were surprised that 3-MC was not a better inducer in the rabbit, and we wished to compare our results with the results of others. Phenobarbital has been evaluated as an inducer of UDP-GT in

rabbits, however, and PB demonstrated no inducing capabilities in one study (17), thus paralleling our results. It appears that in the rabbit a species-related phenomenon is occuring whereby ethanol emerges as the best inducer of p-nitrophenol UDP-GT activity when compared to PB and 3-MC.

ACKNOWLEDGEMENTS

We would like to acknowledge the skilled technical assistance of Beverly A. Conner and Scott R. Moore. This work was supported by a Pharmaceutical Manufacturers Association Foundation starter grant and by funds provided by the State of Washington Initiative Measure No. 171 for medical and biological research on alcoholism.

REFERENCES

1. Koop, D.R., Morgan, E.T., Tarr, G.E., and Coon, M.J. (1982) J. Biol. Chem. 257, 8472-8480.
2. Dutton, G.J. (1980) in Glucuronidation of Drugs and Other Compounds, pp. 135-147, CRC Press, Boca Raton, FL.
3. Hakim, J., Feldmann, G., Troube, H., Boucherot, J., and Boivin, P. (1972) Path.-Biol. 20, 277-285.
4. Dioguardi, N., Idéo, G., Del Ninno, E., and de Franchis, R. (1970) Lancet, 1063.
5. Sinclair, J.F., Sinclair, P.R., Smith, E.L., Bement, W.J., Pomeroy, J., and Bonkowsky, H. (1981) Biochem. Pharmacol. 30, 2805-2809.
6. Fevery, J., de Groote, J., and Heirwegh, K.P.M. (1976) in Frontiers of Gastrointestinal Research, Vol. 2, van der Reis, L., ed., pp. 243-292, S. Karger, Basel.
7. Idéo, G., de Franchis, R., Del Ninno, E., Cocucci, C., and Dioguardi, N. (1971) Enzyme 12, 473-480.
8. Lilienblum, W., Walli, A.K., and Bock, K.W. (1982) Biochem. Pharmacol. 31, 907-913.
9. Pelkonen, O. and Sotaniemi, E. (1982) Pharmacol. Ther. 16, 261-268.
10. Lieber, C.S. (1981) Clin. Gastroenterol. 10, 315-342.
11. Sotaniemi, E.A., Anttila, M., Rautio, A., Stengard, J., Saukko, P., and Järvensivu, P. (1981) Clin. Pharmacol. Ther. 29, 705-710.
12. Coon, M.J., van der Hoeven, T.A., Dahl, S.B., and Haugen, D.A. (1978) in Methods in Enzymology, Vol. 52, part C, Fleischer, S. and Packer, L., eds., pp. 109-117, Academic Press, N.Y.
13. Dutton, G.J. (1980) in Glucuronidation of Drugs and Other Compounds, pp. 200-201, CRC Press, Boca Raton, FL.
14. Magdalou, J., Antoine, B., Ratanasavanh, D., and Siest, G.(1982) Enzyme 28, 41-47.
15. Teschke, R., Moreno, F., and Petrides, A.S. (1981) Biochem. Pharmacol. 30, 1745-1751.
16. Lieber, C.S. and DeCarli, L.M. (1970) Amer. J. Clin. Nutr. 23, 474-478.
17. Gram, T.E., Hansen, A.R., and Fouts, J.R. (1968) Biochem. J. 106, 587-591.