PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 271–274 (1975)

The Effect of Chlordiazepoxide Hydrochloride on the Isolated Perfused Rat Liver (38786)

CHARLES O. ABERNATHY, STEWART SMITH AND H. J. ZIMMERMAN

Liver Research Unit, Veterans Administration Hospital, Washington, D. C. 20422

The extensive employment of chlordiazepoxide·HCl (CDZ) for treatment of anxiety and related conditions is generally attended by few complications. However, several reports (1–5) have implicated CDZ as the cause of hepatic injury including jaundice.

Several other drugs which are known to cause hepatic injury in humans as the result of presumed hypersensitivity or other idiosyncratic reaction have been shown to decrease biliary flow and the excretion of sulfobromophthalein (BSP) by the isolated, perfused rat liver (6–10). Accordingly, studies were implemented to ascertain the effects of CDZ on bile flow and biliary clearance of BSP. In order to test the possibility that a metabolite of CDZ, rather than the parent compound, might lead to liver damage, the effect of pretreatment with phenobarbital (Pb) which has been shown to cause a threefold increase in the production of the main metabolite (11) of CDZ was examined.

Methods and Materials. Female Sprague–Dawley rats (240–260 g) were obtained from Charles River Laboratory and given free access to food and water. They were not starved prior to sacrifice. For the Pb studies, rats received Pb sodium (100 mg/kg, ip) once a day for 5 days, while control rats received saline only.

The liver perfusion technique of Penhos *et al.* (12) was used with several modifications. Rats were anesthesized with pentobarbital sodium (50 mg/kg) and the abdomens shaved to remove hair. The abdominal cavity was opened and 500 units of heparin injected into the vena cava. The bile duct and portal vein were cannulated with PE-10 and PE-205 tubing (Intramedic, Clay-Adams), respectively. After cannulation, the liver was rinsed with a modified Krebs–Henseleit [each 100 ml of buffer also contained 240 mg glucose, 0.83 g bovine serum albumin, 2000 units heparin, and, depending on the experiment, CDZ (5×10^{-4} *M*) or no drug] buffer (pH 7.4) until blood was cleared from the liver (20–30 ml). The liver was then carefully removed from the animal and placed on a plastic platform which drained into a reservoir. The platform and resevoir were in a perfusion chamber (Metalloglass, Boston) maintained at 37 ± 1°. To prevent transient anoxia, the buffer had been oxygenated prior to sacrifice and O_2:CO_2 (95:5) was bubbled through the perfusate during the entire experiment. Sodium taurocholate (1 μM/min) was infused into the perfusate throughout the 90 min experiment and the hydrostatic pressure on the portal vein was maintained at 17 cm water.

After a 30 min equilibrium period (CDZ or carrier was present for the entire 90 min), during which time bile flow was measured to assess the condition of the liver, 20 mg of BSP was added to the perfusate. To insure adequate mixing, the perfusate container was mounted on a magnetic stirrer.

At appropriate intervals during the following 60 min, bile and perfusate samples were taken to determine the bile flow, biliary clearance of BSP, and the hepatic uptake of BSP. The bile flow rate was measured by determining the time needed to collect 20 μl of bile. The concentration of BSP in the bile and perfusate was measured spectrophometrically using appropriate standards.

To obtain maximal bile flow in this model system, several precautions needed to be taken. We found that; (1) the bile cannula should be positioned slightly distal to the lobes of the liver where the bile ducts converge, (2) care must be exercised to position the liver so that neither the portal vein nor the bile duct is constrained, (3) proper tension of the bile duct must be maintained (this is accomplished by taping the bile cannula to the side of the perfusion

Copyright © 1975 by the Society for Experimental Biology and Medicine
All rights reserved.

TABLE I. THE EFFECTS OF CHLORDIAZEPOXIDE HCl AND PHENOBARBITAL ON BILE FLOW AND BILIARY CLEARANCE OF SULFOBROMOPHTHALEIN (BSP).

Treatment[a]	Total Bile ml	Total BSP mg	Bile Flow μl/min/g liver	BSP Excreted μg/min/g liver	BSP/Bile mg/ml	BSP Uptake/g liver mg/g
Controls	1.27 ±0.04	12.10 ±0.55	2.38 ±0.07	23.1 ±1.23	9.57 ±0.53	1.65 ±0.07
CDZ (5×10^{-4} *M*)	1.07 ±0.05[b]	8.37 ±0.39[b]	2.07 ±0.09[b]	16.20 ±0.82[b]	8.01 ±0.43[b]	1.32 ±0.05[b]
Phenobarbital	1.54 ±0.08[b]	12.97 ±0.63	2.32 ±0.09	18.3 ±1.41[b]	8.60 ±0.39	1.39 ±0.11[b]
Phenobarbital × CDZ (5×10^{-4} *M*)	1.19 ±0.05[d]	7.77 ±0.60[b, d]	1.86 ±0.08[b, c, d]	12.1 ±0.83[b, c, d]	6.49 ±0.37[b, c, d]	0.98 ±0.04[b, c, d]

[a] 15 animals in each treatment group. Values given as mean ± SE.
[b] Significantly different than controls. $P < 0.05$.
[c] P (CDZ vs. Phenobarbital × CDZ) < 0.05.
[d] P (Phenobarbital vs. Phenobarbital × CDZ) < 0.05.

chamber), and (4) the angle between the bile and hepatic cannulas should be approximately 45°.

A two-way analysis of variance was calculated and where significance occurred, the means were compared using Students *t* test as outlined by Snedecor (13).

Results. Bile flow was decreased by approximately 20% by the addition of CDZ to the perfusate. The total bile flow in the controls was 1.27 ml (7.69 μl/min/100 g body wt) and that of the CDZ treated group was 1.07 ml (6.42 μl/min/100 g body wt) (Table I).

Bile flow from livers of animals which had been pretreated with Pb was significantly ($P < 0.05$) greater than that of the controls, when expressed as total bile, but expressed according to liver weight, however, there was no difference (Table I).

BSP excretion expressed as total clearance or concentration in the bile, was approximately equal in the control and Pb groups. However, livers from the CDZ and Pb × CDZ groups cleared only 8.37 and 7.77 mg, respectively. These values were significantly lower ($P < 0.05$) than those of their respective controls. CDZ caused a significant reduction (16.6%) in the ability of the livers from control animals to concentrate BSP into bile and a 24% reduction in liver preparations from Pb pretreated rats (Table I).

Calculation of these data on the basis of liver weight also showed that the Pb group excreted less dye, 18.3 μg/min/g liver, than did the controls (23.1). The Pb group was also less efficient than the control group in removing BSP from the perfusate (1.39 vs. 1.65 mg/g liver, respectively. The addition of CDZ to the perfusate resulted in a significant reduction in BSP excretion and its effects were similar in either control or Pb pretreated animals (Table I).

Discussion. The results of the present investigation have demonstrated an adverse effect of CDZ on the isolated perfused rat liver. The deleterious effects of CDZ on bile flow, concentration of BSP into bile and hepatic uptake of BSP were 50% greater when livers from Pb pretreated animals were used than when controls were used. However, the adverse effects of CDZ on BSP excretion were similar in both control and Pb pretreated groups. The complex interrelationship between the effects of Pb and CDZ on control livers did not permit distinction of the role of CDZ from that of a metabolite on hepatic function.

There have been several reports that Pb pretreatment enhances bile flow, plasma disappearance and biliary clearance of various chemicals (14–25). While other microsomal enzyme inducers increase the biliary clearance of chemicals, (22, 23), the ability of Pb to stimulate bile flow is a property not shared by most other enzyme inducers (16). However, ethanol, an inducer

when given chronically (24, 25), also can cause an increase in bile flow (26). The results of the present study suggest that Pb pretreatment stimulates bile flow per animal but not when corrected for the increase in liver weight induced by Pb. Paumgartner *et al.* (19), however, reported that Pb caused a significant increase in bile flow even when the data were calculated on the basis of liver weight. However, their control bile flow rates were lower than those of the present study (4.7 vs 7.7 μl/min/100 g body wt; 19; Results), a difference that could explain the disparate results. Our results parallel those of Boyer (26), who found that chronic ethanol administration led to an increase in bile flow when the data were expressed according to body weight, but not when expressed on a liver weight basis.

There are several possible explanations for the differences between our work and that of other investigators. We utilized an isolated rat liver preparation, whereas other studies were conducted with an intact animal (15–23). Our perfusate was completely synthetic and did not contain heparinized rat blood. We also infused a constant amount of bile salt during the experiment. These procedures enabled us to control several experimental variables, but may have affected certain parameters which, in the intact animal, may influence bile flow. Since the effects of Pb on the liver are multiple (27), the influence on bile flow may be related to other effects. Indeed, Klaassen (16) has shown that there is no simple relationship between increased liver weight and bile flow after Pb treatment. Reconciliation of the apparent diverse reports on the relationship of Pb to hypercholeresis awaits additional work.

The relevance, to reports of hepatic injury caused by CDZ (1–5), of the adverse of this drug on hepatic function observed in the present study remains to be demonstrated. These observations are consistent with, but less than proof of, the hypothesis that the production of hepatic injury by a drug in humans as the result of presumed hypersensitivity depends on some intrinsic, though mild, hepatotoxicity of the respective agent (28). However, the level of CDZ utilized in this study was higher than those generally found in patients under therapeutic conditions. In clinical usage, the level of CDZ in plasma reaches a concentration as high as 6×10^{-6} *M* (29) and CDZ can be concentrated eight-fold by the rat liver (30). Assuming the human liver can concentrate CDZ in a similar fashion, the resulting concentration (4×10^{-5} *M*) would still be approximately ten-fold less than that which reduced bile flow and BSP excretion in these experiments. Accordingly, the relevance of these observations to drug-induced hepatic injury remains to be demonstrated.

Summary. The effects of chlordiazepoxide·hydrochloride (CDZ) on the isolated perfused rat liver were examined. CDZ administration decreased bile flow, biliary excretion of sulfobromophthalein (BSP) and hepatic uptake of BSP. The addition of CDZ to the perfusate of livers obtained from phenobarbital (Pb) pretreated rats led to 50% greater reductions in bile flow, concentration of BSP in bile and hepatic uptake of BSP. The adverse effects of CDZ on BSP excretion per g liver, however, did not appear to be enhanced by Pb pretreatment. The complex nature of the interrelationship of the effects of Pb and of CDZ on the control liver prevented differentiation of the role of CDZ from that of a metabolite on the adverse effect on liver function.

1. Cacioppo, J., and Merlis, S., Amer. J. Psychiat. **117**, 1040 (1961).
2. Abbruzzese, A., and Swanson, J., New Engl. J. Med. **273**, 321 (1965).
3. Pickering, D., New Engl. J. Med. **274**, 1449 (1966).
4. Winkelmayer, R., Delaware Med. J. **30**, 334 (1966).
5. Lo, K.-J., Eastwood, I. R., and Eidelman, S., Amer. J. Dig. Dis. **12**, 845 (1967).
6. Bassan, H., Kendler, J., Harinasuta, U., and Zimmerman, H. J. Biochem. Pharmacol. **20**, 1429 (1971).
7. Bassan, H., Kendler, J., and Zimmerman, H. J., Proc. Soc. Exp. Biol. Med. **137**, 852 (1971).
8. Kendler, J., Bassan, H., and Zimmerman, H. J., Proc. Soc. Exp. Biol. Med. **137**, 1168 (1971).
9. Kendler, J., Bowry, S., Seeff, L. B., and Zimmerman, H. J., Biochem. Pharmacol. **20**, 2439 (1971).

10. Kendler, J., Anuras, S., Laborda, O., and Zimmerman, H. J., Proc. Soc. Exp. Biol. Med. **139,** 1272 (1972).
11. Garattini, S., Marcucci, F., and Mussini, E., Drug Metabol. Rev. **1,** 291 (1972).
12. Penhos, J. C., Wu, C. H., Daunas, J., Reitman, B. A., and Levine, R., Diabetes **15,** 740 (1966).
13. Snedecor, G. W., *in* "Statistical Methods", 5th Ed., p. 237, Iowa State University Press (1966).
14. Fujimoto, J. M., Eich, W. F., and Nichols, H. R. Biochem. Pharmacol. **14,** 515 (1965).
15. Klaasen, C. D., and Plaa, G. L., J. Pharmacol. Exp. Ther. **161,** 361 (1968).
16. Klaassen, C. D., J. Pharmacol. Exp. Ther. **168,** 218 (1969).
17. Klaassen, C. D., Biochem. Pharmacol. **19,** 1241 (1970).
18. Klaassen, C. D., J. Pharmacol. Exp. Ther. **176,** 743 (1971).
19. Paumgartner, G., Horak, W., Probst, P., and Brabner, G., Nauyn-Schmidebeig Arch. Pharmakol. **270,** 98 (1971).
20. Hart, L. G., Guarino, A. M., and Adamson, R. H., Amer. J. Physiol. **217,** 46 (1969).
21. Berthelot, P., Erlinger, S., Dheumeaux, D., and Preaux, A. M., Amer. J. Physiol. **219,** 809 (1970).
22. Levine, W. G., Millburn, P., Smith, R. L., and Williams, R. T., Biochem. Pharmacol. **19,** 235 (1970).
23. Levine, W. G., J. Pharmacol. Exp. Ther. **175,** 301 (1970).
24. Rubin, E., Hutterer, F., and Lieber, C. S., Science **159,** 1469 (1968).
25. Rubin, E., and Lieber, C. S., Science **162,** 690 (1968).
26. Boyer, J. L., Gastroenterology **62,** 294 (1972).
27. Erlinger, S., and Dhumeaux, D., Gastroenterology **66,** 281 (1974).
28. Zimmerman, H. J., Perspect. Biol. Med. **12,** 1 (1968).
29. Vesell, E. S., and Passananti, G. T., Clin. Chem. **17,** 851 (1971).
30. Koechlin, B. A., Schwartz, M. A., Krol, G., and Oberhansli, W., J. Pharmacol. Exp. Ther. **148,** 399 (1965).

Received December 16, 1974. P.S.E.B.M. 1975, Vol. 149.