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Inhibitory Effects of Scillaren and Dinitrophenol on Bilirubin Excretion by the Isolated Perfused Rat Liver[1] (39253)

JOSEPH R. BLOOMER,[2] JOAN ZACCARIA, AND GERALD KLATSKIN

Department of Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510

The hepatic excretion of bilirubin involves its uptake from plasma, conjugation, and transport across the canalicular membrane into bile. Studies in animals indicate that the maximal rate at which bilirubin can be excreted into bile is determined by the rate at which conjugated bilirubin can be transported across the canalicular membrane (1, 2), although conjugation is rate-limiting when there is a marked deficiency in conjugating activity (1, 3). Bile flow may be an additional factor that regulates the transport of bilirubin across the canalicular membrane, thus influencing the maximal excretion rate (2).

We have studied the effects of scillaren and dinitrophenol on bilirubin excretion by the isolated perfused rat liver. Both compounds have been shown to inhibit bile flow in this system (4). Scillaren is a cardiac glycoside that inhibits the bile acid-independent formation of bile (4). Dinitrophenol appears to alter this fraction as well (4), although it may also influence other processes that are required for hepatic transport since it uncouples oxidative phosphorylation.

Materials and methods. The perfusion technique was essentially that of Miller and coworkers (5). Male Wistar rats (CAMM Research Institute, Wayne, New Jersey) weighing 350–400 g were used as liver donors. Ninety milliliters of perfusing medium, which consisted of sheep red blood cells dispersed to a hematocrit of 20 to 30% in Krebs–Ringer bicarbonate buffer, pH 7.4, containing 3 g/100 ml of bovine albumin and 100 mg/100 ml of glucose, was perfused through the liver at a rate of 20 ml/min in each study. The total duration of the perfusion was 3 hr. Bilirubin was infused into the perfusing medium (6) at two different rates, 18.8 μg/min/g of liver or 7.9 μg/min/g of liver, starting 30 min after the perfusion was initiated. The higher infusion rate exceeded the maximal bilirubin excretion rate that could be attained under the conditions of perfusion (14.4 ± 1.2 μg/min/g of liver as determined in 11 studies). Sodium taurocholate (Schwarz-Mann, Orangeburg, N.Y.) was infused simultaneously at a rate of 30 μmole/hr in order to mimic the physiological condition (7). Sixty minutes later, either 25 mg of scillaren (Sandoz Pharmaceuticals, Basel, Switzerland) or 16.6 mg of 2,4-dinitrophenol (Fisher Scientific Co., Fair Lawn, N.J.), dissolved in 0.6 ml of propylene glycol, was administered to the perfusing medium. These amounts produced initial perfusate concentrations previously shown to inhibit bile flow in this system, whereas propylene glycol alone does not (4). The effects of these compounds on bilirubin excretion were compared with control studies by Student's t test. Three livers were studied at each of the six conditions.

The total bilirubin concentration in bile was measured by the method of Malloy and Evelyn (8), and unconjugated and conjugated bilirubin levels in the perfusate were measured by the method of Weber and Schalm (9). The total hepatic bilirubin concentration at the conclusion of the study was estimated from the difference between the amount of bilirubin infused and that recovered in bile and perfusing medium. The activity of homogenates of the perfused livers in conjugating bilirubin (hepatic bilirubin UDPGT activity) was measured by the method of Black and coworkers (10).

Results. At both rates of bilirubin infusion, scillaren and dinitrophenol reduced bile flow and bilirubin excretion (Table I) compared to those in control studies ($P < 0.01$). Although the effects of these compounds were similar during the first 30 min

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[2] Dr. Bloomer is an investigator, Howard Hughes Medical Institute.

after administration, there was a marked difference during the last hour of the perfusion. Following scillaren administration, the bilirubin concentrations in bile rose over the values observed in the control studies (Fig. 1). Thus, bilirubin excretion was reduced by a lesser degree than was bile flow. After dinitrophenol, however, the bilirubin concentration in bile fell during the last hour of perfusion as compared with the control studies (Fig. 1), indicating that there was a greater effect on bilirubin excretion than on bile flow.

At both infusion rates of bilirubin, dinitrophenol inhibited the hepatic removal of unconjugated bilirubin from the perfusing medium, as indicated by the fact that the unconjugated bilirubin concentration in the perfusate increased by a greater degree than occurred during the control infusions (Fig. 2 and Table I). This effect was not observed after scillaren administration. The inhibition of hepatic unconjugated bilirubin removal by dinitrophenol could not be attributed to its greater effect on bile flow, since following bile duct ligation in three studies during the high bilirubin infusion rate, the unconjugated bilirubin concentration in the perfusing medium increased at the same rate as during the control studies. It was also un-

TABLE I. EFFECT OF SCILLAREN AND DINITROPHENOL (DNP) ON BILIRUBIN EXCRETION.

Bilirubin infusion rate	Liver weight (g)	Biliary excretion[a]		Total perfusate bilirubin[b] (mg/100 ml)	Total hepatic bilirubin[b] (mg/g of liver)
		Flow (μl/min/g of liver)	Bilirubin (μg/min/g of liver)		
18.8 μg/min/g of liver					
Control	12.4 ± 1.3	1.33 ± 0.09	13.67 ± 0.88	6.41 ± 1.31	0.66 ± 0.30
Scillaren added	12.4[c] ± 1.5	0.80 ± 0.11	11.11 ± 1.22	6.45 ± 1.21	0.75 ± 0.19
DNP added	11.8 ± 1.6	0.51 ± 0.20	4.67 ± 1.67	10.82 ± 2.03	1.23 ± 0.34
7.9 μg/min/g of liver					
Control	13.5 ± 1.2	1.21 ± 0.10	7.00 ± 1.00	2.36 ± 0.86	0.20 ± 0.17
Scillaren added	14.8 ± 1.4	0.80 ± 0.21	4.89 ± 0.56	2.36 ± 0.74	0.29 ± 0.04
DNP added	14.0 ± 1.7	0.31 ± 0.09	1.44 ± 0.78	7.00 ± 0.51	0.40 ± 0.06

[a] Biliary excretion values are average rates for the interval 90–180 min of perfusion. Scillaren and DNP were administered at 90 min.

[b] Perfusate and hepatic bilirubin concentrations were determined at the end of the perfusion (180 min).

[c] Mean ± SD (three studies).

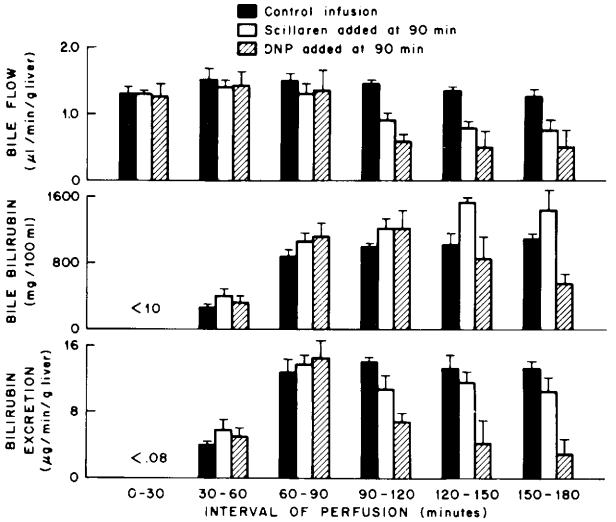

FIG. 1. Effects of scillaren and 2,4-dinitrophenol (DNP) on bile flow and bilirubin excretion by the isolated perfused rat liver are shown at the bilirubin infusion rate of 18.8 μg/min/g of liver. The bilirubin infusion was started after 30 min of the perfusion, and scillaren and DNP were administered at 90 min. Mean ± SD of data from three studies each are shown.

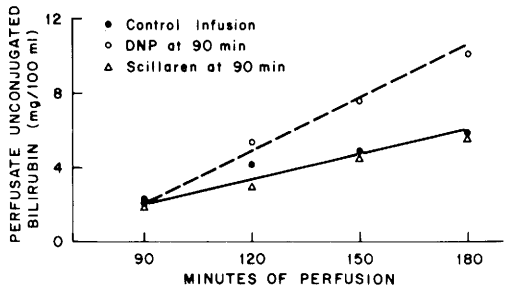

FIG. 2. Effects of scillaren and 2,4-dinitrophenol (DNP) on perfusate unconjugated bilirubin concentrations are shown at the bilirubin infusion rate of 18.8 μg/min/g of liver. The bilirubin infusion was started after 30 min of the perfusion, and scillaren and DNP were administered at 90 min. Mean values from three studies each are shown.

TABLE II. EFFECT OF SCILLAREN AND DINITROPHENOL (DNP) ON HEPATIC WATER AND BILIRUBIN CONJUGATING (UDPGT) ACTIVITY.

	Tissue H_2O (% of wet wt)	Bilirubin UDPGT activity (μg of bilirubin conjugated/min/g of liver)
Control infusions	70.3 ± 3.1	25.1 ± 8.4
Scillaren added	70.2[a] ± 1.7	24.8 ± 3.2
DNP added	72.8 ± 3.1	29.2 ± 7.5

[a] Mean ± SD.

likely to be due to an inhibition of bilirubin conjugation, since the ability of homogenates of the perfused livers to conjugate bilirubin at the termination of the study was not impaired (Table II). Rather, it probably reflects impairment of the initial uptake and/or storage of unconjugated bilirubin by the perfused liver.

Small amounts of conjugated bilirubin were detected in the perfusate during control infusion and after scillaren or dinitrophenol administration (less than 1 mg/100 ml), but there was no significant difference among the various conditions. Conjugated bilirubin did appear in the perfusate in greater amounts (6.32 ± 1.70 mg/100 ml) following bile duct ligation. The difference in conjugated bilirubin metabolism during bile duct ligation, as compared with that following scillaren or dinitrophenol administration, may be due to the difference in degree, and type, of impairment of biliary excretion (i.e., complete mechanical obstruction versus chemically induced partial obstruction).

Discussion. The mechanism of bilirubin movement to the canaliculus, following its conjugation at the smooth endoplasmic reticulum, and subsequent secretion into the biliary channels is poorly understood. Studies with the heterozygous Gunn rat, which has a partial deficiency in the ability to conjugate bilirubin, suggest that conjugation and biliary secretion may be linked (11). This could occur if there were anatomic continuity between the conjugating portions of the endoplasmic reticulum and the transport system for biliary secretion of bilirubin.

Bile flow appears to influence the excretion rate of bilirubin under certain conditions. In sheep, increases in bile flow produced by increasing rates of bile acid infusion cause an elevation in the excretion rate of bilirubin, with no change in the maximal bile bilirubin concentration (2). This suggests that the bilirubin concentration in bile reaches a maximal level, and that bile flow then becomes a rate limiting factor in the amount of bilirubin which can be excreted. Although our studies demonstrate that scillaren and dinitrophenol inhibit both bile flow and bilirubin excretion, indicating that these processes may be associated, the relative effects on bile flow and bilirubin excretion were different. The scillaren studies indicate that the bilirubin concentration in bile may increase further during maximal excretion rates as bile flow is impaired. Dinitrophenol impaired bilirubin excretion to a greater degree than bile flow. Thus, the effects of these compounds on bilirubin excretion must be at least partially independent of their effects on bile flow. Presumably, they inhibit bile water formation by blocking a Na^+-K^+-ATPase system in the canalicular membrane (4, 12). They may affect bilirubin excretion by altering the canalicular membrane in a different manner, or by altering bilirubin transport at a precanalicular level.

The effect of dinitrophenol on bilirubin excretion by the isolated, perfused rat liver is more complex than just an inhibition of bilirubin transport from the hepatocyte into bile. The hepatic removal of unconjugated bilirubin is also altered. Recent evidence suggests that the hepatic uptake of bilirubin

may proceed via a carrier-mediated transport process in the plasma membrane (13), as has been postulated for BSP (14). If so, this may require energy and thus be inhibited by dinitrophenol. On the other hand, dinitrophenol may simply compete with bilirubin for binding to the intracellular hepatic organic anion-binding proteins (15), thus inhibiting bilirubin intake by a means other than uncoupling oxidative phosphorylation.

Summary. The effects of scillaren and dinitrophenol on bilirubin excretion by the perfused rat liver were studied. Both compounds inhibited bile flow, scillaren by 20 to 40%, and dinitrophenol by 60 to 80%. Bilirubin excretion was also impaired. However, the effect of scillaren on bilirubin excretion was less than that on bile flow, as indicated by an increase in the bile bilirubin concentration, whereas dinitrophenol had a greater effect on bilirubin excretion than on bile flow. Dinitrophenol also inhibited the hepatic removal of unconjugated bilirubin from the perfusate, probably because it impaired the initial uptake and/or storage of unconjugated bilirubin by the perfused liver.

1. Arias, I. M., Johnson, L., and Wolfson, S., Amer. J. Physiol. **200,** 1091 (1961).
2. Upson, D. W., Gronwall, R. R., and Cornelius, C. E., Proc. Soc. Exp. Biol. Med. **134,** 9 (1970).
3. Schmid, R., and Hammaker, L., J. Clin. Invest. **42,** 1720 (1963).
4. Boyer, J. L., Amer. J. Physiol. **221,** 1156 (1971).
5. Miller, L. L., Bly, C. G., Watson, M. L., and Bale, W. F., J. Exp. Med. **94,** 431 (1951).
6. Weinbren, K., and Billing, B. H., Brit. J. Exp. Pathol. **37,** 199 (1956).
7. Boyer, J. L., Scheig, R. L., and Klatskin, G., J. Clin. Invest. **49,** 206 (1970).
8. Malloy, H., and Evelyn, K., J. Biol. Chem. **119,** 481 (1937).
9. Weber, A. P., and Schalm, L., Clin. Chim. Acta **7,** 805 (1962).
10. Black, M., Billing, B. H., and Heirwegh, K. P. M., Clin. Chim. Acta **29,** 27 (1970).
11. Robinson, S. H., Yannoni, C., and Nagasawa, S., J. Clin. Invest. **50,** 2606 (1971).
12. Erlinger, S., Dhumeaux, D., Berthelot, P., and Dumont, M., Amer. J. Physiol. **219,** 416 (1970).
13. Scharschmidt, B. F., Waggoner, J. G., and Berk, P. D., J. Clin. Invest. **56,** 1280 (1975).
14. Goresky, C. A., Amer. J. Physiol. **207,** 13 (1964).
15. Levi, A. J., Gatmaitan, Z., and Arias, I. M., J. Clin. Invest. **48,** 2156 (1969).

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