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INFLUENCE OF CLOFIBRATE ON HEPATIC TRANSPORT OF BILIRUBIN AND BROMOSULFOPHTHALEIN IN RATS

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Summary : The hepatobiliary transport of two cholephilic anions, bilirubin and bromosulfophthalein, is compared in the rat following the administration of clofibrate. In the treated rats, the bilirubin transport maximum (on a whole liver basis) increased by 84%. This increase is related to a higher excretion rate of conjugated bilirubin in bile. Hepatic unconjugated bilirubin is not modified. On the contrary, bromosulfophthalein transport decreased slightly but significantly. These results suggest that clofibrate acts primarily on bilirubin hepatic transport by stimulating the conjugating enzyme activity.

INTRODUCTION

Treatment of rats with clofibrate (Ethyl-4-chlorophenoxy-isobutyrate) a hypolipidemic drug, produces a great increase in liver mass (1,2), proliferation of the smooth endoplasmic liver reticulum (3,4) and acceleration of certain metabolisms, such as those of steroid hormones (5) and drugs like aminopyrin or pentobarbital (6,7). Recently, we reported the dramatic effect of clofibrate on the activity of bilirubin-UDP-glucuronyltransferase (BGT), paralleling an increase in the hepatic content of cytosolic Z protein (8). The purpose of this paper is to study the consequences of these effects on

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ABBREVIATIONS: BGT: bilirubin-UDP-glucuronyltransferase; BSP: bromosulfophthalein; B.W.: body weight; Tm: transport maximum C.B.: conjugated bilirubin; U.C.B.: unconjugated bilirubin.

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the hepatic transport of the cholephilic anions bilirubin and BSP, which bind to cytosolic proteins Y and Z both in vitro and in vivo.

MATERIALS AND METHODS

Animal preparation : Male Sprague-Dawley rats (Charles River France), aged six weeks, fed with a controlled diet were given clofibrate (ICI, FRANCE) (20 mg per 100 g B.W. in propylenglycol) per os, once daily for ten days. Controls received propylenglycol by the same route for the same period. 24-h after the last drug dose, animals were anesthetized intraperitonally with sodium pentobarbital (Nembutal, Abbott Laboratories) 5 mg/100 g B.W. Rectal temperature was monitored throughout the experiments at 38°C ± 0.5 ; a jugular vein and a carotid artery were cannulated with polyethylene catheters, PE-50 and PE-10, respectively ; the artery was used for blood sampling. The common bile duct was cannulated with a PE-10 catheter. Bile was then collected for 10 min-periods, in ice-cooled containers in the dark. The ensuing loss of water and electrolytes was compensated by an 0.15 M NaCl intrajugular infusion, 6.61 ml/h, which also contained a pentobarbital maintenance dose of 1.65mg/h per 100 g B.W.

Experimental procedures : Infusion of either bilirubin (Merk, Darmstadt, Germany) or BSP (SERB, Paris) started after a 30-min control period of bile collection. After a priming dose of 1.5 mg/100 g B.W. for bilirubin or 1.8 mg/100 g B.W. for BSP, these substances were infused intravenously after appropriate dilution in 0.15 M NaCl ; the infusion doseswere 150 and 180 µg/min per 100 g B.W. These rates were chosen because they were approximately 1.5-2 times the mean corresponding values of biliary Tm in control rats. In each infused animal, we checked that the plasma concentration of bilirubin or BSP was increasing with time. Tm was calculated from the concentration of bilirubin or BSP measured in the bile samples between the 30th and 60th min of infusion. At the end of the experiments,the liver was removed, washed immediately and weighed. It was then frozen until analysis of its bilirubin content and determination of BGT activity.

Analytical methods : Bile volume was determined gravimetrically assuming a density of 1.0g/ml. BSP concentration in plasma and bile was determined by the spectrophotometric method after appropriate dilution with 0.05 N NaOH. Total bilirubin concentration(T.B.) in plasma and bile was determined by the method of Jendrassik and Grof (9), and conjugated and unconjugated bilirubin (C.B. and U.C.B.) were assayed by the method of Weber and Schalm (10). Hargreaves' method (11) was used to determine total and conjugated bilirubin concentrations in liver. Liver homogenate BGT activity was measured according to Black et al . (12).

The Student t test was used for statistical analysis of results.

TABLE I: Effect of clofibrate on the apparent maximal transport rates (Tm) for bilirubin and BSP.

	Control		Clofibrate treated		P
	N	Mean ± S.E.M.	N	Mean ± S.E.M.	
Bilirubin					
Liver weight					
g/100 g B.W.	9	4.11 ± 0.17	13	5.16 ± 0.19	< 0.001
Bile flow					
µl/mn/100 g B.W.	9	6.3 ± 0.3	13	7.1 ± 0.3	N.S.
Tm					
µg/mn/100 g B.W.	9	76 ± 3	13	140 ± 4	< 0.001
BSP					
Liver weight					
g/100 g B.W.	6	4.45 ± 0.17	7	5.78 ± 0.20	< 0.001
Bile flow					
µl/mn/100 g B.W.	6	10.2 ± 0.5	7	9.6 ± 0.5	N.S.
Tm					
µg/mn/100 g B.W.	6	132 ± 4	7	112 ± 5	< 0.01

The bile flow and anion excretions are the means obtained between the 30th and 60th min of infusion. N.S. indicates $p > 0.05$

RESULTS AND DISCUSSION

Table I shows that in rats pretreated with clofibrate, maximum biliary excretion of bilirubin increased significantly by about 84% ($p < 0.001$) compared to the controls. In contrast BSP Tm was slightly but significantly reduced. In both experiments, bile flow was similar during infusion in treated and control animals. However, in the case of BSP a choleretic effect was observed, in accordance with previous findings (13). The mean dye concentration in bile diminished in the clofibrate-treated rats (11.7 ± SEM 0.6 g/l) compared to the controls (13.0 ± 0.6 g/l), but this decrease was not significant. Since clofibrate treatment increases liver weight by 25%, the Tm of BSP exhibited a highly significant drop in clofibrate-treated rats when results were expressed per g of

TABLE II : Effect of clofibrate on biliary excretion of bilirubin

		Bilirubin excretion µg/mn/100 g B.W.		
Groups	N	Total	Conjugated	Unconjugated
Control	9	76 ± 3	58 ± 3	18 ± 1
Clofibrate treated	13	140 ± 4	124 ± 4	16 ± 1
P		< 0.001	< 0.001	N.S

Values are the means ± S.E.M. obtained between the 30th and the 60th min of infusion. N.S. indicates $p > 0.05$

liver (control rats : 30.0 ± 1.7 µg/mn/g liver ; clofibrate-treated rats : 19.4 ± 0.7 µg/mn/g liver ; $p < 0.001$). On the contrary, the biliary excretion of bilirubin per g of liver rose in clofibrate-treated rats by 47.5%. The increased Tm of bilirubin under the influence of clofibrate treatment was related to the higher excretion rate of conjugated bilirubin observed in bile. U.C.B. excretion remained unchanged. Consequently, there was a marked increase in the conjugated/total bilirubin ratio in bile. Results are shown in Table II. These findings are consistent with the stimulated BGT activity observed (control rats : 1.74 ± 0.09 mg C.B./h/g liver ; clofibrate-treated : 4.01 ± 0.13 mg C.B./h/g liver ; $p < 0.001$) and also with our previous results (8).

In spite of the marked increase of the biliary excretion of bilirubin, the hepatic bilirubin content measured at the end of the infusion experiments was significantly higher in the treated animals compared to the controls (Table III). These results support the contention that clofibrate enhances the

TABLE III : Hepatic content of total , conjugated and unconjugated bilirubin after bilirubin infusion.

		Bilirubin ($\mu g.liver^{-1}$)		
Groups	N	Total	Conjugated	unconjugated
Control	9	272 ± 18	129 ± 10	143 ± 9
Clofibrate treated	13	408 ± 26	231 ± 16	177 ± 13
P		< 0.001	< 0.001	N.S.

Results are means ± means S.E.M. N.S. indicates $p > 0.05$

bilirubin uptake by the liver. It should be noted that there is an accumulation of C.B. in the liver. This observation agrees with the fact that the amount of C.B. excreted by the liver (Tm bilirubin : 27 ± 1 µg/mn/g liver) is lower than the amount which the liver is capable of conjugating per unit of time (BGT : 67 ± 2 µg/mn/g liver). Hepatic bilirubin transport in the rat is therefore enhanced by clofibrate treatment. The question arises of wether this enhancement is related to the increased hepatic level of Z protein and/or to the stimulated BGT activity observed. Several drugs are known to increase BGT activity, among them phenobarbital, which also acts on cytosolic proteins Y and Z. These proteins are thought to facilitate both the uptake and storage of organic anions by the liver (14) thus allowing their subsequent conjugation. Although clofibrate, like phenobarbital, increases both the disappearence rate of bilirubin from plasma (15) and the uptake and maximal bilirubin excretion by the liver, the mechanisms of action seem different, as follows :

1°) phenobarbital treatment enhances Tm for both BSP (13)

and bilirubin (15), whereas clofibrate administration enhances Tm for bilirubin only. These observations are in agreement with a growing number of reports concerning the multiplicity of hepatic transport pathways (16-18).

2°) the enhanced excretion of bilirubin into the bile seen in phenobarbital-treated animals is due to an increase in bile volume rather than an increase in the capacity to secrete bilirubin (15). Clofibrate, on the contrary, induces a higher rate of conjugated bilirubin secretion without changing the unconjugated rate. The difference in the effect of the two drugs on the hepatic transport of bilirubin and BSP might be explained by their different action on cytosolic proteins : phenobarbital increases Y whilst clofibrate increases Z. Similarities were recently noted in molecular weight and binding properties between proteins Y (19) and a glutathion-transferase, S-aryltransferase B responsible for the conjugation of BSP. Consequently, even if Z were involved in BSP transport, its role would not limit the overall process, and the increase in Z alone would not be able to enhance hepatic transport. On the contrary, the phenobarbital-induced rise in Y increases BSP Tm. Thus, maximal biliary excretion of either bilirubin or BSP only appears to be enhanced when the conjugation enzyme activity is stimulated.

Our results suggest that clofibrate acts primarily on bilirubin hepatic transport by stimulating BGT activity ; however, other mechanisms may be involved. A clinical application of the effect of clofibrate on the different steps of hepatic bilirubin transport (uptake, metabolism and excretion) should be possible in the treatment of neonatal jaundice.

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