Biochemical Pharmacology, Vol. 38, No. 16, pp. 2739–2741, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

SHORT COMMUNICATIONS

Biliary excretion of organic anions in clotrimazole-treated rats

(*Received* 25 *November* 1988; *accepted* 10 *February* 1989)

The imidazole-containing antifungal agents have been shown to present both inhibitory and inductive effects on cytochrome P-450 mediated reactions in many mammalian tissues [1, 2]. Very recently it has been reported that some of these agents also induce in the rat Phase II conjugating enzymes such as UDP-glucuronyl transferases or glutathione *S*-transferases [3].

The glutathione *S*-transferases are a family of enzymes playing a central role in the conjugation of many toxic compounds with glutathione. Enzyme induction or inhibition modify the hepatic excretion of compounds conjugated with glutathione [4–6], although the rate of removal of such compounds from plasma also depends on other hepatic factors. Given the absence of information on the effects of N-substituted imidazoles in the disposition of compounds excreted by the liver, we have explored the influence of one of such agents, clotrimazole, on the biliary excretion of two cholephils, sulfobromophthalein (BSP) and dibromosulphthalein (DBSP), in the rat. The fact that BSP is conjugated with glutathione while DBSP is excreted unchanged in this species, allows to distinguish factors affecting hepatic transport from those interfering with conjugation.

Materials and methods

Chemicals. 1-Chloro-3,4-dinitrobenzene (CDNB), clotrimazole, 5,5′-dithiobis-(2-nitrobenzoic acid), glutathione and glutathione reductase were purchased from Sigma Chemical Co. (St Louis, MO). 3,4-Dichloronitrobenzene (DCNB) was obtained from Aldrich Chemical Co. (Steinheim, F.R.G.). Dibromosulphthalein was from SERB (Paris, France).

Animals and experimental procedures. Male Wistar rats (Panlab, Barcelona, Spain) weighing 260–320 g were housed in cages in a temperature controlled room (21°) under a 12 hr dark/light cycle. The animals were fed with commercial pelleted food (Panlab) and water *ad lib*. Clotrimazole (75 mg/kg) suspended in 4 ml/kg of polyethylene glycol 400 was given i.g. daily for 3 days. Experiments were carried out 48 hr after the final dose of clotrimazole [3]. Controls received polyethylene glycol.

Rats were anaesthetized with sodium pentobarbitone (Claudio Barcia, Madrid, Spain; 65 mg/kg). The right carotid artery and right jugular vein were catheterized and the bile duct cannulated with PE-50 tube. Rectal temperature was maintained at 37° by means of a heat lamp. After collecting two basal 15 min samples of bile, rats were injected intravenously with BSP or DBSP (120 μmol/kg, 3 ml/kg) and bile was collected for eight additional 15 min-intervals. Blood samples were obtained via the carotid artery at 4, 8, 15, 22, 37 and 52 min following injection of the organic anions. At the end of the experiments the animals were killed by exsanguination.

Analytical methods. The concentration of BSP and DBSP in plasma and bile was determined spectrophotometrically at 580 nm after appropriate dilution with 0.1 M NaOH. Liver content of BSP was determined by the method of Whelan and Combes [7]. The proportion of unconjugated and conjugated BSP in bile was estimated by ascending chromatography on Whatman n.1 paper using butan-1-ol/acetic acid/ethanol/water (120/1/20/40). Total liver glutathione concentration was determined by the method of Tietze [8]. Hepatic cytosolic glutathione *S*-transferase activity was measured with CDNB and DCNB according to the method of Habig *et al.* [9] and with BSP according to Goldstein and Combes [10]. Pharmacokinetic parameters of plasma BSP and DBSP disappearance were calculated by a two compartment open model with elimination from the peripheral compartment [11, 12]. The significance of the differences among the groups was analyzed by the Mann–Whitney U-test.

Results and discussion

Treatment with clotrimazole induced a significant increase in liver weight (14.1 ± 0.7 g vs 9.5 ± 0.4 g in the controls; $P < 0.05$) and in the liver weight/body weight ratio (4.81 ± 0.44 g/100 g vs 3.37 ± 0.40 g/100 g; $P < 0.05$).

The effect of the antifungal agent on liver glutathione concentration and glutathione *S*-transferase activity is shown in Table 1. Our data indicate that glutathione *S*-transferase activity was enhanced, not only toward the general substrate CDNB, as previously described [3], but also toward DCNB and BSP, that are substrates with a higher isoenzyme specificity.

Given the influence that such modifications could have on the hepatobiliary transport of compounds conjugated with glutathione in the liver, we investigated the influence of clotrimazole on the hepatobiliary transport of one of those compounds, the cholephilic dye BSP. Our data indicate that the maximal biliary excretion (T_m) of BSP was higher and more rapidly reached in clotrimazole-treated rats than in the controls (Fig. 1). A linear relationship between transferase activity and the T_m of BSP was evident both in controls and in treated animals (Fig. 1). Analysis of the bile corresponding to this period indicated that excretion of conjugated BSP was similar in both groups. The contribution to T_m of conjugated BSP, however, was significantly increased following clotrimazole treatment (Table 2), as also was the cumulative excretion of the conjugated dye (22.38 ± 1.07 μmol vs 27.36 ± 2.86 μmol in the controls; $P < 0.05$). Hepatic BSP concentration at 30 min of dye administration was significantly reduced with respect to the controls in rats receiving clotrimazole (Table 2). No significant effect on the T_m of DBSP was found in clotrimazole-treated animals (37.3 ± 3.1 nmol/min. g liver vs 36.5 ± 2.3 nmol/min. g liver in the controls).

Conjugation with glutathione is known to facilitate biliary excretion of BSP because BSP-glutathione is excreted at a faster rate than unconjugated BSP and because the conjugate eliminates the parent compound which is a strong inhibitor of BSP glutathione excretion [13]. The data above support the hypothesis that the increase in the glutathione conjugating capacity would be the mechanism by which clotrimazole enhances in our experiments the hepatobiliary transport of BSP. Different studies suggest that conjugation could be an important factor determining maximal biliary excretion of BSP [4–6]. The close relationship between enzyme activity and the T_m of BSP found in this study further emphasizes the role of glutathione *S*-transferase as a limiting factor in the hepatobiliary transport of this organic anion.

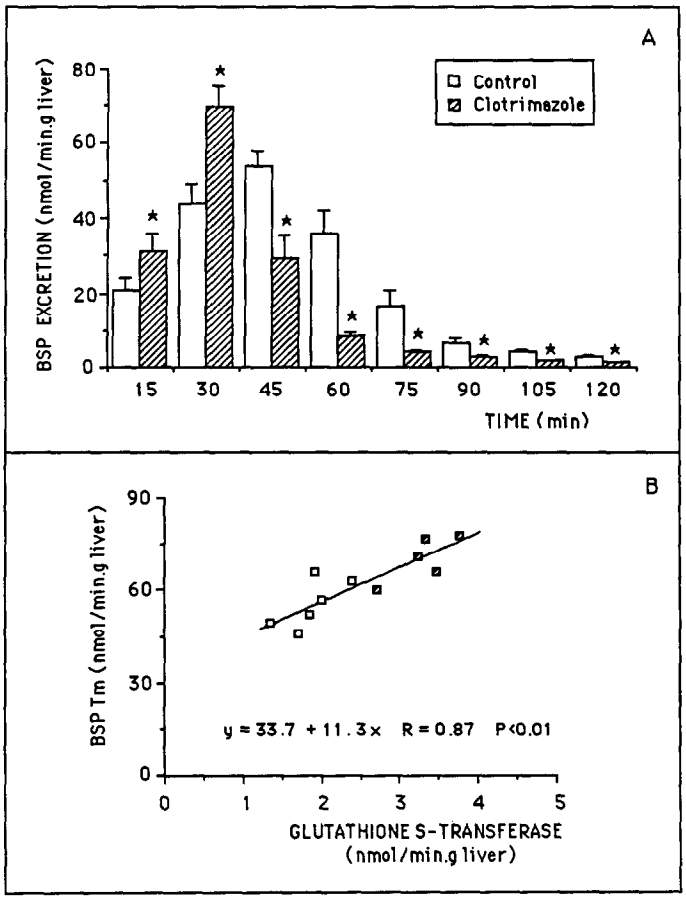

Fig. 1. (A) Biliary excretion of BSP in control and clotrimazole-treated rats. Means ± SE for 5–6 rats. *$P < 0.05$ significantly different from controls. (B) Relationship between the individual values of BSP T_m and hepatic BSP glutathione *S*-transferase activity in control and clotrimazole-treated rats.

Table 1. Effect of clotrimazole treatment on hepatic glutathione concentration and glutathione *S*-transferase activity

	Glutathione (μmol/g liver)	Glutathione *S*-transferase (nmol/min. g liver) CDNB	DCNB	BSP
Control	3.9 ± 0.3	59.58 ± 4.08	2.56 ± 0.08	1.90 ± 0.08
Clotrimazole	3.7 ± 0.3	90.57 ± 10.03*	3.62 ± 0.16*	3.07 ± 0.17*

Values are means ± SE for 5–6 rats.
* $P < 0.05$ significantly different from controls.

Table 2. Effect of clotrimazole treatment on the biliary excretion of unconjugated and conjugated BSP and on liver BSP concentration

	Unconjugated BSP (nmol/min. g liver)	Conjugated BSP	Liver BSP (μmol/g liver)
Control	5.01 ± 0.49	48.80 ± 3.79	480 ± 43
Clotrimazole	5.29 ± 1.55	64.40 ± 4.30*	183 ± 37*

Values are means ± SE for 5–6 rats.
* $P < 0.05$ significantly different from controls. Excretory rates indicate the contribution to T_m of unconjugated and conjugated BSP. Liver BSP concentrations were obtained at 30 min of BSP administration.

Not only conjugation could contribute to the increase in BSP biliary excretion induced by clotrimazole, but also other factors related to hepatic transport. A trend toward a higher value for the initial plasma clearance (K_a) of BSP (0.211 ± 0.063/min vs 0.144 ± 0.49/min in the controls) and a significant increase in that of BSP (0.233 ± 0.030/min vs 0.138 ± 0.037/min; $P < 0.05$) were found in our experiments. This suggests that clotrimazole could modify hepatic blood flow and/or delivery of the organic anions into the liver. In any case, given the absence of changes of DBSP biliary excretion following clotrimazole treatment, effect of this agent on the process of hepatic uptake must be ruled out as a factor contributing to the higher excretion of BSP.

Department of Physiology, Pharmacology and Toxicology
University of León
24007 León
Spain

M. J. TUÑON
P. GONZALEZ
V. MANRIQUE
L. A. GARCIA-PARDO
J. GONZALEZ*

REFERENCES

1. Lavrijsen K, Van Houdt JV, Thijs D, Meuldermans W and Heykants J, Induction potential of antifungal containing and imidazole or triazole moiety. *Biochem Pharmacol* **35**: 487–491, 1986.
2. Rodriguez AD, Gilson GG, Ioannides C and Parke DV, Interactions of imidazole antifungal agents with purified cytochrome P-450 proteins. *Biochem Pharmacol* **36**: 4277–4281, 1987.
3. Ritter JK and Franklin MR, Induction and inhibition of rat hepatic drug metabolism by N-substituted imidazole drugs. *Drug Metab Dispos* **15**: 335–343, 1987.
4. Siddik ZH, Drew R and Gram TE, Metabolism and biliary excretion of sulfobromophthalein in vitamin A deficiency. *Biochem Pharmacol* **29**: 2583–2588, 1980.
5. Foliot A, Touchard D and Celier C, Impairment of hepatic glutathione *S*-transferase activity as a cause of reduced biliary sulfobromophthalein excretion in clofibrate-treated rats. *Biochem Pharmacol* **33**: 2829–2834, 1984.
6. Mariscal MA, Muñoz ME, Collado PS, Esteller A and Gonzalez J, Impairment of sulfobromophthalein biliary excretion and inhibition of gluthatione *S*-transferase activity induced by perhexiline maleate in rats. *Biochem Pharmacol* **37**: 3461–3465, 1988.
7. Whelan G and Combes B, Competition by unconjugated and conjugated sulfobromophthalein sodium (BSP) for transport into bile. Evidence for a single excretory system. *J Lab Clin Med* **78**: 230–244, 1971.
8. Tietze F, Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Applications to mammalian blood and other tissues. *Anal Biochem* **27**: 502–522, 1969.
9. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
10. Goldstein J and Combes B, Spectrophotometric assay of the liver enzyme that catalyzes sulfobromophthalein-glutathione conjugation. *J Lab Clin Med* **67**: 863–872, 1966.
11. Brown RD and Manno JE, ESTRIP, a basic computer program for obtaining polyexponential parameter estimates. *J Pharm Sci* **67**: 1687–1691, 1978.
12. Jansen PL, Peters WHM and Meijer DKF, Hepatobiliary excretion of organic anions in double-mutant rats with a combination of defective canalicular transport and uridine 5'-diphosphateglucuronyltransferase deficiency. *Gastroenterology* **93**: 1094–1103, 1987.
13. Gregus Z, Fischer E and Varga F, Inhibition by bromsulphthalein of the biliary excretion of its glutathione conjugate. *Biochem Pharmacol* **26**: 1951–1952, 1977.

* Author for correspondence: Prof. Javier González, Departamento de Fisiología, Farmacología y Toxicología, Universidad de León, Campus de Vegazana, 24007 León, Spain.

Biochemical Pharmacology, Vol. 38, No. 16, pp. 2741–2744, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Effect of fenofibrate treatment on linoleic acid desaturation in liver of obese Zucker rats

(*Received* 6 *December* 1988; *accepted* 10 *February* 1989)

Fenofibrate, isopropyl [(chloro-4 benzoyl) 4 phenoxyl]-2 methyl-2 propionate, is a clofibrate-related compound which has been used as a hypolipidaemic drug in humans since 1975 [1]. The lowering of triglyceridaemia and cholesterolemia *in vivo* [2] is related to a decrease in HMG–CoA reductase activity [3, 4], increase of post-heparin lipase activity [5] and inhibition of very low density lipoprotein (VLDL) secretion [6]. An increase in the oxidative capacity of liver mitochondria and peroxisomes can also participate in the hypolipidaemic effect of fenofibrate [7], as well as of clofibrate [8–10]. The clofibrate acid-feeding in rats has been reported to increase the proportion of octadecenoic acid (18:1 n-9) in lipids from hepatic homogenates and microsomes by increasing the activity of microsomal stearyl–CoA–Δ9 desaturation [11–13]. Such an increase was also observed in diabetic, hyperthyroid and hypothyroid rats [11]. However, little is known about the effect of hypolipidaemic drugs on the biosynthesis of arachidonic acid (20:4 n-6). It is now well established that, in animals, this acid is biosynthesized from dietary linoleic acid (18:2 n-6) through two microsomal desaturation steps and one elongation step.

Recently, we have shown that the proportion of 20:4 n-6 in liver lipids was lower in genetically obese Zucker rats (fa/fa) than in their lean littermates [14]. This resulted mainly from a decreased Δ5 desaturation of dihomo-γ-linolenic acid (20:3 n-6) into 20:4 n-6 in liver microsomes whereas Δ6 desaturation of 18:2 n-6 into γ-linolenic acid (18:3 n-6) was only slightly modified.

In this context, the aim of the present study was to investigate the effect of fenofibrate on the conversion of linoleic acid into arachidonic acid in the liver of obese Zucker rats both *in vitro* and *in vivo*. The rate of Δ6 desaturation of linoleic acid and the rate of Δ5 desaturation