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SHORT COMMUNICATION

Effects of Gemfibrozil and Clofibric Acid on the Uptake of Taurocholate by Isolated Rat Hepatocytes

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ABSTRACT. Clinical use of fibrate hypolipidaemic agents has been associated with an increased incidence of hepatobiliary dysfunction including increased bile lithogenicity, gallstone formation, and cholestasis. The hepatic transport of bile acids plays an important role in bile formation and flow, and interference with the hepatocellular transport of bile acids may result in hepatobiliary dysfunction. The aim of this study was to investigate the effects of gemfibrozil and clofibric acid on the uptake of taurocholate by rat isolated hepatocytes. In control hepatocyte preparations (N = 5) at 37°, the uptake of taurocholate was described by saturable Michaelis–Menten kinetics with a mean (±SD) K_m of 44.1 ± 10.2 μM and V_{max} of 62.0 ± 23.0 nmol/10^6 cells/min. In the presence of 200 μM clofibric acid, there was no significant change in the kinetics of taurocholate uptake. However, in the presence of 200 μM gemfibrozil there was a statistically significant ($P < 0.05$) decrease in the V_{max} of taurocholate uptake (32.0 ± 18.2 nmol/10^6 cells/min, N = 5) and no change ($P > 0.05$) in K_m (48.5 ± 29.5 μM, N = 5). Gemfibrozil behaved as a non-competitive inhibitor of taurocholate uptake, with a K_i of 144 μM, which is approximately 50 times higher than the unbound gemfibrozil concentrations achieved clinically in humans. Thus, gemfibrozil and clofibric acid did not appear to directly alter the hepatic uptake of taurocholate at clinically relevant concentrations. BIOCHEM PHARMACOL **54**;1:215–218, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. gemfibrozil; clofibric acid; taurocholate; hepatic uptake; fibrates; bile acids

The hepatic transport of bile acids plays a critical role in bile formation and flow. Bile acids and many organic compounds are transported across the liver by carrier-mediated mechanisms that exhibit overlapping specificities between compounds. The uptake of bile acids into hepatocytes is mediated by a relatively specific Na^+-dependent transporter and also by an Na^+-independent transporter with a broader substrate specificity that may be involved in the uptake of non-bile acid organic anions [1]. An ATP-dependent transport system appears to mediate the canalicular secretion of bile acids [1]. Interference with any steps in hepatocellular bile acid transport may result in hepatobiliary dysfunction [1, 2], and such a mechanism has been suggested to contribute to the cholestatic effects of ethinyl oestradiol [3], oestradiol-17β-(β-D-glucuronide) [4], and cyclosporin A [5].

Fibrate hypolipidaemic agents, such as clofibrate and gemfibrozil, have been associated with hepatobiliary dysfunction, including increased bile lithogenicity and gallstone formation [6] as well as cholestasis [7]. In humans and rats, clofibric acid and gemfibrozil are taken up by the liver where they undergo metabolism and excretion into bile [8, 9]. The hepatic transport of fibrates may also be carrier mediated, and potential interference with the transport of bile acids by these agents may play a role in mediating their side-effects. The aim of this study, therefore, was to investigate the effects of clofibric acid and gemfibrozil on the uptake of taurocholate by hepatocytes.

MATERIALS AND METHODS

Isolated hepatocytes were prepared from adult male, hooded Wistar rats (300–400 g) according to the method of Seglen [10] and resuspended at a final concentration of 0.5 × 10^6 cells/mL in Tris–HCl buffered balanced salt solution [11]. Viability was assessed by Trypan blue exclusion and lactate dehydrogenase leakage, and only preparations with viabilities > 90% were used in subsequent transport experiments.

Taurocholate uptake experiments were carried out using aliquots (1 mL) of freshly isolated rat hepatocytes preincubated for 15 min at 37° in a shaking water bath. Uptake was initiated by the addition of 25 μL of taurocholic acid to achieve final concentrations of 1.5 to 200 μM (containing 80 nCi/mL [^{14}C]-taurocholic acid/incubation). Hepatocytes were also preincubated with clofibric acid or gemfibrozil (200 μM) for 5 min prior to the addition of taurocholate. Taurochenodeoxycholic acid (200 μM), a known compet-

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itive inhibitor of taurocholate uptake, was used as a positive control. All incubations were carried out in duplicate, and two aliquots (150 μL) were sampled 45 sec after the addition of taurocholic acid. The cells were separated from the medium using rapid silicone oil centrifugation [12]. The amount of [^{14}C]-taurocholic acid present within the cells and in the incubation medium was determined by scintillation counting, and the concentrations of taurocholate were determined based on the specific activity for each incubation. Non-specific binding of taurocholate to hepatocytes was determined from similar incubations carried out at 0°. Previous pilot experiments had established that taurocholate uptake was linear with respect to incubation time and hepatocyte cell numbers.

Kinetic analysis of taurocholate uptake was based on a saturable Michaelis–Menten process described by a single specific transporter system using Multifit (Day Computing, Cambridge, U. K.). In the event that inhibition of taurocholate uptake was observed in the presence of either 200 μM clofibric acid or gemfibrozil, further incubations were carried out over a range of inhibitor concentrations, in order to determine the nature of inhibition. These data were fitted to competitive, non-competitive, and uncompetitive models of inhibition, using computer regression analysis (Regression, Blackwell, U. K.). Statistical analysis was performed using the Wilcoxon Signed Ranks test, a paired non-parametric analysis (SYSTAT Inc. 1990, U. S. A.).

RESULTS AND DISCUSSION

The uptake of taurocholate by isolated rat hepatocytes was a saturable process best described by Michaelis–Menten kinetics (Fig. 1A) with an apparent K_m of 44.1 ± 10.2 μM (mean ± SD, N = 5), and a V_{max} of 62.0 ± 23.0 nmol/10^6 cells/min. These results are similar to those reported in previous studies using comparable incubation conditions [12–15]. The measured taurocholate uptake in this and similar studies reflects total uptake that may be mediated by both Na^+-dependent and Na^+-independent bile acid transporters, and the K_m value may therefore be a composite value, incorporating the contribution of both pathways. However, it has been proposed that the majority of taurocholate uptake (75–80%) by isolated hepatocytes is mainly due to Na^+-dependent transport [14, 15]. A putative Na^+-dependent taurocholate transporter protein has been cloned recently from rat liver with an apparent K_m of 25 μM following expression in *Xenopus* oocytes [1].

The presence of 200 μM clofibric acid (Fig. 1B) did not appear to alter the uptake of taurocholate (K_m of 41.0 ± 27.5 μM, $P > 0.05$, N = 5; V_{max} of 51.7 ± 31.8 nmol/10^6 cells/min, $P > 0.05$, N = 5). In contrast, in the presence of gemfibrozil (Fig. 1C), while there was no significant change in the K_m of taurocholate uptake (48.5 ± 29.5 μM, $P > 0.05$, N = 5), V_{max} was decreased significantly to 32.0 ± 18.2 nmol/10^6 cells/min ($P < 0.05$, N = 5). The effect of gemfibrozil on taurocholate uptake was best described by a non-competitive model of inhibition, which

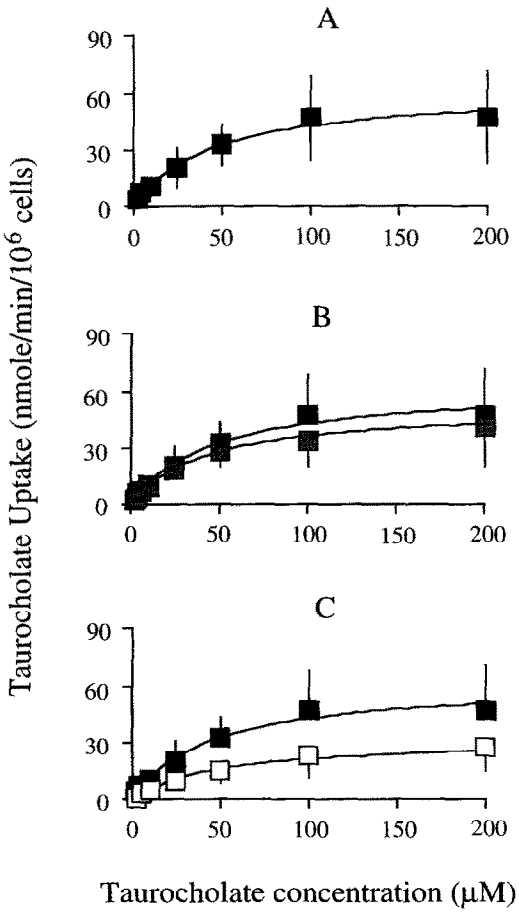

FIG. 1. Uptake of taurocholate (A) alone (■), (B) in the presence of 200 μM clofibric acid (▨), and (C) in the presence of 200 μM gemfibrozil (□) by isolated rat hepatocytes. Results are means ± SD, N = 5. All panels show the control curve for taurocholate uptake.

estimated a K_i of 144 μM for gemfibrozil (Fig. 2), and provided a second estimate of the K_m and V_{max} of taurocholate uptake as 45.1 ± 6.9 μM and 54.5 ± 5.5 nmol/10^6 cells/min, respectively. Consistent with previous reports, taurochenodeoxycholate significantly inhibited the uptake of taurocholate by 88% from 39.1 ± 11.8 to 4.6 ± 3.1 nmol/10^6 cells/min ($P < 0.05$, N = 5) [15]. Neither gemfibrozil nor clofibric acid reduced cell viability (results not shown).

The lack of effect of clofibric acid on taurocholate uptake should be interpreted with caution given the small number of experiments carried out. However, the non-competitive inhibition of taurocholate uptake by gemfibrozil and the apparent lack of effect of clofibric acid suggest that neither of these compounds are substrates for the carrier systems that mediate taurocholate transport at the hepatocyte sinusoidal membrane. The K_i for non-competitive inhibition of taurocholate uptake by gemfibrozil falls within the range of total gemfibrozil plasma concentrations achieved clinically in humans [16]. However, in plasma, gemfibrozil is highly bound to albumin (approximately 98%) so that the maximum unbound gemfibrozil concentrations would

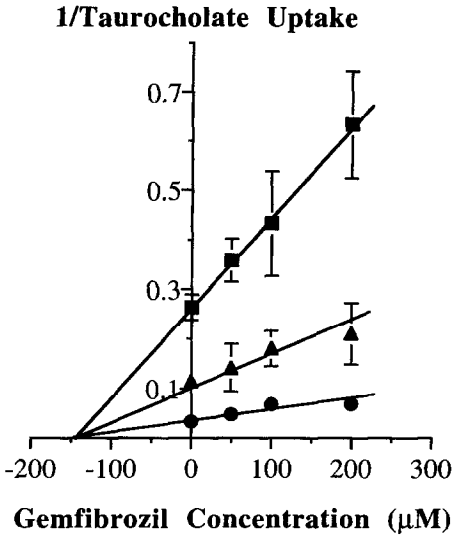

FIG. 2. Dixon plot of the effect of gemfibrozil on the uptake of 3 μM (■), 10 μM (▲), and 50 μM (●) taurocholate by isolated rat hepatocytes. Each point is the mean ± SD of 4 replicates. Standard deviations for the 50 μM data are too small to be seen over the symbol for the data points.

approximate 3–6 μM [17]. Therefore, the *in vitro* inhibition of taurocholate uptake exerted by gemfibrozil occurred at concentrations up to 50 times higher than the unbound concentrations achieved clinically.

The mechanism of the non-competitive inhibition of taurocholate uptake by gemfibrozil is unclear. Some compounds may alter membrane transport functions by interacting with membrane lipids and altering membrane fluidity, thus decreasing the mobility of the bile acid carrier and affecting the activity of the Na^+-K^+-ATPase pump [2]. This mechanism has been proposed for the inhibition of taurocholate uptake in ethinyl oestradiol-pretreated rats [3]. It is of interest to note that carboxylic acid drugs, including fibrates, are metabolized to CoA thioesters [18], which have been shown to act as substrates for lipid synthesis and become incorporated into triglycerides that form an integral component of the membrane lipid bilayer [19, 20]. In addition, carboxylic acid drugs are also metabolized to acyl glucuronides, which have been shown to mediate covalent binding to hepatic membrane proteins [21]. Thus, either of these factors may contribute to alterations of membrane fluidity. In such a case, long-term clinical exposure to fibrate hypolipidaemic agents may result in cumulative membrane damage that may, with time, contribute to clinical alterations in hepatocellular transport.

The observed effect of gemfibrozil may also have resulted from alterations to the canalicular transport of taurocholate. Since this study measured initial velocity of uptake by quantitating the intracellular concentrations of taurocholate, increased efflux of taurocholate from the cells would also have been observed as a decrease in intracellular concentration, and hence, sinusoidal uptake. However, such a mechanism could not be verified in this study as efflux was not measured.

In summary, the hepatobiliary dysfunction observed *in vivo* in both humans and rats administered fibrate hypolipidaemic agents does not appear to be related to direct alterations in the hepatic uptake of conjugated bile acids, since neither clofibric acid nor gemfibrozil inhibited the uptake of taurocholate into rat isolated hepatocytes at clinically relevant concentrations.

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