METABOLIC DISPOSITION OF CLOFIBRATE

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ABSTRACT

In this review, various aspects of the metabolic disposition of clofibrate are described. Clofibrate circulates in the blood of laboratory animals and man in the form of its active metabolite clofibric acid (CPIB). Levels of CPIB in serum (or plasma), urine or tissues can be measured by ultraviolet spectrometry, a method which provides reliable and reproducible data provided the samples do not contain non-specific UV absorbing substances or interfering drugs. Otherwise, gas-liquid chromatography or high-pressure liquid chromatography are specific and sensitive techniques to assay CPIB. In man, virtually all of an oral dose of clofibrate is absorbed and excreted in the urine. There is some evidence for enterohepatic circulation. CPIB is eliminated from the serum in two phases, with half-lives of 0.5 hr and 14-22 hr, resp. CPIB is excreted unchanged, as the glucuronide, and as other as yet unidentified metabolites. Bioavailability studies have been conducted with several marketed brands of clofibrate as well as with compounds which release CPIB in vivo; none of the CPIB derivatives offers any advantage over clofibrate from the point of view of CPIB bioavailability. CPIB is highly protein bound, and has a volume of distribution (8-9 liters) slightly greater than the volume of plasma albumin; at least some CPIB is taken up into tissue cells. In patients with nephrotic syndrome or chronic renal failure, the percentage of unbound CPIB is increased. The bioavailability of CPIB is unaltered when clofibrate is administered with the anion exchange resins cholestyramine or colestipol. Although CPIB is capable of displacing warfarin from its protein-binding sites, the studies reported to date imply that the enhanced anticoagulant activity of warfarin in the presence of clofibrate is the result of a pharmacodynamic rather than pharmacokinetic interaction. Although clofibrate is an enzyme inducer in rats, there is no evidence that it induces liver microsomal enzymes in man or in monkeys.

Species differences in the disposition of clofibrate are reviewed. The elimination half-life of CPIB varies from 2 hr in mice to 40-50 hr in beagle dogs, and the plasma protein binding ranges from 97% in man to 35% in mice.

INTRODUCTION

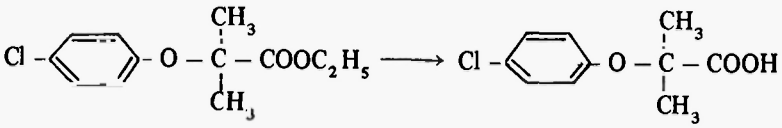
Since its introduction in 1962, clofibrate (ethyl 2-[4-chlorophenoxy]-2-methylpropionate) has become established as the most widely used

antihyperlipidemic drug. The appearance of an effective and non-toxic lipid lowering agent was met with great enthusiasm, and a symposium on clofibrate was held in England in 1963 /1/. More recently, an extensive monograph on the drug has appeared /2/. A compound with a rather simple structure, clofibrate has become the prototype for the development of other antihyperlipidemic drugs as well as for investigations on structure-activity relationships. Surprisingly, the drug has remained stubbornly resistant to definitive elucidation of its mode of action. Clofibrate was first marketed in combination with androsterone since it was believed that the two agents acted synergistically to reduce elevated cholesterol levels /3/; this was soon proven untenable /4, 5/, and androsterone was removed from the formulation. Thorp /6/ suggested that the drug displaces thyroxine from its albumin binding sites, thereby shunting thyroxine to the liver where the hormone would inhibit lipid biosynthesis; subsequent experimentation dispelled this theory. Later, Barrett and Thorp /7, 8/ proposed that, by inhibiting lipid mobilization, clofibrate decreases fatty acid transport, thereby reducing the hepatic availability of fatty acids for lipid biosynthesis. In 1970, Steinberg /9/ considered various options and concluded that the antihyperlipidemic activity of clofibrate is mediated mainly by the drug's ability to inhibit hepatic cholesterol synthesis and to decrease the rate of hepatic release of lipoproteins. More recently, enhanced catabolism of very low density lipoproteins has also been indicted as one of the actions of clofibrate /10, 11/. It has become apparent that clofibrate has myriad effects on lipid biosynthesis, liver enzyme activity and lipoprotein turnover, but the "magic bullet" which initiates these events has eluded definition.

The classic paper by Thorp in 1962 /12/ remained the bible on the disposition of clofibrate for twelve years, a period which witnessed virtually no new studies on this aspect of the properties of the drug. Since 1974, there has been a resurgence of interest in the metabolic fate of clofibrate. The potential role of drug disposition studies in the understanding of the mode of action of the drug remains to be established. However, many questions have been asked, such as the relationship of blood levels to therapeutic activity, the effect of disease on the pharmacokinetics of the drug, the possibility of tissue uptake, the role of protein-binding in drug response, and variation in the disposition of the drug in species used to study its pharmacology. In this paper, the state-of-the-art on the disposition of clofibrate in laboratory animals and man is reviewed.

HISTORICAL BACKGROUND: J.M. THORP

Thorp /12/ studied various aspects of the disposition of clofibrate in the rhesus monkey and in man. He found that the ester is rapidly hydrolyzed by tissue and serum esterases, both *in vivo* and *in vitro,* to the corresponding acid, which circulates in the blood and is responsible for the pharmacological activity of the drug.



Clofibrate Clofibric acid (CPIB)

CPIB circulates only as the free acid; Thorp found no glucuronide in the serum. CPIB (pKa = 3) is almost wholly ionized in serum, and is 96% bound to bovine albumin at a concentration of 200 μg/ml /12/. In monkeys, the apparent volume of distribution was reported to be 10-15% that of the body weight, and no CPIB was detected in muscle, fat, heart, spleen, bile or cerebrospinal fluid. At oral doses greater than 100 mg/kg, CPIB was detected in the liver, the concentration corresponding to 20-40% that of serum. In man, the elimination half-life of CPIB in the serum was reported to be 12 hr; 92-98% of an oral dose was excreted in the urine, mainly as the CPIB-glucuronide. The rate of urinary excretion also corresponded to an elimination half-life of 12 hr.

In the rat, Thorp reported in 1963 that only 50-60% of the compound excreted in the urine was present as the glucuronide, and that the efficiency of glucuronide conjugation of CPIB increased from the rat to monkey to man /13/; at hypolipidemic doses, no CPIB was found in rat liver. Rabbit foetal serum contained a higher concentration of CPIB than did maternal serum /13/.

ANALYTICAL MEASUREMENT

Studies on the bioavailability and pharmacokinetics of clofibrate or its derivatives depend upon the measurement of CPIB. A method involving ultraviolet spectrometry was first described by Barrett and Thorp /7/. Acidified serum or plasma is extracted with isooctane:

ethanol (95:5), and the optical density is read at 226 mμ. Although the technique lacks specificity, it is capable of providing reliable and reproducible data on circulating CPIB levels provided that measurements are not made on sera containing non-specific UV absorbing substances, (eg. from patients with certain diseases) or on samples containing interfering drugs /14/.

The measurement of CPIB by gas liquid chromatography (GLC) has been described by numerous investigators /14-28/ and has been recently reviewed /29/. The primary advantage of the GLC assays of CPIB is increased specificity. Most of the methods involve the separation and purification of CPIB by various extraction procedures or by column or thin-layer chromatography. Prior to injection onto the GLC column, CPIB is usually converted to the methyl ester or, rarely, to the trimethylsilyl derivative /16/ or butyl ester /25/. The GLC methods are also well adapted for the measurement of CPIB in bile, urine, saliva and tissues. In general, the sensitivity of the various methods is approximately 1 μg/ml which is sufficient for most clinical and pharmacological studies; this sensitivity can be improved by increasing sample size, decreasing volume of extracting solvent, etc. The use of an electron capture detector has been claimed to produce a sensitivity of 1 pg CPIB/sample injected into the instrument /28/.

Recently, several high-pressure liquid chromatography (HPLC) methods have been reported for the measurement of CPIB in biological fluids /30-32/. The technique does not involve derivatization and would appear to be more rapid and convenient than the GLC methods. Excellent agreements have been attained when the UV method was compared with the GLC /14/ or HPLC /31-32/ assays for CPIB. It has been suggested that in a well-controlled study, with adequate serum blanks and in the absence of interfering drugs, the UV procedure is the one of choice because of speed, simplicity and accuracy /14/; otherwise, the GLC or HPLC methods should be used to provide reliable data.

The use of gas chromatography-mass spectrometry (GC-MS) provides a very high degree of sensitivity and specificity, and has been applied to the assay of clofibrate and structurally related impurities in commercial preparations of the drug /33, 34/. Hignite and Azarnoff /35/ used GC-MS to quantitate the amount of CPIB in the effluent of a sewage disposal plant in Kansas City, Missouri. The concentration of CPIB averaged 7 μg/ℓ, and it was calculated that approximately one-half of the clofibrate consumed in the area was discharged into the Missouri River at an average rate of 2 kg CPIB/day.

A synthesis of [^{14}C] - labelled clofibrate and CPIB has been described by Ferdinandi /55/.

PHARMACOKINETICS IN MAN

Clofibrate is generally prescribed at 2 g/day divided into 4 equal doses. More recently, the trend has changed to dosing at 1 g every 12 hours. Most bioavailability and pharmacokinetic studies have therefore involved the use of 500 or 1000 mg of the drug, or equimolar amounts of clofibrate derivatives which also release CPIB in the serum. Whether the study be conducted with clofibrate, other esters of CPIB or with various salts of CPIB, bioavailability is assessed by measuring plasma or urinary levels of CPIB. Urinary measurements are valid estimates of bioavailability because, as will be discussed, virtually all of an oral dose of clofibrate is excreted in the urine as free or conjugated CPIB. No unchanged clofibrate has been detected in the blood or urine by GLC /23/.

The absorption half-life of clofibrate in both healthy volunteers /23, 36/ and hyperlipidemic subjects /37/ is approximately 1½ hr. After an oral dose of [^{14}C] clofibrate, no radioactivity is excreted in the feces while 99-100% of the dose is recovered in the urine within 6 days /38/; thus, all of the oral dose is absorbed. The time to peak (t_{max}) of CPIB after an oral dose of 500 or 1000 mg clofibrate ranges from 3-6 hr /23, 36-41/. The peak CPIB concentration (C_{max}) after a single oral dose of clofibrate has been reported to be 35-50 μg/ml after 500 mg /23, 39/ and 55-100 μg/ml after 1000 mg /36-37, 40-41/.

Thorp /12/ originally calculated the apparent plasma elimination half-life (t½) of CPIB to be 12 hr. It now appears that the t½ of CPIB is somewhat longer. Several investigators have reported a t½ of 14-15 hr /23, 38, 42/. Sedaghat and Ahrens /38/ gave an intravenous injection of a tracer dose of [^{3}H]CPIB to hyperlipidemic patients at 3 hours following an oral dose of 1 g clofibrate, which was part of their lipid-lowering therapy. By measuring plasma radioactivity at several early time intervals, these workers were able to detect a first exponential (up to approximately 1½ hr after the dose) with a t½ of 0.45 hr preceding the terminal phase of the curve, with a t½ of 15 hr. In our experience, we routinely find an average terminal t½ in a given study to be 18-22 hr (range 14-35 hr). When we examine the pharmacokinetics of CPIB at different times, the t½ of CPIB may vary by up to 7 hr in the same subject. Thus, there is some day-to-day variation in the rate of

CPIB elimination in individuals. Hartlapp and Gugler /43-44/ studied the pharmacokinetics of CPIB in volunteers given single oral doses of 500, 1000 and 2000 mg clofibrate, and after multiple doses of 1000 mg twice daily for 12 days. The t½ varied between 12 and 25 hr with a mean value of 17 hr, but no effect of dose was seen; the t½ after multiple dosing was similar to the single dose values. Unlike Hartlapp and Gugler /43, 44/, Cailleux *et al* /40/ unexpectedly found that a decrease in the t½ of CPIB occurred when the dose of clofibrate was increased, thus: for 500 mg, t½ = 29 hr; for 1000 mg, t½ = 22 hr; and for 1500 mg, t½ = 14 hr.

There does not appear to be any sex difference in the pharmacokinetics of CPIB in either normal volunteers /23, 36, 39, 41/ or in hyperlipidemic patients /37, 38/. However, no study has been conducted on a direct comparison of the disposition of clofibrate in normal and hyperlipidemic subjects using the same experimental design. In patients with familial hypercholesterolemia (Type 11 hyperlipoproteinemia), Pichardo *et al* /37/ found that the pharmacokinetics of CPIB in subjects who responded to the cholesterol-lowering effects of clofibrate were different than in those in whom clofibrate did not lower serum cholesterol. For example, the t½ was significantly longer in responders (23 hr) than in non-responders (15 hr). In addition, the area under the plasma concentration-time curve (AUC) of CPIB was significantly greater in responders when subjects were given a single oral dose of clofibrate 3 weeks after cessation of treatment. The reasons for these differences in the handling of clofibrate by sensitive and resistant patients are not known, but point out the need to compare in the same study such factors as protein-binding, conjugation, renal clearance, etc. both in hyperlipidemic patients and in normal subjects.

Assuming complete absorption, the plasma clearance of total CPIB is 400-500 ml/hr /37, 42/, and is independent of dose up to 1000 mg /44/; clearance values are somewhat higher in the steady-state /44/.

Pharmacokinetic data obtained from a single dose - blood level study can be used to predict the steady-state concentration of a drug /45/. However, the steady-state CPIB levels, as well as the AUC in the steady-state, are markedly lower than values calculated on the basis of single dose data /40, 43, 44/. The reason for this discrepancy is not known, although it has been suggested that there may be a decrease in the protein binding at the higher plasma concentrations achieved in the steady-state /40, 43, 44/.

The pharmacokinetics of CPIB after intravenous administration of CPIB to man /38/ as well as to rats /46/ have been described according to a two-compartment model. Houin *et al* /23/ also used a two-compartment model to interpret plasma CPIB data after oral administration of clofibrate. Since the plasma level decay after oral dosing appears to be monophasic (the rapid initial elimination phase is not detectable upon oral administration), most other investigators have interpreted plasma CPIB curves by a one-compartment open model /37, 40-44/.

The steady-state levels of circulating CPIB in subjects receiving 1000 mg of clofibrate twice daily have been in the range of 100-123 μg/ml in samples withdrawn prior to dosing /38, 40, 47, 48/, and 131-162 μg/ml at the peak /17, 41, 48/. An inverse correlation between body weight and the steady-state plasma CPIB concentration has been found /37, 49-50/, and the slightly lower CPIB levels in males found by Taylor *et al* /48/ may be due to the lower mean body weights of the females. Steady-state plasma CPIB concentrations were shown to be inversely proportional to circulating cholesterol levels in patients who responded to the cholesterol-lowering effects of the drug, but not in non-responders /37/. No study has been reported on the relationship between plasma CPIB and circulating triglycerides in hypertriglyceridemic subjects.

The apparent volume of distribution (V_d) of total CPIB has been reported to range from 5 to 9 liters, or 8-14% of the body weight /23, 38, 43/. The plasma volume of human albumin is approximately 10% of the body weight. CPIB is strongly bound to plasma albumin, a property which would limit diffusion of the drug into tissues. However, a V_d in the range of 8-9 liters /38, 43/ would imply that at least some tissue uptake takes place. Thorp /12/ was unable to detect any CPIB in muscle, fat, heart or spleen of monkeys given up to 500 mg/kg/day of clofibrate; low amounts of CPIB were detectable only in the liver, the concentration being 20-40% that of serum. On the other hand, upon oral administration of [^{14}C] clofibrate to rats, Cayen *et al* /51/ found substantial tissue levels of radioactivity, albeit lower than that of serum. Five percent of the dose was present in the liver 3 hours after dosing. Substantial liver CPIB levels in rats have also been reported by other investigators /52-54/. The CPIB which enters the cell localizes primarily in the cytosol fraction, with only small amounts associated with subcellular organelles /53-54/. Only trace amounts of CPIB are present in adipose tissue of patients undergoing clofibrate therapy /38/; however, fat is not an ideal tissue to study CPIB uptake as tissue dis-

tribution studies in rats have shown that the concentration of CPIB in epididymal fat is substantially lower than other tissues such as liver, heart, kidneys, lungs and adrenals /51/. Indeed, based on calculations of CPIB pool size, it has been concluded that at least some CPIB must find its way into tissue cells of man /38/.

BIOAVAILABILITY STUDIES

Studies have been conducted on the comparative bioavailability of several marketed brands of clofibrate /36, 39, 56-57/. Normal subjects are usually given a single oral dose of each preparation with a suitable time interval between doses, and plasma or serum CPIB levels are measured. In all instances, including one study in which four clofibrate preparations were compared /39/, the blood level patterns of CPIB were virtually identical, thus demonstrating bioequivalence of the various clofibrate formulations.

Several compounds which release CPIB *in vivo* have been prepared, but none offer any advantage over clofibrate from the point of view of the bioavailability of CPIB. This is not surprising, as clofibrate is completely absorbed and has a half-life sufficiently long to allow for a twice-a-day administration. Thus, the basic aluminum salt of clofibrate (alufibrate) gave CPIB levels with lower C_{max} and AUC but higher t_{max} and apparent t½ than after an equivalent dose of clofibrate /39,42,58/, indicating slow and incomplete absorption; after twice a day dosing with clofibrate or an equivalent amount of alufibrate for 5-10 days, the serum levels /39, 58/ and urinary excretion /39/ of CPIB were significantly lower after alufibrate than clofibrate. Single and multiple doses of simfibrate (1,3-propanediol-bis[p-chlorophenoxymethylpropionate]) gave lower circulating levels of CPIB than did equimolar doses of clofibrate /47/. Harvengt and Desager compared the bioavailability of CPIB after single /42/ and multiple /58/ doses of clofibrate and clofibride, the CPIB ester of γ-hydroxy-N,N-dimethylbutanoic acid amide. After a single dose, the C_{max} was higher with clofibride, but the AUC (0-24 hr) were the same for both drugs; curiously, the steady-state levels of CPIB were approximately 30% higher after clofibrate than clofibride. Other clofibrate analogues which have been shown to be bioequivalent to clofibrate include pyridoxine clofibrate /40/, magnesium clofibrate in a preparation containing meso-inositol hexanicotinate /59/, and calcium clofibrate /41,48/.

BIOTRANSFORMATION AND EXCRETION

In man, CPIB is excreted in the urine /23, 38, 39/ with only trace amounts found in the faeces /38/. Because of its low pK_a, CPIB is always ionized in urine, and thus its pattern of excretion is independent of urinary pH. After a single dose, 60% of the dose is excreted in 24 hr /39/ and more than 90% in 48 hr /23, 59/. Approximately 60% of the urinary CPIB is conjugated /23, 38, 43/, mainly as the glucuronide /12, 43/. Houin *et al* /23/ found that after a 500 mg capsule given to normal volunteers, the urine contained the following (percent of dose): 32% free CPIB, 28% glucuronide conjugate of CPIB, and 33% of some other conjugate which withstood β-glucuronidase hydrolysis. This second major conjugate is also an ester of CPIB, since it is readily hydrolysed by dilute alkali /60/. When rats and dogs are given a single oral dose of [^{14}C] clofibrate, all of the serum radioactivity in rat serum is due to CPIB, while in dog serum, 20% of the serum radioactivity is due to other metabolites /51/. An average of 5% of the orally or intravenously administered dose is excreted in the faeces of rats, while the rest is recovered in the urine /51/. With dogs, approximately 21% of oral [^{14}C] clofibrate is found in the faeces but this is due in part to incomplete absorption /51/. In rat urine, virtually all the CPIB is present in either the free form or conjugated with glucuronic acid /51, 61/. Although it was originally reported that the glucuronide was the only conjugate of CPIB in dog urine /16/, recent studies have shown the presence of an alkali hydrolyzable conjugate, in addition to the glucuronide, in both the urine and bile of dogs /51/.

Thorp /12/ did not find any CPIB in the bile of clofibrate treated monkeys. Also, Mannisto *et al* /39/ did not detect any CPIB in bile of nine subjects with cholecystectomies within 24 hr after a 500 mg dose of clofibrate. On the other hand, Sedaghat and Ahrens /38/ reported that the CPIB concentration of bile from patients treated with 2 g/day of clofibrate averaged 55 μg/ml, which was approximately one-third the level in plasma; conjugated CPIB accounted for 40% of the total. It was calculated that at least 5% of the daily dose appears in the bile /38/. Studies in rats have shown that 48% of an oral dose of [^{14}C] clofibrate appears in the bile /61/, and that 42% of the radioactivity from [^{14}C] CPIB perfused into the liver appears in the bile /54/. CPIB and its glucuronide are also present in the bile of dogs treated with clofibrate /16/. Since negligible amounts of CPIB are excreted in the faeces of rats /51/ or man /38/, these data imply that CPIB to some

extent undergoes enterohepatic circulation.

No conjugated CPIB has been detected in human plasma /38/, and only 3% of circulating CPIB in rats is in the conjugated form /51/. Although it has been suggested that the primary site of CPIB conjugation is in the kidney /38, 51/, the finding of conjugated CPIB in the bile of rats /54/, dogs /16, 51/ and man /38/ implies that the liver is also capable of conjugating CPIB. It is possible that a portion of the CPIB which is conjugated in the liver is secreted into the bile and undergoes hydrolysis in the gastrointestinal tract; the free CPIB thus formed is then reabsorbed. However, the finding that, in Gilbert's Syndrome, a condition characterized by diminished activity of hepatic UDP-glucuronyl transferase, the urinary excretion of CPIB- glucuronide is substantially reduced /62/, suggests that the liver plays a role in the formation of urinary CPIB-glucuronide. Thus, the relative contribution of the kidney and liver to the conjugation of CPIB eliminated in the urine remains to be elucidated.

ANIMAL STUDIES

The t½ of CPIB shows marked species variation, ranging from 2 hr in the mouse to 40-50 hr in the dog /51, 61, 63/. The t½ in the rat after oral and intravenous administration of clofibrate and CPIB, respectively, is 5-7 hr /51, 63, 64/; the value of 19 hr for the rat /46/ appears to be an overestimate. We have compared the extent of protein binding of CPIB in plasma from various species using a stainless steel multi-cell equilibrium dialysis apparatus. The range of percentage bound was from 35% in the mouse to 97% in man (Table 1). It was interesting to note that, with the exception of the dog, the longer the t½, the greater the percentage bound. The nature of the relationship, if any, between protein binding and the t½ remains to be evaluated.

The rat is the species most often used to study the pharmacology of clofibrate. It is customary to treat rats with a diet containing 0.25-0.3% (250-300 mg/kg/day) clofibrate, a dose which produces a serum CPIB level of approx. 80-100 μg/ml after 1 week of treatment /65, 66/. The CPIB level tends to decrease if treatment is prolonged to 3-4 weeks. Hypolipidemic activity in rats can also be demonstrated at lower doses, eg. 0.075% of the diet for 1 week /51/. Clofibrate lowers serum cholesterol and/or triglycerides in virtually all species examined. In our experience, the mouse is relatively resistant to the lipid lowering activity of clofibrate; in this species, a diet containing 0.3% (300 mg/kg/day) of clofibrate is the minimum dose which would consistantly lower

TABLE 1. *Disposition of clofibrate: species differences*

Species	*Percent Free CPIB* [a]	*Serum t½ (hr)*
Man	3	18-22
Rhesus monkey	5	7
Dog	15	40-50
Guinea Pig	20	-
Rat	25	5-7
Mouse	65	2

[a] CPIB concentration = 100 μg/ml

serum lipids. Serum CPIB is approx. 90 μg/ml in mice killed within 2 hr after food withdrawal following 1 week treatment with 0.3% clofibrate. Since this level is similar to that found in rat serum after similar treatment, the greater sensitivity of clofibrate in rats may be related to the longer t½ in rats than in mice.

PROTEIN-BINDING

Normal Values

The original observation by Thorp /12/ that CPIB is highly protein-bound has been confirmed in several studies. Between 3 and 8% of the plasma CPIB is in the free form both in patients undergoing clofibrate therapy /17, 38/ and in normal volunteers given a single /44, 67/ or multiple /49/ doses of the drug. Gugler and Hartlapp /44/ found that the protein-binding of CPIB varied with the concentration. Thus, up to a concentration of 50 μg/ml, the protein-binding of CPIB was constant at about 98%; as the concentration of CPIB rose to 300 μg/ml, the percentage bound decreased to about 92%. When total plasma clearance was calculated from total plasma CPIB, the value was 340 ml/hr for a single dose of 500 or 1000 mg, but increased to 410 ml/hr for a 2000 mg dose and was even higher (490 ml/hr) in the steady-state (1000 mg twice daily). However, when the calculations were based on the unbound plasma CPIB concentration, the plasma clearance as well as the V_d were identical at all doses. It was concluded that these findings, as well as the lower than predicted (i.e. from a single dose) steady-state

levels of CPIB, were due to concentration dependent changes in protein-binding.

Since the unbound portion of certain drugs is in equilibrium between serum and saliva, the levels of CPIB in saliva can be used to estimate the unbound portion in the plasma. Values of 4% /25/ and 1% /30/ free CPIB have been reported by measuring salivary levels in human volunteers; the latter value appears to be on the low side. It is necessary to validate the approach for clofibrate by comparing in the same subjects values obtained both by equilibrium dialysis of the plasma and by assay of salivary CPIB.

Kidney Diseases

Hyperlipoproteinemia is a common feature of the nephrotic syndrome and of chronic renal failure, and patients with these kidney disorders have been treated with clofibrate to lower the elevated lipid levels. It has been reported that approximately 20% of the plasma CPIB is free in patients with nephrotic syndrome /49, 67/ and renal failure /68/. The reasons for the decreased binding are due to changes in plasma albumin. The nephrotic syndrome is characterized by extensive protein loss through the kidneys; the albumin concentration in plasma can be decreased to as low as 1 g/100 ml from a normal value of 4.5 g/100 ml. Functionally, the remaining plasma albumin molecules are normal and thus the decreased binding of CPIB is due to the lowered plasma albumin concentration. In contrast, renal failure does not result in partial depletion of circulating albumin, but is associated with abnormal albumin molecules with lower capacity for binding acidic drugs /69/. The two conditions produce different effects on the pharmacokinetics of CPIB. In clofibrate-treated subjects with nephrotic syndrome but normal renal function, the $t_{1/2}$ of CPIB is reduced from 17-20 hr (in normal volunteers) /49, 70/ to 6-11 hr /49, 70-72/. In patients given 1 g clofibrate twice daily, the steady-state plasma concentration of total CPIB is lower in nephrotics than in controls (48 vs 140 μg/ml) /49/. This, coupled with the increased percentage unbound CPIB in the nephrotic subjects, results in virtually identical plasma concentration of free drug in both groups (5 μg/ml) at the steady-state. Also, the excretion pattern of free CPIB and CPIB glucuronide is similar in both nephrotics and controls /49/. It has therefore been suggested that modification of the daily dose of clofibrate is not necessary in patients with nephrotic syndrome but normal renal func-

tion /49/. This is in contrast to an earlier recommendation that the total daily dose of clofibrate should not exceed 500 mg for each gram of albumin/100 ml of serum /67/.

On the other hand, since in patients with chronic renal failure, drug elimination through the kidneys is impaired, the steady-state levels of CPIB are higher and the t½ substantially longer than in normal subjects. For example, the steady-state CPIB level in uremic subjects given 200 mg clofibrate/day was 77 μg/ml, the same as in nonuremic hypertriglyceridemic patients treated with 2 g clofibrate daily /72/. Other investigators have reported similar findings /68, 73/; thus, in a uremic patient given 500 mg clofibrate twice daily, a steady-state CPIB level of 500 μg/ml was found /68/. The t½ of CPIB in patients with renal failure has been reported to range from 45 hr to 7 days /70, 72-75/. In renal impairment, the percentage of urinary CPIB conjugated to glucuronic acid is lower /67, 75/. Presumably, renal damage impairs the rate of conjugation of CPIB in the kidney.

Uremic patients treated with clofibrate tend to experience elevated creatine phosphokinase (CPK) levels, and muscular pain and stiffness /67, 68, 72, 74/. This appears to be due to impaired protein binding coupled with higher steady-state blood levels and prolonged t½, resulting in high levels of free CPIB which can cross cellular membranes into skeletal muscle. With this in mind, the adjustment of the clofibrate dose to as low as 200 mg/day can produce the desired lowering of plasma lipids without side effects /72, 74/. From these considerations, it would appear more critical to monitor unbound CPIB rather than total CPIB in patients with kidney disease.

When consideration is being given to drug-protein binding, the classical concept is that the pharmacologically active portion is the unbound drug, since it is free to cross capillary membranes to reach the site of action. It is interesting that, in the earlier literature on clofibrate, the mode of action of the drug was thought to be due to the high degree of CPIB binding to plasma albumin. CPIB displaces thyroxine from its albumin binding sites /63/, and this was one of the first suggestions as to its mode of action. As mentioned in the introduction, CPIB competes with free fatty acids (FFA) for the albumin binding sites, and it has been proposed that the availability of FFA for hepatic lipid biosynthesis is thus reduced /8/. Recent studies have shown that as the FFA chain length increases, displacement by CPIB becomes more pronounced (stearate > palmitate > myristate) /76/, and that the displacement is a competitive one on a high affinity site of

bovine serum albumin (BSA) /77/. Binding studies have shown that CPIB is bound at a high affinity site on BSA involving strong hydrophobic bonding, and also at several sites of lower affinity /77,78/. The differences in the binding characteristics of CPIB between bovine, rat and human serum albumins are relatively small /79/, and thus BSA can be used to study the protein binding properties of CPIB. Nevertheless, the precise role of protein binding of CPIB to the therapeutic activity of clofibrate still remains to be elucidated.

DRUG INTERACTIONS

There has been considerable interest in recent years in the combined use of clofibrate and other antihyperlipidemic drugs in the control of elevated lipid levels. The rationale for combination therapy derives from the premise that drugs which affect lipoprotein disposition by different mechanisms may produce, when given together, additive or possibly synergistic decreases in elevated plasma lipids. Numerous studies have been reported on therapy with clofibrate combined with nicotinic acid (or its analogues) as well as with the anion exchange resins cholestyramine and colestipol.

The uptake into the blood of [^{14}C] from a single oral dose of [^{14}C] clofibrate is not altered in rats fed chronically with cholestyramine /61/ or colestipol /80/. Also, the steady-state plasma CPIB levels, 24-hr urinary CPIB excretion and various other pharmacokinetic parameters are virtually unaltered in hyperlipidemic human patients treated chronically with both clofibrate (1 g twice daily) and cholestyramine (4 g four times daily), as compared to the same dose of clofibrate alone /38/. The absence of pharmacokinetic interaction between clofibrate and the anion exchange resins in human volunteers has been reported by others /81,82/.

Although combined therapy with clofibrate and nicotinic acid has been successful in normalizing elevated serum lipids, the possibility of pharmacokinetic interaction of the two drugs has not been studied in man. However, studies in rats have shown that the peak level of serum nicotinic acid is higher and the AUC is 59% greater when nicotinic acid is given with CPIB than when given alone /64/. Also, pretreatment for 1 wk with nicotinic acid increases t½ of intravenously injected CPIB from 5 to 7 hr; the presence of nicotinic acid in rat serum does not affect the protein-binding of CPIB /64/. It was suggested that the

presence of CPIB may have prolonged the renal clearance of nicotinic acid.

Diosgenin, an agent which in rats inhibits cholesterol absorption without enhancing bile acid elimination /83/, has also been tested with clofibrate. The t½ and AUC of CPIB after a single oral dose of clofibrate were similar in rats pretreated with diosgenin and in untreated controls /84/.

Clofibrate and warfarin are frequently given together to patients with cardiovascular disorders. On occasion haemorrhages /85-87/ from this interaction have been reported. Since both drugs are highly protein bound, it was initially believed that a competition for the protein-binding sites took place, since it has been found *in vitro* that CPIB competitively inhibits the binding of [^{14}C] warfarin to human serum albumin /88-90/. However, other investigators have been unable to displace warfarin from human albumin by the addition of CPIB /91,92/. Pharmacokinetic studies in man have shown that combined administration of sodium warfarin and clofibrate for 21 days augmented the hypoprothrombinemia found in long-term therapy with warfarin alone, but had no effect on steady-state plasma warfarin levels /93/. In another study, pretreatment with clofibrate had no effect on the elimination t½ of warfarin after a single oral dose /91/. Bjornsson *et al* /94/ studied the pharmacokinetics of the R- and S-enantiomorphs of warfarin with and without clofibrate treatment. Clofibrate did not alter the t½ of either enantiomorph, but increased the V_d and clearance of the R-enantiomorph. Clofibrate increased the plasma free warfarin by 22%, which was in good agreement with the calculated value based on the increased V_d. However, since the clearance of warfarin is proportional to the free fraction /95/, it was concluded that any effect of plasma protein displacement on the anticoagulant action would be temporary, since with a new steady-state, the concentration of free warfarin would be the same as that present prior to displacement /94/. It therefore appears that although CPIB may displace protein-bound warfarin *in vivo*, this is not the cause of the enhanced anticoagulant activity of warfarin in the presence of clofibrate. It is possible that the effect is the result of some pharmacodynamic rather than pharmacokinetic interaction.

At a concentration range of 0.5-3 μg/ml digitoxin, CPIB (250 μg/ml) displaces digitoxin from human serum albumin *in vitro* /96/. However, when digitoxin is present at therapeutic concentration (25 ng/ml), the slight decrease in digitoxin binding (92% to 87%) produced by CPIB (250 μg/ml) is considered to be insignificant /96/.

Clofibrate potentiates the inhibition of tumour growth by various cytotoxic agents in rat Walker 256 carcinosarcoma /97/. In an *in vitro* tissue culture medium, CPIB potentiates the cytotoxicity of chlorambucil /98/, a highly protein-bound drug. It was also found that CPIB displaces chlorambucil from serum protein; since the concentration of free chlorambucil determines tumour growth inhibition, the effect was ascribed to an interaction at the protein-binding sites /98/.

From the various studies on the protein-binding of CPIB, it is evident that the displacement of other protein-bound drugs by CPIB is theoretically plausible, and can under certain experimental conditions be demonstrated *in vitro* and *in vivo*. However, whether such an interaction is necessarily manifested by a change in activity of the drug is another question, and would depend very much on concomitant changes in volume of distribution and body clearance. As seen with warfarin, a decrease in protein-binding coupled with enhanced hypoprothrombinemia does not mean cause and effect. At this stage of our knowledge on the disposition of clofibrate, it cannot be stated that interaction with CPIB at the protein-binding sites is responsible for augmented therapeutic activity of a concomitantly administered drug.

EFFECT OF CLOFIBRATE ON DRUG METABOLISM

Chronic clofibrate administration to rats causes liver enlargement and proliferation of the smooth endoplasmic reticulum /99/. The drug increases the protein and cytochrome P-450 content of liver microsomes /100/ and induces the formation of microsomal enzymes that metabolize drugs such as testosterone /100/, pentobarbital, ethylmorphine and aminopyrine /101/. Also, clofibrate reduces the pentobarbital and zoxazolamine sleeping times in rats /101/. In contrast to the effect of barbiturates, the increase in liver weight induced by clofibrate in rats does not result in increased capacity to eliminate various organic compounds into the bile /102/; indeed, the liver transport function in clofibrate-treated rats decreases when expressed as per gram of liver /102/. Chronic clofibrate treatment does not affect the pharmacokinetics of antipyrine in rhesus monkeys /103/ or in human volunteers /104/. Thus, although clofibrate is an enzyme inducer in rats, it does not induce liver microsomal enzymes in primates.

CONCLUDING REMARKS

Although the metabolic fate of clofibrate appears at first glance to be relatively uncomplicated (completely absorbed, no known phase 1 biotransformation of CPIB, high protein binding, excretion only in urine), it is still necessary to answer several questions in order to gain a more thorough understanding of the disposition of the drug. For example, the variation in t½ and protein-binding in healthy subjects and hyperlipidemic patients, the relative contribution of the liver and kidney to CPIB conjugation, the identification of other urinary conjugates, and the relationship of therapeutic activity to circulating levels of total or unbound CPIB remain to be assessed. Such studies do not only add to our armamentarium of knowledge but may also help explain the variability in therapeutic response to the drug under different clinical situations.

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