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On the metabolism of clofibride, a hypolipaemic drug

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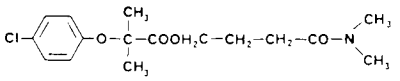
The absorption and metabolism of clofibride, a new hypolipaemic drug of *p*-chlorophenoxyisobutyric type, were investigated in the CD rat, the beagle and the olive baboon monkey. Clofibride is rapidly and massively resorbed and hydrolysed into 4-chlorophenoxyisobutyric acid (CPIB) and 4-hydroxy-*N*-dimethylbutyramide (HMB). CPIB, in free and glucuroconjugated form, and its metabolite, 4-chlorophenol, in the form of the glucuronate ether, are found in the serum of the rat. HMB is rapidly metabolized. The half-life of CPIB, the main active metabolite, in the serum is about 12 h in the rat, 43 ± 9 h in the dog and 6 ± 1 h in the baboon. In the rat, peak hypocholesterolaemic activity occurs late—24 h after administration of the drug and 20 h after peak concentration of CPIB in the blood. The half-life of 4-chlorophenol glucuronate ether in the serum is about 4 h whereas that of HMB is about 3 h. In the rat, the elimination of clofibride takes place mainly via the urine since 70% of the dose administered is found in the form of free or conjugated CPIB, 10% in the form of HMB or one of its metabolites, in 48 h samples of urine. Over the same period, faecal elimination accounts for no more than 2% of the dose ingested. In addition, in this species, the CPIB, 30% of which is secreted via the biliary route without being eliminated in the faeces, undergoes an enterohepatic circulation.

Clofibride (4-hydroxy-*N*-dimethylbutyramide 4-chlorophenoxyisobutyrate)‡ is a new hypolipaemic drug of *p*-chlorophenoxyisobutyric type which has weak toxicity (Da Lage et al 1972) and marked hypocholesterolaemic and hypotriglyceridaemic activity in the laboratory animal (Nordmann et al 1972, 1973). The clinical trials (Leutenegger et al 1974; Drouin et al 1975) and subsequent therapeutic application confirmed the experimental results.



Clofibride

The esters of *p*-chlorophenoxyisobutyric acid (CPIB) are rapidly hydrolysed in the organism and the acid, which is the active principle, appears rapidly in the blood after oral administration (Thorp 1962; Almirante et al 1969). It is to a large extent eliminated in the form of glucuronate ester, almost exclusively via the urine. According to Thorp it cannot be detected in most of the organs of the rhesus monkey, except the liver where it is found in very small quantities relative to the dose administered, and it is not excreted in the bile. Almirante et al could not detect it in the liver, heart, kidneys or adipose tissue of the rat but they did find a metabolite of the acid (probably 4-chlorophenol) in these organs and in the urine. Those authors also reported that CPIB is eliminated in large amounts in the bile.

For the present study of the metabolism of clofibride, experiments were mainly carried out in the rat although supplementary tests were performed in the monkey and in the dog. Clofibride was administered by mouth and both the ester itself and its hydrolysis products were tested for: CPIB and 4-hydroxy-*N*-dimethylbutyramide (HMB) and their conjugated derivatives. Attempts to identify 4-chlorophenol and the metabolites of HMB were also made.

METHODS

Species used and conditions of administration of the substance

The experiments were with male CD rats (Charles River, Elbeuf, France), male and female beagles (D. and C. Appleton Ltd., Carmarthenshire, Wales) and olive baboon monkeys (Southern Animal Farms Ltd., Twickenham, Middlesex).

The rats were treated by oesophageal intubation. They were given either clofibride 400 or 1000 mg kg^{-1}, or HMB 1000 mg kg^{-1}. The substances were administered in suspension or in solution in a 10% mucilage of gum arabic in the constant volume of 5 mg kg^{-1}.

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The dogs and monkeys received clofibride 500 mg kg^{-1}, also by oesophageal intubation, in suspension in a 5% solution of polysorbate 80 in the constant volume of 1 ml kg^{-1}. The metabolism of clofibride was investigated after administration of a single dose in all three species and during chronic treatment in the baboon.

Assay of clofibride, CPIB, 4-chlorophenol and the conjugated derivatives of these two metabolites

Techniques similar to those proposed by Barrett & Thorp (1968) and Almirante et al (1969) were used. Clofibride, CPIB and 4-chlorophenol can be assayed by u.v. absorption after selective extraction by isooctane at different pHs. The conjugated derivatives of the two metabolites, which are insoluble in isooctane, were assayed in the same way following chemical hydrolysis. CPIB and 4-chlorophenol were identified by thin layer chromatography of the isooctane extracts; their respective R_F values are 0·52 and 0·54.

Assay of HMB and its metabolites

HMB and its metabolites were assayed by gas chromatography following extraction with acetone or chloroform and dissolution in dichloromethane. This technique separates HMB or γ-butyrolactone, which have the same retention time, and another unstable metabolite of HMB (compound Y). HMB can be distinguished from γ-butyrolactone by thin-layer chromatography. The R_F values of the HMB and butyrolactone spots, detected by exposing the plates to iodine fumes, are 0·3 and 0·5 respectively. The identification of HMB was also confirmed by mass spectrometry which with infrared spectrometry were used to determine the approximate structure of compound Y. Finally the glucuroconjugated derivatives were tested for in the urine by enzymic hydrolysis with Helicase (beta-glucuronidase and sulphatase preparation from the juice of *Helix pomatia* Industrie Biologique Française, 92231 Gennevilliers, France).

RESULTS

Concentration in the serum

After oral administration of a 400 mg kg^{-1} (1·22 mM kg^{-1}) dose in the rat, clofibride was not found in the serum. This is accounted for by the fact that the compound is rapidly hydrolysed by the serum esterases, as demonstrated in vitro. CPIB and HMB were found in free form and 4-chlorophenol in glucuroconjugated form. γ-Butyrolactone was not detected.

The kinetics of the concentration of CPIB in the serum were compared with those of the hypocholesterolaemic activity (Fig. 1). 30 min after administration of the drug the concentration of CPIB in the serum was 1·6 μmol ml^{-1}; it attained a peak value (2·6 μmol ml^{-1}) towards the third hour and then fell slowly: 1 μmol ml^{-1} after 24 h, 0·05 μmol ml^{-1} after 48 h. CPIB had completely disappeared from the serum after 72 h. From these results it can be estimated that the metabolite has a half-life in the serum of about 12 h. The hypocholesterolaemic activity is not seen until the 6th hour (−18%). It then progressively increases to reach a peak towards the 24th hour (−38%). It is still present after 48 h (−13%) and disappears after 72 h. Comparison of these findings shows that there

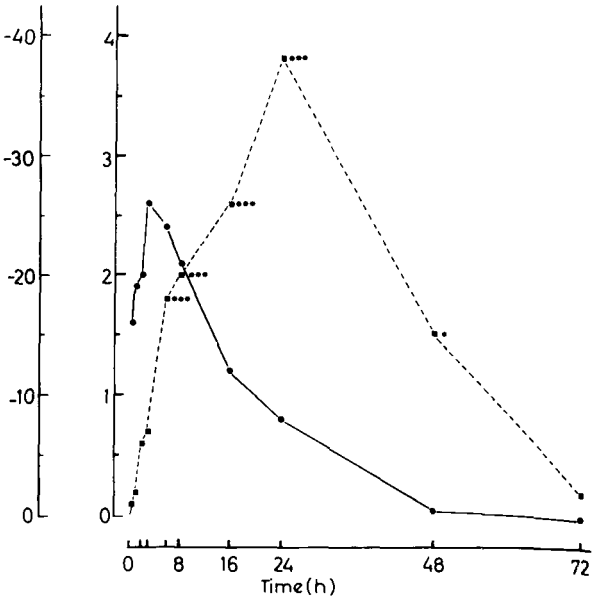

FIG. 1. Concentration of CPIB in the serum and the hypocholesterolaemic activity of clofibride in the rat after oral administration of 400 mg kg^{-1}. ●——● CPIB; ■ - - - ■ hypocholesterolaemic activity. A group of 6 control animals and a group of 6 animals treated with clofibride were killed at each test time. Serum cholesterol was determined by the method of Levine & Zak (1964) and the hypocholesterolaemic activity of clofibride was evaluated by calculating the percentage difference between the mean serum cholesterol in each treated group and the mean serum cholesterol in the corresponding control group. The statistical significance of these differences was ascertained by Student's *t*-test (*: significant for $P = 0{\cdot}05$; ***: significant for $P = 0{\cdot}001$). The concentration of CPIB in the serum was determined for homogeneous pools prepared by mixing aliquots of serum from each group. Ordinates: LH—hypocholesterolaemic activity (%). RH—CPIB concn (μmol ml^{-1}).

is a delay of about 20 h between the maximum concentration of CPIB in the serum and peak pharmacodynamic activity.

Only the glucuroconjugated derivative of 4-chlorophenol was detected, in very small quantities, in the serum of the rat. Its concentration was of the order of 0·05 to 0·1 μmol ml^{-1} between the first and third hours after administration of clofibride. It could no longer be detected after 5 h.

Finally, free HMB was also present in small amounts in rat serum (maximum concentration: 0·15 μmol ml^{-1}). To determine the kinetics of HMB in the serum in more detail, the amide was administered in the high dose of 1000 mg kg^{-1} by mouth. Under these conditions the shape of the curves was the same as that seen with 400 mg kg^{-1} (Table 1). Much larger amounts were found. It appears that the peak concentration of HMB occurs towards the 30th min after administration and that the time required for total elimination of this metabolite is slightly greater than 2 h.

The half-life of CPIB in the serum was also determined for the male and female beagle and baboon monkey following oral administration of clofibride 500 mg kg^{-1}. In both these species it was found to be independent of sex but varied greatly between individuals.

Table 1. Concentration of HMB in the serum of the rat after administration of a single oral dose of clofibride (400 mg kg^{-1}) or of HMB (1000 mg kg^{-1}). A group of 10 control animals, a group of 10 animals treated with clofibride and a group of 10 animals treated with HMB were killed at each test time. The concentrations of HMB in the serum were determined using homogeneous pools prepared by mixing aliquots of serum from each group.

Substance and dose (mg kg^{-1})	HMB in μmol ml^{-1} of serum after administration			
	15 min	30 min	1 h	2 h
Clofibride 400	0·15	0·15	—	0
HMB 1000	4·2	4·3	3·2	0·2

In the dog (Fig. 2), the concentration of CPIB in the serum was about 1 μmol ml^{-1} 30 min after treatment. Peak concentration (2·8 μmol ml^{-1}) was attained towards the second or third hour, and the concentration subsequently fell slowly. Elimination was not complete until between the 6th and 9th days. Half-life was as great as 43 $\pm$ 9 h (n = 6).

In the monkey also (Fig. 3), CPIB appeared rapidly in the serum and attained its maximum concentration (2·6 μmol ml^{-1}) towards the third hour, but subsequently, its elimination was much more rapid than in the dog: 1·8 μmol ml^{-1} after 6 h

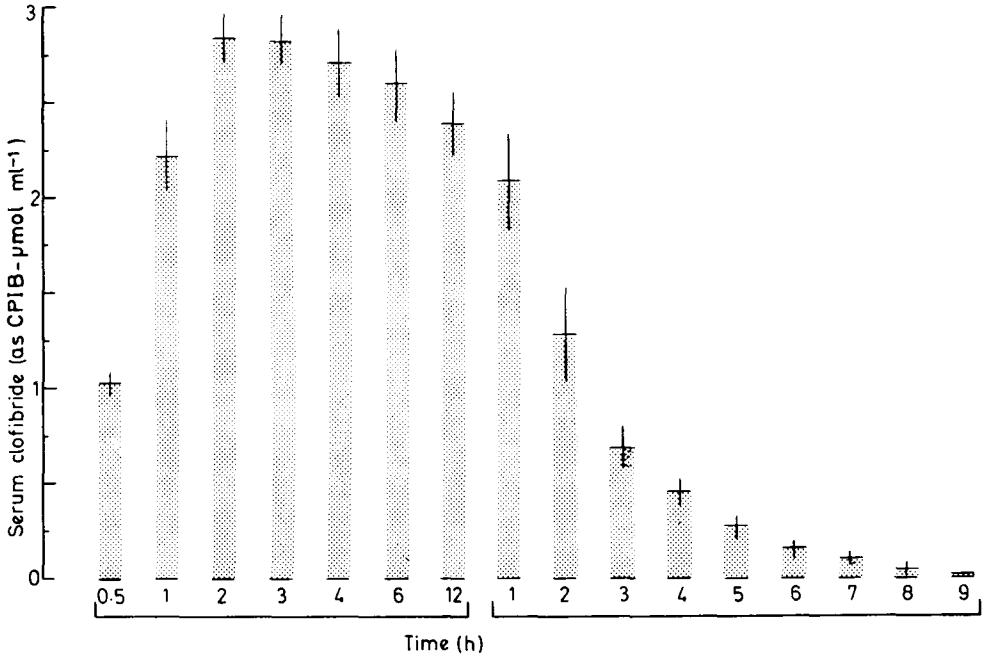

FIG. 2. Concentration of clofibride (as CPIB) in the serum of the dog after administration of a single oral dose of 500 mg kg^{-1}.

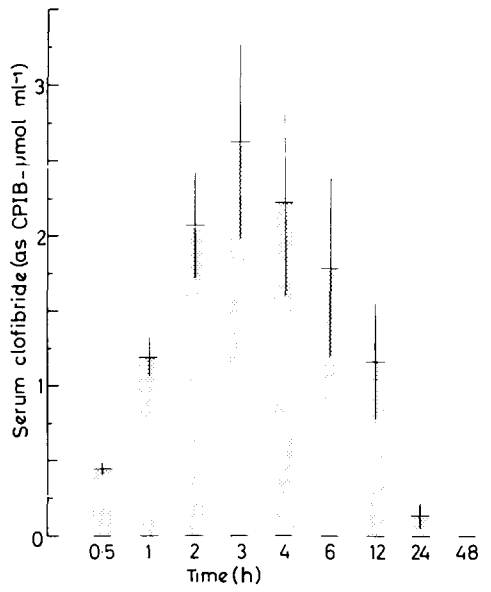

FIG. 3. Concentration of clofibride (as CPIB) in the serum of the monkey after administration of a single oral dose of 500 mg kg^{-1}.

and 0·2 μmol ml^{-1} after 24 h. It could no longer be detected after 48 h. Its half-life was 6 ± 1 h (s.e.m.) n = 8.

The change in the concentration of this metabolite in the serum with time was also investigated in the monkey over several months (Table 2), with clofibride being administered daily at 500 mg kg^{-1} (same dose as for the acute tests). It appeared that the half-life of CPIB in the serum was unaffected by repeated treatment.

Elimination in the urine and faeces in the rat

After administration by mouth of 400 mg kg^{-1}, no clofibride could be found in the urine or faeces. Free and glucuroconjugated CPIB, glucuroconjugated 4-chlorophenol, free HMB and an unstable metabolite (compound Y) of this alcohol-amide were found in the urine. The approximate structure of compound Y was determined. It appears to be a compound of the following type:

$$(H_3C)_2N - CO - CH_2 - CH_2 - CO - N(CH_3)_2$$

The faeces contained free and glucuroconjugated CPIB and free HMB.

60% of the dose of clofibride administered was found in the 24 h urine in the form of CPIB and 10% in the form of 4-chlorophenol. The main metabolite is thus CPIB; it is eliminated in free form (15%) and

Table 2. Concentrations of clofibride (as CPIB, in μmol ml^{-1}) in the serum of the monkey after daily oral treatment in the dose of 500 mg kg^{-1}.

Time after treatment (h)	Male No.	Female No.	Week of treatment 9	18	26
3	1		2·13	1·69	2·59
	2		2·93	3·31	2·10
	3		2·87	2·75	3·27
		4	2·30	3·02	3·95
		5	2·90	1·98	2·44
		6	3·20	4·58	3·02
	Mean ± s.e.m.		2·72 ± 0·17	2·87 ± 0·42	2·90 ± 0·27
24	1		0	0	0
	2		0·02	0	0·04
	3		0·04	0·03	0·03
		4	0	0	0
		5	0	0·03	0
		6	0·02	0·05	0
	Mean ± s.e.m.		0·01 ± 0·01	0·02 ± 0·01	0·01 ± 0·01

especially in conjugated form (45%). 4-Chlorophenol was present solely as the glucuronate ether. Only the glucuronate ester of CPIB (10%) was found in urine collected between the 24th and 48th hour. Overall, 48 h after treatment about 80% of the clofibride administered had been eliminated in the urine in the form of PCIB or its metabolites (Table 3).

In the 48 h urine 2 to 3% of the dose administered was in the form of HMB and 5 to 10% in the form of compound Y. These two compounds were not conjugated.

Finally, the 48 h faeces contained only small amounts of metabolites: 1 to 2% of free and glucuroconjugated CPIB, 0·5% of free HMB, relative to the dose administered.

Table 3. Urinary elimination of CPIB and its metabolites in the rat after oral administration of clofibride in the dose of 400 mg kg^{-1}. For each period the analyses were carried out with pooled urines from 6 treated rats and 6 control rats.

Substance excreted	Urinary elimination (% of the dose administered) 0–24 h	24–48 h	0–48 h (total)
Free CPIB	14	0	14
Glucuroconjugated CPIB	47	10	57
Glucuroconjugated 4-chlorophenol	9	0	9
Total	70	10	80

Biliary elimination and hepatic concentration in the rat
After the 400 mg kg^{-1} dose, clofibride was not found in the bile or the liver. CPIB was eliminated continuously via the bile for 24 h at a rate which remains fairly constant with time. It appeared to be eliminated mainly in glucuroconjugated form (Table 4). The global quantities of free and glucuroconjugated CPIB found in the 24 h bile corresponded to 10 and 20% of the dose of clofibride administered, respectively.

Table 4. Biliary elimination of CPIB and its glucuronate in the rat after oral administration of clofibride in the dose of 400 mg kg^{-1}. After catheterization of the bile duct under short anaesthesia, the rats (unanaesthetized) were placed under conditions of semi-restraint and their bile was collected over 5 consecutive periods. For each period the analyses were carried out on bile pools from 4 treated rats and 4 control rats.

	Biliary elimination (% of the dose administered)					
Substance eliminated	0–2 h	2–4 h	4–6 h	6–8 h	8–24 h	0–24 h (total)
Free CPIB	1·1	1·0	0·8	0·7	5·8	9·4
Glucuro-conjugated CPIB	2·9	3·9	2·6	1·8	8·8	20·0
Total	4·0	4·9	3·4	2·5	14·6	29·4

In the liver, 4 h after treatment, 4% of the administered dose was found in the form of free (3%) and glucuroconjugated (1%) CPIB. The organ contained the same amounts of the two metabolites 8 h after treatment. No HMB was detected.

DISCUSSION

4-Hydroxy-*N*-dimethylbutyramide 4-chlorophenoxyisobutyrate or clofibride is rapidly hydrolysed in the organism to 4-chlorophenoxyisobutyric acid (CPIB) and to 4-hydroxy-*N*-dimethylbutyramide (HMB). The CPIB itself is partially hydrolysed to 4-chlorophenol.

HMB is also partially transformed into a metabolite (compound Y). CPIB is found partly in the form of the glucuronate ester and 4-chlorophenol is found solely in the form of the glucuronate ether.

Clofibride is rapidly resorbed. CPIB is present in the blood of the rat, monkey and dog within 30 min of oral administration. It is resorbed in large quantities since, in the rat, 80% of the dose administered is eliminated via the urine in 48 h whereas faecal elimination is negligible.

After oral administration in the rat of the drug at a hypolipaemic dose of 400 mg kg^{-1}, glucuroconjugated 4-chlorophenol and free HMB are present in the blood in small amounts (maximum concentration around 0·1 μmol ml^{-1}) and for a relatively short period (about 3 h).

The concentration of CPIB in the serum rises rapidly to reach a peak (2·7 μmol) 3 h after treatment; this then falls to 0·05 μmol ml^{-1} by the 48th h. The half-life of CPIB in the serum of the rat is thus about 12 h. In the baboon it is 6 $\pm$ 1 h and does not appear to be altered by chronic treatment with high doses. CPIB does not therefore accumulate in the monkey. In the beagle the metabolite has a long half-life in the serum: 43 $\pm$ 9 h. Its very slow elimination accounts for clofibride's chronic toxicity in the dog in doses that are well tolerated in the rat or the monkey. These results are in agreement with those reported by Thorp (1962) and Platt & Thorp (1966) in their studies of the metabolism of clofibrate, the ethyl ester of CPIB. Those authors also reported that in man this metabolite has a half-life in the serum of about 12 h, similar to that observed in the rat and the monkey.

The kinetics of the hypocholesterolaemic activity of clofibride, determined in the rat following oral administration, at the same time as the determination of the kinetics of the serum concentration of CPIB, showed that the hypocholesterolaemic effect became significant 6 h after treatment; peak activity was seen at the 24th hour and the effect only disappeared after 3 days. There is, however, a long lag between the peak concentration of CPIB in the serum and peak hypocholesterolaemic activity. CPIB may act either directly, by inhibition of the synthesis or activation of the break-down of cholesterol and the triglycerides (Walsh et al 1969; White 1971; Fallon et al 1971), or indirectly, by displacement of the free fatty acids, and of thyroxine especially, from their binding sites on the plasma proteins (Platt & Thorp 1966; Barrett & Thorp 1968).

The metabolism of clofibride, determined from the urinary and faecal elimination of CPIB and 4-chlorophenol, is satisfactory since the global amount found represents about 80% of the dose administered. Urinary elimination predominates whereas little of the substance is eliminated via the faeces. The main metabolite is the glucuronate ester of CPIB. Only small amounts of HMB and its metabolite, compound Y, are eliminated since globally these compounds merely represent 10% of the dose administered. The short half-life of HMB in the blood suggests that this amide and compound Y are rapidly converted into physiological metabolites

which enter into the cycles of intermediate metabolism.

The rat's liver contains no HMB. CPIB is found in this organ in free and conjugated form: its concentration corresponds to only 4% of the dose ingested and remains constant for at least the first 8 h after treatment. Thorp (1962), studying the metabolism of clofibrate in the rhesus monkey, also reported weak concentrations of CPIB in the liver; none was detected in the muscle, adipose tissue, heart or spleen.

CPIB is found in free and conjugated forms in the bile of the rat. The overall amount of CPIB eliminated via the bile during 24 h represents 30% of the administered dose; the rate of elimination varies little with time. Since the hepatic concentration of CPIB remains stable for a long time after treatment and there is little faecal elimination of the metabolite, it appears that both the free and conjugated forms undergo an enterohepatic circulation. The existence of such circulation in the rat was confirmed by Almirante et al (1969) who also detected large amounts of CPIB in the bile of rats treated with esters of this acid. This biliary elimination is not common to all species since Thorp (1962) found no CPIB in the bile of the rhesus monkey. Enterohepatic circulation would help to explain CPIB's therapeutic mode of action, and it would be useful to determine whether such circulation exists in man.

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