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The biliary excretion of acenocoumarol in the rat: stereochemical aspects

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Within 24 h, 50% of a single dose of the acenocoumarol enantiomers was recovered in bile and 20% in urine of Wistar rats. The elimination products were mainly (>90%) the 6- and 7-hydroxyacenocoumarol as conjugates in the bile but free in the urine. Only *R*-acenocoumarol, free and conjugated, was excreted in bile. There were no gross differences between the enantiomers in metabolic pattern or in the amount of metabolites formed. A significant difference was observed for the biliary excretion of the 7-hydroxy metabolite; the ratio of free and conjugated 7-hydroxyacenocoumarol was three times higher for the *S*- than for the *R*-isomer. An unknown third metabolite was recovered in bile in higher amounts with the *S*- than with the *R*-acenocoumarol. Only traces of this metabolite were recovered from urine. The data show an extensive biliary excretion of acenocoumarol and demonstrate stereoselective mechanisms in the excretion processes.

The enantiomers of the 4-hydroxycoumarin acenocoumarol differ in their pharmacokinetics. In the rat, the *S*-enantiomer is cleared four times faster (Thijssen et al 1985), and in man 10 times faster (Godbillon et al 1981; Thijssen et al 1986), than the *R*-isomer. As the drug is eliminated predominantly by biotransformation and as there is hardly any difference in plasma protein binding between the enantiomers (Thijssen et al 1985), the difference in body clearance reflects stereoselective differences in the intrinsic biotransformation rate. For the chemical congener, warfarin, stereochemical differences in the rate as well as in the route of metabolism have been demonstrated (Pohl et al 1976a, b). Little is known about the fate of acenocoumarol. We previously showed in rats that reduction of the aromatic nitro-group of acenocoumarol did not occur (Thijssen et al 1985). In man, two hydroxylated metabolites, i.e. the 6- and 7-hydroxylated derivatives (Fig. 1) were recovered from urine (Thijssen et al 1986). We have investigated the biliary and urinary excretion of single doses of the *R*- and *S*-enantiomers of acenocoumarol in the rat to find whether differences in the metabolic route between the enantiomers explain their pharmacokinetic differences.

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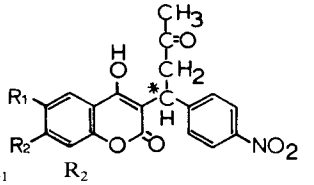
Materials and methods

The optically pure *R*- and *S*-enantiomers of acenocoumarol were a gift from Ciba-Geigy, Basel, Swizerland. Male inbred Wistar rats (Centraal Proefdierenbedrijf TNO, Zeist, The Netherlands), 300–350 g, were used. They had free access to water and food.



R_1	R_2	
H	H	acenocoumarol
OH	H	6-hydroxyacenocoumarol
H	OH	7-hydroxyacenocoumarol

FIG. 1. Structure of acenocoumarol and its 6- and 7-hydroxy metabolite.

To study the stereoselectivity of biliary and urinary excretion in the same rat, a permanent catheter leading from the bile duct to the duodenum was implanted as described by Kuipers et al (1985). After surgery, the rats were allowed to recover for 5–7 days. The patency of the catheter was checked daily for an undisturbed bile flow. The condition of the animals was judged from their physical appearance and from their body weight. Following the subcutaneous administration of 1 mg of one of the acenocoumarol isomers, the rats were placed in a metabolic cage. The bile duct catheter was connected to a polyethylene tube which lead via a swivel joint to a collection vessel. The duodenum catheter was flushed with saline and plugged. Urine and bile were collected for 24 h. After a week, the experiment was repeated with the other isomer.

Concentrations of the drug and its metabolites were assayed by HPLC (Thijssen et al 1985). To separate the metabolites from unretarded matrix compounds, the eluting strength of the mobile phase was reduced; column: Lichrosorb 5RP18 0·46 × 15 cm, Chrompack, The Netherlands; mobile phase: 0·1% acetic acid–acetonitrile (5:3; v/v) brought to pH 4·8 with 1 M ammonia. The amount of the drug and metabolites present as conjugates was assayed after treating urine and bile with β-glucuronidase/arylsulphatase (Boehringer, Mannheim, GFR).

Table 1. Biliary and urinary excretion of *R*- and *S*-acenocoumarol and their metabolites in rats[a].

Compound administered	% of dose recovered	
	Bile	Urine
R-		
Parent compound	0·8 ± 0·4	1·1 ± 0·7
Parent conjugated	5·4 ± 2	1·6 ± 0·5
6-OH metabolite	1·2 ± 0·5	4 ± 1
6-OH conjugated	6·7 ± 2	0·7 ± 0·5
7-OH metabolite	2·8 ± 1	4·5 ± 1·5
7-OH conjugated	25·7 ± 7	3 ± 3
Metabolite X	1·2 ± 1	—[b]
Metabolite conjugated	2 ± 1	—[b]
S-		
Parent compound	0·2[c]	0·1 ± 0·1**
Parent conjugated	0·5[c]	1·8 ± 1
6-OH metabolite	2·4 ± 1	2·7 ± 0·9
6-OH conjugated	5·2 ± 2	0·7 ± 0·7
7-OH metabolite	9·6 ± 4*	8 ± 4
7-OH conjugated	22·5 ± 5	2·7 ± 2·5
Metabolite X	2 ± 0·8**	—[b]
Metabolite conjugated	10·5 ± 7**	2 ± 1·8

[a] The compounds (1 mg) were administered subcutaneously. Bile and urine were collected for 24 h. The data are presented as mean ± s.d. (n = 5).
[b] The compound(s) was (were) not detected.
[c] *S*-Acenocoumarol was recovered only in 2 of the 5 bile samples.
Analysis of differences between *R*- and *S*-isomer by paired *t*-test.
*$P < 0.05$; **$P < 0.01$.

Results

Beside unchanged acenocoumarol, three metabolites were present in bile and urine. Two of these were identical to the 6- and 7-hydroxyacenocoumarol reference samples. The third metabolite was not identical to any of the products of reduction of the ketone group, nor was it identical to the amino or the acetamido metabolite (Fig. 1). The main route of acenocoumarol elimination appeared to be by metabolism and the route of metabolite excretion mainly via the bile. Cumulatively (0–24 h), about 50% of the administered isomers was excreted via the bile and about 20% via the urine. There was no difference in total amount excreted between the enantiomers. Biliary and renal excretion, as individual compounds, is summarized in Table 1. Stereoselective differences were seen in the biliary excretion of unchanged drug. The *R*-isomer was found in the bile, both free and conjugated, of all rats (total excretion amounted to 6% of the dose). *S*-Acenocoumarol was found in the bile of two rats only, and was present in minor quantities (<1% of the dose). There was also a significant difference in the urinary excretion between the isomers, the *S*-isomer being excreted in the free form at <0·1% of the dose. The total, free and conjugated, amount of the drug excreted via the urine was about 2% of the dose for both isomers. The 7-hydroxy metabolite appeared to be the main metabolite for both isomers. About 40% of the drug administered was recovered as the 7-hydroxy metabolite, which, as conjugate(s), was excreted predominantly in the bile. Biliary excretion of the unconjugated 7-hydroxy metabolite, was significantly more as the *S*-isomer than the *R*-isomer. The cumulative amount of the 6-hydroxy metabolite excreted accounted for 10–12% of the administered dose mainly in bile as conjugate (free + conjugate(s) about 8% of the dose). Like the 7-hydroxy metabolite, the 6-hydroxy *S*-isomer in its free form appeared to be more effectively excreted than the *R*-isomer. The difference, however, was not statistically significant ($P = 0.055$). Significant differences between the drug isomers were observed with respect to the formation of the unknown metabolite, higher amounts were recovered after the *S*-isomer than after the *R*-isomer. Its excretion was mainly via bile in the conjugated form. Only the *S*-isomer-derived metabolite in its conjugated form was found in urine.

Discussion

The biliary excretion of the 4-hydroxycoumarins, warfarin and phenprocoumon, and their metabolites has been described in animals and man (Powell et al 1977; Wong et al 1978; Wong & Solomonraj 1980; Toon et al 1985). In the present study we have shown that acenocoumarol, predominantly in the form of its metabolites, is mainly excreted in bile; about 50% of single doses within 24 h. Urinary excretion amounted to

about 20% of the dose. We showed previously that after a single dose of racemic [^{14}C]acenocoumarol rats excreted only 40% of the radioactivity within 5 days—24% in urine and 16% in faeces (Thijssen et al 1983). The amount excreted in urine within the first 24 h was about 90% of the ultimate amount excreted. These data indicate that considerable enterohepatic recycling of acenocoumarol metabolites takes place. Enterohepatic recycling has also been demonstrated for warfarin rats (Remmel et al 1981) and for phenprocoumon in man (Jähnchen & Meinertz 1977).

Hydroxylation of the coumarin skeleton at the 6- and 7-position is a common biotransformation route for 4-hydroxy coumarins (Pohl et al 1976a, b; Dieterle et al 1977; Toon et al 1985) and the formation of these acenocoumarol metabolites in the rat has been described herein. The structure of the third metabolite was not established, but in analogy to warfarin, it could be the 8-hydroxy or the benzylic-hydroxy metabolite. For warfarin, the latter metabolite has been recovered in minor quantities only (Pohl et al 1976a). In bile, 6- and 7-hydroxy acenocoumarol were present predominantly in the form of conjugate(s), whereas in urine the free metabolites were excreted. In human urine these metabolites were also present mainly in the unconjugated form (Thijssen et al 1986). There was no difference between the isomers in the relative amounts of metabolite formed. In this respect, acenocoumarol behaves differently from warfarin in the rat in which 7-hydroxylation of the *R*-isomer predominates (Pohl et al 1976a). Despite the great differences in pharmacokinetics between the acenocoumarol enantiomers (Thijssen et al 1985), this is hardly reflected in the metabolic patterns recovered in bile and urine. This would indicate that either the route(s) of elimination responsible for the non-recovered part (30–40%) of the drug dose is highly stereoselective for the *S*-isomer, or the biotransformation reaction, which is common to both isomers, is faster for the *S*-isomer. Striking differences were observed in the biliary excretion of the drugs; only the *R*-isomer, free and conjugated, being excreted and then in minor amounts. In this respect, acenocoumarol differs from warfarin (free and conjugated) which Powell et al (1977) showed to be, next to 7-hydroxy-warfarin, the main biliary component of a warfarin dose in rats. The reason for the stereoselectivity in biliary excretion or acenocoumarol could be a reflection of the stereoselectivity in conjugation, i.e. only the *R*-isomer is conjugated and subsequently excreted in bile. However, the recovery of conjugated *S*-isomer in urine does not support this. Stereoselectivity in conjugation may be the cause of the differences between the excreted amounts of non-conjugated *R*- and *S*-7-hydroxy acenocoumarol in bile, but also stereoselectivity in biliary excretion may operate. The observations strongly suggest the intervention of one or more carrier-mediated processes in the biliary excretion of acenocoumarol and some of its metabolites. To our knowledge, stereoselectivity in biliary excretion has not been demonstrated before (Simonyi et al 1986).

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