ACTIVE METABOLITES OF ACENOCOUMAROL: DO THEY CONTRIBUTE TO THE THERAPEUTIC EFFECT?

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- 1 The pharmacokinetics and pharmacodynamics of racemic acenocoumarol (AC), the amino (AM) and acetamido (AA) derivative were investigated in healthy volunteers after administration of a single oral (10 mg) dose.
- 2 All three coumarins were rapidly absorbed from the gastrointestinal tract. The elimination half-lives were 10.9, 10.4, and 4.1 h, for AC, AM and AA, respectively.
- 3 After the oral administration of AC, no AM and AA was detected in the plasma, and less than 1% of the dose was recovered in the urine (0–24 h) as AM.
- 4 Renal clearance was the main route of elimination of AM and AA; the former by glomerular filtration, the latter by active excretion.
- 5 Binding to serum proteins was the highest for AC (1.52% free). AM and AA were less tightly bound (about 3% free). AM or AA did not interfere with the AC plasma protein binding.
- 6 The oral administration of AC resulted in a rise in prothrombin time with a maximum effect at 24 to 30 h. No anticoagulant activity was observed upon the administration of AM and AA.
- 7 The results indicate that the compounds AM and AA, if they are formed out of AC at all, do not contribute to the anticoagulant activity of AC.

Keywords acenocoumarol metabolites therapeutic effect

Introduction

The 4-hydroxy coumarin derivative acenocoumarol (AC) is classified as a short-acting anticoagulant (O'Reilly, 1980). Due to its short half-life of elimination ($t_{1/2} = 3\text{--}9\text{ h}$; Dieterle *et al.*, 1977; Godbillon *et al.*, 1981; Houwert-de Jong *et al.*, 1981), the duration of anticoagulation following a single administration is short-lasting 48–72 h (Meinertz *et al.*, 1978), and the recovery from hypocoagulation following cessation of therapy is rapid. On theoretical considerations, the short biological half-life of AC is of disadvantage for its use in chronic anticoagulant therapy, for the extent of inhibition of the vitamin K-dependent clotting factor synthesis will fluctuate, paralleling the fluctuation in AC body content during the daily dose interval. This will be manifest for factor VII plasma levels especially, because the half-life of elimination of this clotting factor from plasma is about 5 h (Owren, 1963). However, the use of AC in chronic anticoagulant therapy has been justified in the supposition that the formation of the active amino (AM) and acetamido (AA) metabolites will prolong the duration of AC action (O'Reilly, 1980; Godbillon *et al.*, 1981). The activity of these metabolites in man,

however, has not been investigated, nor their pharmacokinetic properties.

In this paper, the pharmacokinetics and pharmacodynamics of racemic AC, AM, and AA following a single oral dose in healthy human volunteers is described.

Methods

Subjects

Five healthy volunteers, one female (E) and four males (A, B, C, D), participated in the study. None of them had a history of liver disease or bleeding disorders and their plasma prothrombin times were found to be normal. None of the volunteers was currently using drugs for therapy or whatsoever. Before starting the study, the participants were asked not to use salicylates or pyrazolinon derivatives from 2 days before the experiment, and if applying, to minimize alcohol intake. Subject A (65 kg) participated in all three experiments, subjects B (76 kg) and

C (80 kg) participated in the experiments for AC and AA, subjects D (72 kg) and E (52 kg) received AM only. The time between the experiments for a same subject was 2 months.

Drug administration and sample collection

The coumarin derivatives were administered orally (10 mg) after an overnight fast; AC as tablets (Sintrom mitis®), AM, and AA soluted in 100 ml of tap water. During the first 8 h of the experiment, blood samples were obtained from an indwelling catheter in a forearm vein. Samples on later times were obtained by venepuncture. Immediately after collection, the blood sample (4.5 ml) was mixed with 0.5 ml of 0.1 M trisodium citrate, and centrifuged at 1200 g for 10 min. Plasma was drawn off and transferred in two separate vessels (for prothrombin time assay and for drug assay) and stored at -20°C until assayed.

Materials

AC (Sintrom mitis®) was obtained commercially (Ciba-Geigy, The Netherlands). AM and AA were prepared from AC as described previously (Thijssen *et al.*, 1983a).

¹⁴C-labelled AC was a gift from Ciba-Geigy (Basle, Switzerland) and ¹⁴C-labelled AM and AA were prepared from it.

Analytical procedure

Prothrombin time (PT) was estimated in test plasma after dilution (five times) in barium sulphate adsorbed oxalate pool-plasma. Activation was induced by human thromboplastin (kindly supplied by Prof. Dr C. Hemker, Department of Biochemistry, Medical Faculty, University of Limburg, Maastricht).

Analysis of plasma and urine for the coumarin derivatives were performed by a h.p.l.c. assay as described previously (Thijssen *et al.*, 1983a). Protein

binding of the compounds was determined by equilibrium dialysis using the Dianorm® dialyzer (Diamchemia, Switzerland). Plasma (0.5 ml) containing one of the ¹⁴C-labelled compounds (concentration 1 µg/ml) was dialysed for 5 h at 37°C against 0.5 ml of pH 7.4 phosphate buffer containing 0.9% sodium chloride. To investigate a potential interaction with AC protein binding, plasma equilibrium dialysis of 1 µg/ml of [¹⁴C]-AC was performed in the presence of 5 µg/ml of AM and AA, respectively. All protein binding estimates were performed in triplicate.

Pharmacokinetic analysis

The apparent elimination half-lives were calculated from the terminal segment of the log C-time curve. The area under the concentration-time curve (AUC) was calculated by $AUC = AUC_0^t + Ct \times t_{1/2}/0.693$. AUC_0^t was calculated by the trapezoidal rule. Renal clearance (CL_R) was calculated by U/AUC , where U is the amount cumulative (0–24 h) excreted in urine.

Results

After oral administration, all three coumarin derivatives were rapidly absorbed resulting in peak concentrations at about 2 to 3 h for AC and at about 0.5 to 2 h for AM and AA (Figure 1a,b,c). Peak concentrations amounted to 0.3 (0.05), 1.6 (0.7), and 0.32 (0.13) µg/ml (mean ± s.d.) for AC, AM, and AA, respectively.

Neither AM nor AA was detected in the plasma of the subjects which received AC. In the urine, AM was present, all be it in small quantities (Table 1). Other metabolites were present but were not identified, nor quantitated. Acenocoumarol was excreted in minute amounts (Table 1).

Following the ingestion of AM, AA was found in the plasmas of subjects A and D (Figure 1b). However, the plasma levels were low. Urinary excretion of

Table 1 Cumulative urinary excretion (0–24 h) of AC, AM, and AA in healthy subjects following a single oral (10 mg) dose.

Compound administered Compound excreted Subject	% of the dose excreted						
	AC	AC AM	AA	AM	AA	AA	AA
A	0.1	1	—	29	13.6	31	3.5
B	0.2	0.25	—			28.5	4.6
C	0.2	0.4	—			23	2
D				30	7.6		
E				40	4		
Mean	0.16	0.55		33	8.4	27.5	3.4
±s.d.	0.06	0.4		6	4.8	4	1.3

AM and AA amounted to 30 to 40, and 4 to 14% of the dose, respectively (Table 1).

The coumarin AA was partially deacetylated during its passage through the body (Figure 1c). The fraction of the AA dose excreted in urine as AM was 2 to

4.6%. The renal excretion of the unchanged compound (i.e. AA) amounted to 20 to 30% of the dose administered (Table 1).

The pharmacokinetic constants, individual and mean, evaluated from the experiments are sum-

Figure 1(a) Plasma concentrations of AC after oral administration of (10 mg) racemic AC in subjects A, B, and C; (b) Plasma concentrations of AM (—) and its metabolite AA (----) after the oral administration (10 mg) of AM in subjects A, D, and E; (c) Plasma concentrations of AA (—) and its metabolite AM (----) after the oral administration (10 mg) of AA in subjects A, B, and C.

Table 2 Pharmacokinetic parameters of AC, AM, and AA in healthy subjects following a single oral (10 mg) dose.

Subject	AC		AM		CL_R ($ml\ min^{-1}$)	AA		CL_R ($ml\ min^{-1}$)
	$t_{1/2}$ (h)	AUC ($\mu g\ ml^{-1}\ h$)	$t_{1/2}$ (h)	AUC ($\mu g\ ml^{-1}\ h$)		$t_{1/2}$ (h)	AUC ($\mu g\ ml^{-1}\ h$)	
A	12.0	4.6	8.6	8.6	5.6	3.8	1.2	20.7
B	11.6	4.0				3.4	1.4	33.9
C	9.2	3.2				5.0	1.7	22.5
D			11.5	11.5	4.4			
E			11.0	23.9	2.8			
Mean	10.9	3.9	10.4	14.7	4.3	4.1	1.4	25.7
$\pm$ s.d.	1.5	0.7	1.6	8.1	1.4	0.8	0.25	7.1

Table 3 Plasma protein binding of AC, AM, and AA in healthy subjects

Subject	% Unbound*		
	AC	AM	AA
A	1.34	2.69	2.86
B	1.31	2.96	3.13
C	1.69	2.75	2.74
D	1.52	2.74	
E	1.75	3.70	
Mean	1.52	2.97	2.91
$\pm$ s.d.	0.20	0.42	0.20

*Each individual value is the mean of an estimation in triplicate.

marized in Table 2. The mean apparent half-life of elimination of racemic AC was 10.9 h. The compounds AM and AA showed half-lives of 10.4 and 4.1 h, respectively. The AUCs were 3.9, 14.7, and 1.9 $\mu g\ ml^{-1}\ h$ for AC, AM, and AA, respectively. The renal clearance of AM and AA was 4.3 and 25.7 $ml\ min^{-1}$, respectively.

The extent of plasma protein binding of the three coumarin derivatives is presented in Table 3. The highest protein binding was found for AC (1.52% unbound), whereas the more polar derivatives showed lower protein binding (about 3% unbound). No interaction with AC protein binding was observed in pool-plasma for AM or AA when present in 5 $\mu g/ml$ amounts, 1.41 (0.18), 1.36 (0.73), 1.44 (0.10)% free (mean $\pm$ s.d.; $n = 3$) for AC, AC in the presence of AM, and AC in the presence of AA, respectively.

Upon administration of AC, PT rose to a maximum at 24 h for subjects A and B, and at 30 h for subject C, corresponding with 45, 38, and 32% activity of normal prothrombin complex activity (Figure 2). At 50 h, PT was normalized in subject A, and almost normalized in subject B.

The compounds AM and AA at the dose administered showed no anticoagulant activity in any of the subjects (Figure 2).

Figure 2 Prothrombin (PT) times obtained in subjects that received a single oral dose (10 mg) of racemic AC (subjects A, B, C) and prothrombin times obtained in subject E ($\Delta - \Delta$) upon the administration of AM and in subject B ($\nabla - \nabla$) upon the administration of AA. The number in parenthesis represent the % of normal prothrombin complex activity. The shaded area represents the normal range of prothrombin time.

Discussion

The prime objective of the present study was to investigate the relevance of the potential AC metabolites, AM and AA, for the anticoagulant activity of the former.

Dieterle *et al.* (1977) showed for two subjects that upon oral administration, AC was extensively metabolized, mainly by reduction to AM and subsequent acetylation to AA. Plasma levels of AM exceeded those of AC from beyond 2 h in one subject and from beyond 10 h in the other. Urinary excretion of both the metabolites accounted for 20–30% of the AC dose (Dieterle *et al.*, 1977). Our experiments show complete opposite results. No AM or AA was found in plasma of the subjects that received a single oral dose of AC, and in urine only minor quantities of AM were found. It is no question that these results are by chance, for plasma analyses of patients receiving AC chronically, neither showed the presence of AM or AA (Thijssen *et al.*, 1983a). Experiments with rats also showed that AC, when given subcutaneously or intraperitoneally, either single or chronically, was hardly metabolized by the reductive pathway (Thijssen *et al.*, 1983b). Aromatic nitroreduction has been shown to occur readily in liver systems, i.e. liver homogenates and liver microsomes (Gillette *et al.*, 1968). However, at least in rats, substantial evidence is presented that in those *in vivo* situations where extensive biotransformation by aromatic nitroreduction was observed, the intestinal microflora was responsible for it rather than liver systems (Wheeler *et al.*, 1975; Levin & Dent, 1982). An indication that in man the rate of aromatic nitroreduction is small too, may be given by the low clearance of the benzodiazepine derivative nitrazepam, the elimination of which is believed to depend solely on aromatic nitroreduction (Jochemsen *et al.*, 1983). However, also with respect to this drug, the role of the intestinal microflora has been stressed for its clearance (Hewick & Shaw, 1978).

It might well be that the extensive formation of AM

(and subsequently AA) out of AC, as observed by Dieterle *et al.* (1977), resulted from biotransformation by the intestinal flora. Namely, AC was given to the subjects in capsules, and if these capsules resisted digestion long enough, AC might have reached the large intestine before absorption. The influence of different application forms on AC disposition is currently under investigation.

The coumarin derivatives AM and AA have been shown to be potent anticoagulants in mice (Dieterle *et al.*, 1977) and rats (Thijssen *et al.*, 1983b). The present investigation showed that a 10 mg dose of AM or AA *per os* in man was insufficient to give that high liver concentrations to interfere with the vitamin K-dependent clotting factor synthesis. This indicates that the metabolites AM and AA, if they are formed in the gastrointestinal tract, do not contribute to the anticoagulant activity of AC in man.

Concerning the pharmacokinetics of the three coumarin derivatives, the more rapid absorption from the gastrointestinal tract observed for AM and AA in comparison to AC is probably due to the fact that the former compounds were taken as a solution, whereas AC was taken as tablets. The half-life of elimination observed for racemic AC upon oral administration ($t_{1/2} = 10.9$ h) is somewhat higher than the values presented thus far in literature, $t_{1/2} = 3$ to 9 h (Dieterle *et al.*, 1977; Godbillon *et al.*, 1981; Houwert-de Jong *et al.*, 1981). As unchanged AC was hardly excreted in urine, elimination of AC was almost completely by metabolism. Elimination of AM was by renal excretion and by acetylation. The AM renal clearance of 4.3 ml min^{-1} in combination with the AM plasma protein binding of 97%, points to glomerular filtration only. For AA, the renal clearance of 25.7 ml min^{-1} and the protein binding of 97% points to active renal excretion processes.

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