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Cimetidine potentiates the anticoagulant effect of warfarin by inhibition of drug metabolism*

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Cimetidine (*N*″-cyano-*N*-methyl-*N*′-{2-[(5-methylimidazol-4-yl)methylthio]ethyl}guanidine), a histamine H_2-receptor antagonist [1], has been shown to potentiate the anticoagulant effect of warfarin in man, but the mechanism has not been established [2]. We have therefore studied the effect of cimetidine on the pharmacokinetics and pharmacodynamics of warfarin in the rat and determined the mechanism of the interaction.

Warfarin studies. Twelve male inbred Wistar rats (250 g) were given a single intraperitoneal (i.p.) injection of racemic sodium warfarin (0.22 mg/kg) (Ward Blenkinsop) containing 43 μCi [^{14}C]warfarin/mg (Radiochemical Centre, Amersham, U.K., sp. act. 51 mCi/mmole). They then received i.p. injections of either cimetidine (120 mg/kg) or 0.9% saline at 12-hr intervals, commencing 1 hr after warfarin, to allow for absorption of warfarin. Three further rats were given cimetidine (120 mg/kg) as above but did not receive warfarin. Blood samples (0.9 ml) were taken from the tail artery, under light ether anaesthesia, and added to 3.8% trisodium citrate (0.1 ml). Prothrombin times were measured by the one stage technique and converted to prothrombin complex activity (PCA) by reference to a standard dilution curve [3]. Concentration of [^{14}C]warfarin in plasma was measured by scintillation spectrometry after extraction and thin layer chromatography as previously described [4]. The recovery of warfarin from plasma was 83.9 ± 2.2 per cent (mean ± S.D., N = 9).

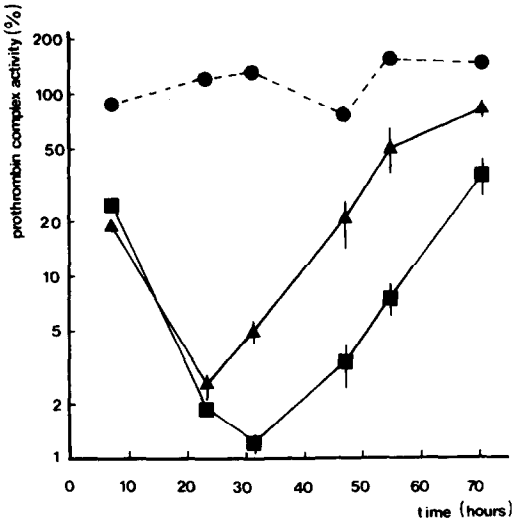

Fig. 1. The effect of i.p. warfarin (0.22 mg/kg) on prothrombin complex activity. ▲—▲; saline treated rats (N = 6), ■—■; cimetidine 120 mg/kg 12 hourly treated rats (N = 6). ●–·–●; cimetidine treated rats which did not receive warfarin (N = 3). Results are expressed as mean ± S.E.M.

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The elimination half lives of warfarin in individual rats were calculated by linear regression of the monoexponential slopes of plasma warfarin concentration vs time [4].

The relationship between plasma warfarin concentration and the rate of synthesis of prothrombin complex was investigated using the mathematical model of Nagashima, *et al.* [5]. The rate constant of degradation of P.C.A. was calculated by dosing 8 rats with warfarin (5 mg/kg) i.p. and measuring P.C.A. 3 hourly until a level of less than 5 per cent had been achieved. (This dose of warfarin inhibits clotting factor synthesis completely in the rat [6].)

Zoxazolamine paralysis times. Paralysis times were determined in groups of 4 male Wistar rats after i.p. injection of zoxazolamine (75 mg/kg) (Aldrich Chemical Company) in dimethylsulphoxide, 30 min after cimetidine (120 mg/kg) or 0.9% saline.

Pentobarbitone sleeping times. Sleeping times were determined in groups of 5 male Wistar rats (250–300 g) after i.p. injection of pentobarbitone (40 mg/kg) (Sagatal, May & Baker) 30 min after i.p. injection of cimetidine (30 mg/kg, 60 mg/kg, 120 mg/kg) or 0.9% saline. In a further experiment sleeping times were determined after i.p. injection of pentobarbitone (40 mg/kg), containing [^{14}C]pentobarbitone (0.3 μCi/mg) (New England Nuclear, sp. act. 56 mCi/mmole), 30 min after cimetidine (120 mg/kg) or 0.9% saline. On waking, blood was obtained by cardiac puncture under ether anaesthesia and the waking concentration of [^{14}C]pentobarbitone measured [7].

Chronic dosing studies. Rats were treated with either cimetidine (120 mg/kg) or 0.9% saline 12-hourly for 4 days with a 24 hr rest before (a) determination of pentobarbitone sleeping time, or (b) measurement of liver microsomal protein concentration [8], cytochrome P-450 content [9] and cytochrome *c*-reductase activity [10].

In vitro metabolism. Liver microsomes were prepared (after phenobarbitone induction) as previously described [11]. The effect of cimetidine on microsomal *N*-demethylation of aminopyrine (2×10^{-3} M) was determined by measuring both formaldehyde and 4-amino-antipyrine formation [12].

Table 1. The effect of acute cimetidine treatment on pentobarbitone sleeping time*

Treatment group (N = 5)	Sleeping time (min)
0.9% Saline (control)	98 ± 9
Cimetidine (30 mg/kg)	208 ± 11†
Cimetidine (60 mg/kg)	326 ± 24‡
Cimetidine (120 mg/kg)	410 ± 21§

* Results are given as mean (N = 5) ± S.E.M. Animals received either saline or cimetidine 30 min before pentobarbitone (40 mg/kg).

† $P < 0.001$. Significance of the difference between groups sequentially; using analysis of variance.

‡ $P < 0.01$.

§ $P < 0.05$.

Table 2. The effect of chronic cimetidine treatment on liver enzyme activity and pentobarbitone sleeping time*

	Saline	Cimetidine
Body weight (g)	319 ± 17	286 ± 18
Liver weight (g/100g body wt)	4.19 ± 0.10	4.13 ± 0.07
Microsomal protein (mg/g liver)	25.5 ± 1.9	28.1 ± 1.8
Cytochrome *c* reductase (nmoles/mg/min)	48.1 ± 3.5	58.9 ± 6.8
Microsomal P-450 (nmoles/mg)	0.54 ± 0.02	0.72 ± 0.01†
Pentobarbitone sleeping time (min)	82 ± 3	84 ± 11

* Results are given as mean (N = 4) ± S.E.M. Animals received either saline or cimetidine (120 mg/kg) twice daily for four days prior to determinations.

† Statistical difference from saline treated using Student's *t*-test, $P < 0.001$.

Results and discussion. From Fig. 1 it can be seen that cimetidine potentiates the anticoagulant effect of warfarin in the rat. Cimetidine alone had no anticoagulant effect. Cimetidine produced a significant ($P < 0.02$) increase in the elimination half life of warfarin from 11.1 ± 0.6 to 13.9 ± 0.7 hr (mean ± S.E.M.), but did not affect the plasma concentration of warfarin required to produce maximum inhibition of clotting factor synthesis (cimetidine-treated rats 0.14 ± 0.01 μg/ml: controls 0.13 ± 0.02 μg/ml) indicating that the interaction is purely pharmacokinetic [5].

We therefore investigated the effect of cimetidine on the *in vivo* metabolism of other drugs. There was a significant ($P < 0.05$) increase in zoxazolamine paralysis time from 229 ± 39 to 395 ± 39 min. Cimetidine increased pentobarbitone sleeping time in a dose-dependent manner (Table 1), but waking concentrations of [^{14}C] pentobarbitone were not significantly different in the rats treated with cimetidine or saline (cimetidine-treated 15.3 ± 1.1 μg/ml; controls 11.7 ± 1.2 μg/ml). Chronic pretreatment with cimetidine did not affect the sleeping time (Table 2), indicating that the inhibition was reversible.

Furthermore, there was no change in hepatic microsomal cytochrome *c* reductase activity or in microsomal protein concentration and there was in fact a small (33 per cent) increase in microsomal cytochrome P-450 content. Finally, we found that cimetidine inhibited the *in vitro* demethylation of aminopyrine and the I_{50} was 1.5×10^{-3} M.

Our studies demonstrate that cimetidine enhances the anticoagulant effect of warfarin in the rat, as it does in man, and that this interaction is due to enzyme inhibition. These results are in agreement with the preliminary report of Puurunen and Pelkonen [13]. Cimetidine is a 4(5)-substituted imidazole and Wilkinson *et al.* [14] have demonstrated that such compounds may be potent inhibitors of hepatic microsomal enzyme activity.

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