Interaction between warfarin and propranolol

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- 1 The interaction between propranolol and warfarin was investigated in six healthy volunteers using a low dose warfarin protocol and in New Zealand white rabbits.
- 2 Warfarin concentration ($C_{\min}$) in the volunteers increased by 14.7% on addition of propranolol ($P < 0.02$). There was no significant change in prothrombin time.
- 3 In the rabbit study, elimination half-life was shorter in the propranolol treated animals (8.20 ± 0.44 h) than in controls (10.89 ± 0.62 h). This was due to a fall in its volume of distribution from 1.13 ± 0.10 l kg^{-1} in controls to 0.81 ± 0.08 l kg^{-1} in the propranolol treated group. Clearance was not significantly altered.
- 4 The small change in warfarin concentration could be of clinical importance in some patients because warfarin has a low therapeutic index. This requires further investigation.

Keywords warfarin propranolol drug interaction

Introduction

β -adrenoceptor blocking drugs are now widely used both in the secondary prevention of ischaemic heart disease and in the treatment of patients with angina. In recent years there has been a resurgence in the use of oral anticoagulants in the management of ischaemic heart disease largely as a result of the Dutch over 65 study (Sixty-plus Reinfarction Study Research Group, 1980). Further trials of secondary prevention with these drugs are in prospect. It thus seems likely that a sizeable number of patients will be given β -adrenoceptor blockers and oral anticoagulants concurrently and it is obviously important to define whether there is a clinically important interaction between them.

Propranolol has been shown to reduce anti-pyrene clearance (Greenblatt *et al.*, 1978), and it has been suggested that the elimination of other drugs metabolised by the mixed function oxidases might also be impaired by β -adrenoceptor blockers, especially lipid soluble members of this group, such as propranolol.

The aim of this study was to investigate

whether there is either a dynamic or a kinetic interaction between propranolol and warfarin in man using a protocol previously used to study drug interactions with oral anticoagulants (Serlin *et al.*, 1979, 1980, 1981). Studies have also been carried out in the rabbit.

Methods

Human study

Six healthy male volunteers aged 22–46 years, mean 32.5 years) were selected for study. No subject was on drug treatment or had a contraindication to either warfarin or propranolol therapy. Written informed consent was obtained and approval granted by the Mersey Region Ethical Committee.

The dose of racemic warfarin necessary to increase prothrombin time by around 3 s above the control time was determined for each subject as previously described (Serlin *et al.*, 1979). This dose (range 1.5–4 mg daily; mean 3.2 mg) was then maintained throughout the study, which continued for 42 days. Warfarin was administered

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at the same time each day. Propranolol hydrochloride (80 mg twice daily) was administered in addition to warfarin from day 14 to day 27 inclusive as illustrated in Figure 1.

Blood samples were withdrawn prior to administration of warfarin and on days 3, 4, 7, 9, 11, 14, 15, 16, 18, 21, 23, 25, 28, 30, 32, 35, 37, 39 and 42 of the study. Prothrombin time and plasma warfarin concentration were measured on each day. In addition, plasma was obtained for protein binding studies on days 9 and 11 (warfarin alone) and 21 and 25 (warfarin plus propranolol). Two 24 h urine collections for measurement of warfarin metabolites were made during the second week of the warfarin alone treatment period and again during the second week of warfarin and propranolol treatment. The ratio of warfarin alcohols: 7-hydroxywarfarin reflects the relative contribution of these pathways to warfarin elimination. A selective inhibition of hydroxylation by propranolol would increase the ratio compared with the control period.

Exercise testing (bicycle; individual load for each subject) was used to assess whether the dose of propranolol given had produced adequate β -adrenoceptor blockade.

Rabbit study

Male New Zealand White rabbits were used. Control prothrombin times were determined for each individual animal as previously described (Park *et al.*, 1979). A group of four animals received propranolol (20 mg kg^{-1}day) intraperitoneally in saline (1.7 ml kg^{-1}) throughout the study while a control group received saline alone. The dose of propranolol was selected to

give a similar dose ratio to that used in the human study. Thirty minutes after the fourth propranolol or saline only injection, all animals were given a single intravenous dose of warfarin (0.63 mg kg^{-1}). Blood samples (2 ml) were obtained from the marginal ear vein 5 min before and at 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 56 and 72 h after warfarin administration for measurement of prothrombin time and plasma warfarin concentration. For comparative purposes prothrombin complex activity (PCA) was expressed as a percentage of its own control, taking 100% as the beginning of each experiment.

Analytical methods

Prothrombin time was measured by a one stage procedure using Manchester comparative thromboplastin and an automated coagulometer.

Plasma total warfarin concentration was measured by high performance liquid chromatography using a modification of the method described by Shearer (1983). Warfarin was extracted from plasma (100 μl) using methyl tert butyl ether (200 μl) and 5N HCl (50 μl). Acenocoumarin (100 ng) was added before extraction as internal standard. A reversed phase spherisorb-nitrile column (5 μm ; h.p.l.c. technology) was used. Mobile phase was hexane: isopropanol: dichloromethane: glacial acetic acid (85: 10: 5: 1). Detection was by u.v. spectrophotometer at 313 nm (Altex M160). Limit of detection was 50 ng ml^{-1} of plasma. Coefficient of variation was 4% at 100 ng ml^{-1} of warfarin. The major urinary metabolites of warfarin (warfarin alcohols and 7-hydroxywarfarin) were also measured by this method.

Warfarin protein binding was determined using [^{14}C]-labelled warfarin by equilibrium dialysis using MSE Dianorm apparatus.

Figure 1 Prothrombin times and plasma warfarin concentrations (mean and s.e. mean) in six volunteers maintained on a constant warfarin dose and given propranolol from day 14 to 27 inclusive.

Table 1 Individual plasma warfarin concentrations ($C_{\min}$ ng ml⁻¹) during treatment with warfarin (W) alone or in combination with propranolol (Prop). Values shown are the mean of four concentrations obtained during the second week of each treatment period. *t* values refer to differences between treatment periods.

	W alone	W + Prop	W alone
AW	449	467	416
ML	610	708	566
AS	419	481	444
PG	434	502	448
AB	753	931	968
MO	686	754	763
Mean values ± s.e. mean	558 ± 72	640 ± 94	601 ± 111
<i>t</i>		3.751	1.561
<i>P</i>		<0.02	NS

Calculations

In the rabbit study warfarin elimination half-life was determined using linear regression analysis. Apparent volume of distribution was calculated by dividing the dose administered by the extrapolated concentration at time zero. AUC was determined by the trapezoidal rule. Clearance was calculated from the equation

$$CL = \frac{0.693 \times V}{t_{1/2}}$$

and checked by dividing the dose by AUC.

Statistical analysis was by Student's *t*-test.

Results

Human study

The mean values for prothrombin time and plasma warfarin concentration are shown in Figure 1. Warfarin concentration shows a small but statistically significant increase after addition of propranolol but there was no significant change in prothrombin time. Steady state warfarin concentration measurements (days 7–14, 21–28, 35–42) are given in Table 1. There was a mean 14.7% increase in warfarin concentration on addition of propranolol ($P < 0.02$). After withdrawal of propranolol, warfarin concentration had returned to baseline within 2 weeks in four of the volunteers.

The ratio (mean ± s.e. mean) of warfarin alcohol: 7-hydroxywarfarin in the 24 h urine collections was 0.34 ± 0.06 for warfarin alone and 0.40 ± 0.09 for warfarin plus propranolol (no significant difference $P > 0.1$). Total urinary excretion of warfarin alcohols was not significantly altered by propranolol (warfarin alone— $271 \pm 64 \mu\text{g day}^{-1}$; warfarin + propranolol— 278

$\pm 60 \mu\text{g day}^{-1}$). Similarly there was no significant change in 7-hydroxywarfarin excretion (warfarin alone— $675 \pm 90 \mu\text{g day}^{-1}$; warfarin + propranolol— $610 \pm 60 \mu\text{g day}^{-1}$). Warfarin metabolite recovered in urine represented $29 \pm 3\%$ of daily dose.

Propranolol had no significant effect on warfarin protein binding. The free warfarin fraction was $0.78\% \pm 0.03\%$ for warfarin alone and $0.88\% \pm 0.12\%$ for warfarin in the presence of propranolol. The concentration of free warfarin was $4.5 \pm 1.2 \text{ ng ml}^{-1}$ when given alone and $5.4 \pm 1.2 \text{ ng ml}^{-1}$ when coadministered with propranolol ($P > 0.1$).

All six volunteers showed inhibition (mean 19.7%) of exercise tachycardia after administration of propranolol. Exercise pulse (mean ± s.e. mean) was $137 \pm 2 \text{ beats min}^{-1}$ for warfarin alone and $110 \pm 2 \text{ beats min}^{-1}$ on addition of propranolol ($P < 0.001$).

Rabbit study

The pharmacokinetic data are shown in Table 2. Propranolol had no significant effect on warfarin clearance but elimination half-life was significantly shorter in the propranolol treated group because of a reduction in volume of distribution. There was no significant difference in PCA at any time point between the two groups as shown in Figure 2. The time to reach 50% recovery was also similar— $57.5 \pm 5.9 \text{ h}$ in the control group and $57.8 \pm 4.8 \text{ h}$ in the propranolol treated group.

Discussion

Propranolol had a small but statistically significant effect on steady state plasma warfarin concentrations in the six volunteers on the low

Table 2 Warfarin pharmacokinetic parameters (mean value $\pm$ s.e. mean) in rabbits pretreated with propranolol or saline (controls). *t* values refer to differences between propranolol treated and controls.

	<i>CL</i> (ml min^{-1})	<i>t</i> _{1/2} (h)	<i>V</i> (l kg^{-1})
Propranolol treated	3.08 ± 0.19	8.20 ± 0.44	0.81 ± 0.08
Controls	3.15 ± 0.20	10.89 ± 0.62	1.13 ± 0.10
<i>t</i>	0.223	3.058	2.109
<i>P</i>	NS	<0.05	<0.1

dose warfarin protocol. However, this degree of change did not have a measurable dynamic effect as shown by the lack of significant change in prothrombin time. The basis of this interaction has not been established.

Propranolol is known to inhibit the elimination of several drugs which are metabolised by the hepatic mixed function oxidase system—antipyrine (Greenblatt *et al.*, 1978; Bax *et al.*, 1981), lignocaine (Conrad *et al.*, 1983), theophylline (Conrad & Nyman, 1980) and diazepam (Hawthornth *et al.*, 1983). The impact of an enzyme inhibitor on elimination of a particular drug depends on the proportion of drug cleared via the pathway(s) susceptible to inhibition by that agent (Rowland, 1975). This is illustrated in the following equation:—

$$\text{Ratio (R)} = \frac{C_{ss} \text{ inhibited}}{C_{ss} \text{ normal}} = \frac{1}{f_m \left(1 + \frac{I}{K_i}\right) + (1-f_m)}$$

I = amount of inhibitor

K_i = inhibitor constant

This model demonstrates that when all the drug is eliminated by the inhibited route (*f_m* = 1) the ratio increases dramatically with increasing

inhibitor concentration. If *f_m* < 0.5 the maximum increase in the ratio is less than two-fold. A two-fold increased in *C_{ss}* is only likely to be of clinical importance for drugs with a low therapeutic index.

The metabolism of warfarin has not been extensively investigated in man. Present evidence suggests that the S-enantiomer is mainly metabolised to 7-hydroxywarfarin and the R-enantiomer to warfarin alcohols (Lewis & Trager, 1970; Lee *et al.*, 1981). It is possible that propranolol may inhibit the hydroxylation of warfarin but the effect on reduction reactions is not known. Warfarin was administered as a racemic mixture of its R and S enantiomers because it is this form which is used in clinical practice. The *f_m* for warfarin 7-hydroxylation will therefore be less than 0.5 which indicates that a major change in *C_{ss}* is not possible. However, warfarin has a low therapeutic index and even a small change in *C_{ss}* could be clinically important.

The low dose warfarin protocol used in this study enables volunteers to be investigated under controlled conditions. Subtherapeutic warfarin concentrations are used to avoid the risk of haemorrhage. Such a study is more likely to detect a small change in warfarin kinetics than

Figure 2 Prothrombin complex activity (PCA) over 72 h in rabbits treated with 0.63 mg kg^{-1} warfarin i.v. following pretreatment with propranolol ($n = 4$; ●) or saline ($n = 4$; ▲).

a change in prothrombin time because the volunteers are on the lower shallow part of the dose-response curve. The mean increase in warfarin C_{ss} was 14.7% but this ranged from 4%–24%. Patients taking therapeutic doses of warfarin are on the steep part of the dose-response curve. For those patients with prothrombin times at the upper end of the therapeutic range a 20% increase in warfarin concentration could cause a clinically important increase in prothrombin time and consequent risk of haemorrhage. It is clearly important to study this interaction in patients but such studies are difficult to perform under controlled conditions.

The major metabolites of warfarin are measurable in urine, though 7-hydroxywarfarin is partly excreted in the bile (Lewis *et al.*, 1974). If propranolol simply inhibited warfarin hydroxylation the amount of 7-hydroxywarfarin in urine should decrease with respect to warfarin alcohols. There would therefore be an increase in the ratio of warfarin alcohol: 7-hydroxywarfarin. This was not observed in the present study. Similarly there was no significant effect on total daily excretion of these metabolites. However, there was a trend towards a reduction in excretion of 7-hydroxywarfarin in the presence of propranolol. Whether or not there is a true effect of propranolol would be more easily demonstrated in patients treated with therapeutic doses of warfarin. Furthermore there was no significant protein binding interaction between propranolol and warfarin, presumably

because warfarin binds mainly to albumin and propranolol to α_1 -acid glycoprotein.

The animal model was used to study this interaction using higher warfarin doses. Again no significant dynamic interaction was observed and there was no change in systemic clearance of warfarin. This suggests that propranolol in the dose used not inhibit warfarin metabolism in the rabbit. Metabolism of many drugs in the rabbit is faster than in man. A higher dose of propranolol might have an effect, but has not been investigated. However, there was a shortening of elimination half-life in the propranolol group but this was due to a fall in volume of distribution. The fall in V_d cannot be explained at present, but one possibility is that propranolol might inhibit warfarin uptake into the liver. Warfarin hepatic uptake and hepatic metabolism have been shown to be distinct processes in the rat (Covell *et al.*, 1983).

The clinical importance and mechanism of the warfarin/propranolol interaction have not been established. The magnitude of change in our study is similar to that seen using a shorter period of treatment with a higher dose of warfarin (Bax *et al.*, 1984). Until results from further controlled studies in patients are available it is advisable to monitor carefully the prothrombin time of any patient on anticoagulants who is given concurrent treatment with propranolol. This may also apply to other lipophilic β -adrenoceptor blockers.

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