Br. J. clin. Pharmac. (1991), **31**, 715–718 ADONIS 0306525191001307

Ponalrestat does not cause a protein binding interaction with warfarin in diabetic patients

R. F. W. MOULDS[1], R. O. FULLINFAW[1], R. W. BURY[1], W. E. PLEHWE[2], N. JACKA[3], K. M. McGRATH[3] & F. I. R. MARTIN[4]

Departments of Clinical Pharmacology and Therapeutics[1], Haematology[3] and Diabetes and Endocrinology[4], Royal Melbourne Hospital, and Department of Medicine[2], Prince Henry's Hospital, Melbourne, Victoria, Australia

Ponalrestat (Statil, ICI; Prodiax, Merck Sharp and Dohme) is an aldose reductase inhibitor which is highly protein bound. Ponalrestat markedly displaced warfarin from its protein binding *in vitro* at a concentration of 500 μg ml^{-1}, but not at a concentration of 50 or 100 μg ml^{-1}. Twelve diabetic patients (six males), age range 38–65 years, in receipt of chronic stable warfarin therapy, were given ponalrestat (600 mg daily) for 2 weeks in an open trial. A matching placebo tablet was administered for 1 week before and after the active treatment period. Patients were seen ten times (four times during the ponalrestat phase), and during the ponalrestat phase, plasma samples were also taken before and at 3 h after the daily dose of ponalrestat. At none of the visits was there any significant change in prothrombin ratio (INR), plasma total or unbound warfarin concentrations, or percentage protein binding of warfarin. No clinical complications of combination treatment were detected. The maximum ponalrestat concentration observed in the patients was approximately 100 μg ml^{-1}. We conclude that no significant interaction between these drugs occurs at the doses of ponalrestat studied.

Keywords warfarin protein binding interaction ponalrestat diabetes

Introduction

Warfarin is commonly administered to patients with a wide variety of medical disorders, including diabetes. Warfarin is also a drug which undergoes many interactions with other drugs by a variety of mechanisms, and because of its narrow therapeutic index, such interactions can be of clinical importance. Warfarin is 99% bound to plasma proteins (albumin) and one of the potential interactions of warfarin is with other drugs that are highly protein bound. Theoretically, however, displacement of warfarin from its protein binding sites should only lead to a short term increase in free plasma warfarin concentrations (MacKichan, 1984).

Aldose reductase inhibitors have been shown in acute experimental diabetes to prevent biochemical and structural abnormalities which are not prevented by insulin therapy (Cohen *et al.*, 1985; Dyck *et al.*, 1980; Mayer & Tomlinson, 1983). These agents are therefore of potential benefit in the prevention of complications in diabetic patients (Pirart, 1977a, 1977b). Ponalrestat, a phthalazinone compound, is a potent inhibitor of both human and rat aldose reductase (Stribling *et al.*, 1985) and is highly bound (99%) to human albumin. Thus it has the potential to displace warfarin and to produce a clinically significant drug interaction.

This *in vivo* and *in vitro* study was undertaken to determine whether ponalrestat displaces warfarin from plasma protein and, if displacement occurs, whether it is of clinical significance.

Methods

Measurement of bound and unbound plasma warfarin concentrations

Blood samples were collected into heparinized tubes and centrifuged for 10 min at 1500 *g*. The plasma was separated and stored at −70° C until analysis. An initial *in vitro* study (see Results) showed that these storage conditions did not have any significant effect on plasma drug binding.

Prior to assay samples were equilibrated at 37° C and then analysed using a combination of high performance liquid chromatography (h.p.l.c.) and radiolabelled techniques. Total warfarin was measured directly by h.p.l.c. (Ueland *et al.*, 1985), while unbound warfarin was determined using the method of Mungall *et al.* (1984). The

Correspondence: Dr R. F. W. Moulds, Department of Clinical Pharmacology and Therapeutics, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

protein-free fraction was obtained by ultrafiltration at ambient temperature using Centrifree® filters (Amicon Scientific, Australia).

The within day and between day coefficients of variation for measurment of percent unbound warfarin were 18.6% and 19.1%, respectively ($n = 5$).

Measurement of ponalrestat concentrations and INR

Plasma ponalrestat concentrations were measured by h.p.l.c. following protein precipitation of 100 μl samples with an equal volume of acetonitrile. Prothrombin times were measured using a Coag-a-mate × 2 automated coagulometer and human placental thromboplastin (Thromborel S, Behring). The results were converted by the standard formula to INR (International Normalised Ratio).

In vitro *study*

An *in vitro* study was performed to establish that warfarin is displaced by ponalrestat from plasma protein. Phenylbutazone was used as a control displacing agent.

Whole blood (20 ml, 7.5 ml plasma) was obtained from six normal volunteers. Each plasma sample was spiked with warfarin to give a concentration of approximately 2 μg ml^{-1} and was then divided into five aliquots. One aliquot was the control, and to the other four was added ponalrestat 50 μg ml^{-1}, ponalrestat 100 μg ml^{-1}, ponalrestat 500 μg ml^{-1}, or phenylbutazone 200 μg ml^{-1}. Each sample was then assayed for total warfarin concentration and percent protein binding of warfarin.

Clinical study

Eligible patients were aged between 18 and 65 years and had clinically stable insulin-dependent or non-insulin dependent diabetes for at least 6 months. All had stable anticoagulant control on warfarin (defined as a variation of INR and daily warfarin dosage during the three months prior to entry within ± 25% of values obtained at entry).

The study was an open 4 week trial, with 1 week placebo run-in and washout periods separated by 2 weeks of treatment with ponalrestat (Statil, ICI), 600 mg once daily given as two 300 mg tablets. Two placebo tablets were given on each day of the appropriate placebo periods. Trial medication was taken each morning, together with the patients' usual other medications. Warfarin was taken each evening except in one patient (5) who took it each morning.

Patients were examined at the initial visit after giving written informed consent and were then seen at intervals of 2 to 5 days on nine further occasions over 4 weeks. Two visits were during the initial placebo phase, four during the ponalrestat treatment phase, and three during the second placebo (washout) phase. Blood was taken at each visit before ingestion of that day's dose of ponalrestat for estimation of prothrombin ratio (INR) and serum total and unbound warfarin concentrations. On the four visits during the ponalrestat phase, another blood sample was taken 3 h after ingestion of the ponalrestat dose for measurement of INR, total warfarin and unbound warfarin concentrations.

The study was approved by the institutional Ethics Committees at the two participating hospitals.

Statistical analysis

For analysis of data obtained during the initial placebo phase, the ponalrestat phase and the final placebo phase, Student's paired *t*-test was applied to the mean of the data from the eight patients who were on a constant daily warfarin dose throughout the trial (Patients 1, 2 and 5 were taking different warfarin doses on different days of the week, and patient 6 had a dose reduction).

For statistical analysis of data obtained before and after the ponalrestat dose during the ponalrestat phase, data from all 12 patients were used.

Results

In vitro *studies*

a) The results of the initial study to determine the effects of freezing showed that, on 24 samples from three individuals, there was no significant difference in the percentage protein binding of warfarin before and after freezing at −70° C ($P < 0.05$).

b) The results of the *in vitro* displacement study are shown in Figure 1. There was no significant displacement of warfarin at ponalrestat concentrations of 50 or 100 μg ml^{-1}, but considerable displacement at the highest ponalrestat concentration of 500 μg ml^{-1}. Significant displacement of warfarin was also produced by phenylbutazone 200 μg ml^{-1}.

Clinical study

Values of the percentage of unbound warfarin are shown in Table 1. There was no significant difference between the means of the data obtained during any of the phases, nor between the before and after ponalrestat measurements during the ponalrestat phase. There was a similar lack of effect on ponalrestat on INR, total warfarin and free warfarin concentrations as no significant differences

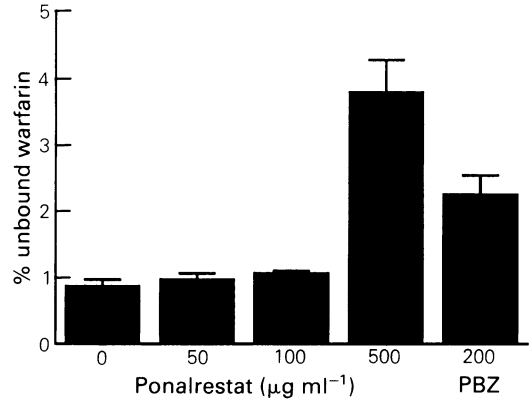

Figure 1 Mean (± s.e. mean) percentage unbound warfarin in plasma samples from six normal volunteers in the presence of increasing concentrations of ponalrestat, and a single concentration of phenylbutazone (PBZ).

Table 1 The effect of ponalrestat on the plasma binding of warfarin

	Mean % unbound warfarin			
		Ponalrestat phase		
Patient number	*Initial placebo phase (mean of 3 visits)*	*before ponalrestat (mean of 4 visits)*	*3 h after ponalrestat (mean of 4 visits)*	*Final placebo phase (mean of 3 visits)*
1	0.31	0.31	0.34	0.22
2	0.38	0.44*	0.47*	–
3	0.92	1.11*	0.84*	0.96
4	0.57	0.48	0.71	0.48
5	0.45	0.44	0.59	0.33
6	0.41	0.37#	0.46#	–
7	0.92	1.05	0.99	1.17
8	0.78	0.67	0.76	0.65#
9	0.78	0.72	0.83	0.69
10	0.98	0.97*	0.95*	0.85
11	1.11	1.06	1.08	1.06
12	0.96	0.91	0.85	0.85

* Mean of 3 visits only (see text); # mean of 2 visits only (see text).

were observed when those data were analysed individually. Patient 2 took a potentially interacting drug (diclofenac) from the end of the ponalrestat treatment phase, and no further results from this patient were included. A single missed dose of warfarin occurred at visit 9 in patient 8; the results for that patient were not included for that visit. After examination of the plasma ponalrestat concentrations it was assumed that a missed dose of ponalrestat occurred at visit 5 in patient 3 and that the before and after ponalrestat samples were inadvertently mislabelled at visit 6 in patient 10. The results for these patients, on those particular visits, were therefore not included. One patient (6) also had a dose reduction of warfarin at visit 6 because of a single increased INR reading. Therefore, all results from visit 6 onwards were excluded for this patient.

With the possible exception of this one patient in whom a dose reduction of warfarin was made there was no significant effect of ponalrestat on any of the measurements. In particular, there was no significant difference in the percent unbound warfarin before and 3 h after the dose of ponalrestat.

Plasma ponalrestat concentrations

Pre- and post-dose plasma concentrations of ponalrestat showed that little drug accumulation occurred with daily dosing over 2 weeks. The maximum plasma drug concentration observed in any of the patients was approximately 100 $\mu g\ ml^{-1}$.

Side effects

No significant side effects were observed with ponalrestat, and there were no significant changes in routine serum haematological or biochemical measurements in any of the patients.

Discussion

This study has demonstrated that, over the course of 2 weeks, there was no significant interaction between ponalrestat in a dose of 600 mg day^{-1} and therapeutic doses of warfarin.

However, it should be noted that one patient had a dose reduction of warfarin during the ponalrestat phase. This patient had the highest INR of all the patients before the study commenced, was aware of the clinical significance of the result, and requested the dose reduction. It was also only a single INR measurement which was elevated, and when the INR was repeated 3 h after the elevated level was obtained, the level was lower. We therefore do not think the dose reduction in this one patient was a reflection of a significant interaction.

Ponalrestat is highly bound in plasma (99%), and warfarin is equally, or more highly, bound to human albumin. However, a protein binding interaction with warfarin should not have a long term effect on free plasma warfarin concentrations since warfarin is restrictively cleared by the liver, i.e. only the free drug is cleared. Therefore although displacement of warfarin from plasma binding sites may cause a transient increase in the free warfarin concentration, this should revert to its previous level, with a time course which will depend on the half-life of warfarin (approximately 36–48 h, Kelly & O'Malley, 1979). Thus, any transient increase in free warfarin concentrations should largely disappear after approximately 7–10 days (or five half-lives). Therefore, the question of a significant protein binding interaction with warfarin only arises during the first few weeks of coadministration. However, this was the time over which this study was performed, and we have actually measured the percent unbound warfarin (and derived the free warfarin concentration) both before and after ponalrestat doses. Therefore we should have detected any significant displacement of warfarin.

It should be noted that we calculated the free concentration of warfarin from its total concentration and the percentage protein binding, and that the coefficient of variation for the measurement of free drug concentration was between 15% and 20%. Although we cannot be certain what change in free warfarin concentration would result in a significant change in the INR, it is likely that a change of more than 20% would be required. Thus, even with this lack of precision in the measurement of free

warfarin concentration, a clinically significant acute displacement should have been detected.

The most likely reason for the lack of a protein binding interaction is that sufficiently high concentrations of ponalrestat were not reached *in vivo*.

We are grateful to ICI Pharmaceuticals for financial support for this study, and assistance in the design of the study and analysis of the results.

We are also grateful to Ann Marie Sherman for supervision and venesection of the patients.

References

Cohen, M. P., Dasmahapatra, A. & Shapira, E. (1985). Reduced glomerular sodium-potassium adenosine triphosphatase activity in acute streptozotocin diabetes and its prevention by oral sorbinil. *Diabetes*, **34**, 1071–1074.

Dyck, P. J., Sherman, W. R., Hallcher, I. M., Service, J., O'Brien, P. C., Gevia L. A., Palumbo, P. J. & Swanson C. J. (1980). Human diabetic endoneurial sorbitol, fructose and myoinositol related to sural nerve morphometry. *Ann. Neurol.*, **8**, 590–596.

Kelly, J. G. & O'Malley, K. (1979). Clinical pharmacokinetics of oral anticoagulants. *Clin. Pharmacokin.*, **4**, 1–15.

MacKichan, J. J. (1984). Pharmacokinetic consequences of drug displacement from blood and tissue proteins. *Clin. Pharmacokin.*, **9** (Suppl. 1), 32–41.

Mayer, J. H. & Tomlinson, D. R. (1983). Prevention of defects of axonal transport and nerve conduction velocity by oral administration of myo-inositol or an aldose reductase inhibitor in streptozotocin-diabetic rats. *Diabetologia*, **25**, 433–438.

Mungall, D., Wong, Y. Y., Talbert, R. L., Crawford, M. H., Marshall, J., Hawkins, D. W. & Ludden, T. M. (1984). Plasma protein binding of warfarin: methodological considerations. *J. pharm. Sci.*, **73**, 1000–1001.

Pirart, J. (1977a). Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973 (2nd part). *Diabete et Metabolisme*, **3**, 173–182.

Pirart, J. (1977b). Diabetes mellitus and its degenerative complications: a prospective study of 4,400 patients observed between 1947 and 1973 (3rd and last part). *Diabete et Metabolisme*, **3**, 245–256.

Stribling, D., Mirrlees, D. J., Harrison, H. E. & Earl, D. C., (1985). Properties of ICI 128,436, a novel aldose reductase inhibitor, and its effects on diabetic complications in the rat. *Metabolism*, **34**, 336–344.

Ueland, P., Kvalheim, G. & Lonning, P. E. (1985). Determination of warfarin in human plasma by high performance liquid chromatography and photodiode array detector. *Ther. Drug Monit.*, **7**, 329–335.

(Received 30 November 1990, accepted 4 February 1991)