Transpulmonary pharmacokinetics of an ACE inhibitor (perindoprilat) in man

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- 1 The transpulmonary pharmacokinetics of the intravenous diacid ACE inhibitor perindoprilat were studied in 10 male patients undergoing diagnostic cardiac catheterisation for the management of ischaemic heart disease.
- 2 Following successful completion of diagnostic cardiac catheterisation and ventriculography, subjects received a low dose (1 mg) constant rate infusion of perindoprilat over 20 min with co-infusion of the intravenous marker dye indocyanine green (0.5 mg kg⁻¹). Simultaneous transpulmonary blood sampling was conducted for 1 h and subsequent peripheral venous blood samples were collected for 20 h.
- 3 No acute changes in blood pressure or heart rate were noted despite rapid and marked inhibition of central circulation plasma ACE activity persisting in peripheral venous blood for 20 h. A delayed rise in plasma renin activity was noted.
- 4 Transpulmonary passage during early accumulation of the drug was seen to incorporate an early delay. Concurrent ICG measurements suggested that this was entirely due to circulatory delay and not to binding of the drug. Thus, despite the suggested high concentration of tissue ACE activity in the pulmonary circulation, transpulmonary passage of perindoprilat was not measurably influenced by binding at this site under the conditions studied.

Keywords transpulmonary perindoprilat indocyanine green tissue ACE pharmacokinetics

Introduction

Inhibitors of angiotensin converting enzyme (ACE) are increasingly being recognised as major agents in the management of both essential and renovascular hypertension and chronic cardiac failure (Johnston, 1988; Williams, 1988). The mechanism of action of these agents on acute and chronic administration is complex and involves a range of primary and secondary effects of ACE inhibition including reduction in circulating and local concentrations of the pressor peptide angiotensin II (Ehlers & Riordan, 1989). The pulmonary circulation has traditionally been associated with the generation of angiotensin II from angiotensin I by the action of ACE. For many years it was felt that circulating AII was the product of pulmonary ACE activity and that this was in turn a major endocrine function of the lungs (Ng & Vane, 1968; Said, 1982). It has become clear, however, that ACE activity and AII generation occur in many organs and a tissue based renin angiotensin system performs autocrine, paracrine and possibly endocrine functions in conjunction with its circulating counterpart (Campbell, 1987; Dzau, 1988). Considerable interest

has arisen in the relative importance of this tissue based system both in generating the haemodynamic effects of angiotensin and in defining the pharmacodynamic response to ACE inhibition. The relative importance of tissue and plasma based ACE in generating the haemodynamic effects of AII or ACE inhibitor drugs is unclear.

In theory uptake of drug into tissue sites should alter the plasma concentration-time profile of ACE inhibitors. Non-linear pharmacokinetic models appear to describe ACE inhibitor disposition better than linear ones (Francis *et al.*, 1987; Lees *et al.*, 1989). The parameters of the pharmacokinetic model include terms which relate to the affinity and extent of binding. Since binding is believed to be primarily to ACE, these pharmacokinetic parameters could indirectly give an index of tissue ACE activity and/or inhibition. Changes in these indices may therefore predict the haemodynamic response better than the conventional total drug concentration-effect relationship.

In the present study we have examined the transpulmonary extraction of the intravenous diacid ACE

inhibitor perindoprilat (MacFadyen *et al.*, 1990). As the pulmonary circulation is theoretically a rich source of ACE activity, uptake by the lung might be expected to influence the pharmacokinetics of the ACE inhibitor.

Methods

Patients

The study was conducted in an open design in ten male patients undergoing diagnostic cardiac catheterisation for the investigation of chest pain. The demographic details of the study population are given in Table 1. Each patient had stable clinical symptoms, normal findings on physical examination and normal biochemical and haematological indices. Patients were selected such that they had no clinical, biochemical nor radiological

evidence of cardiac failure and had not received diuretic therapy in the 2 months prior to study. The protocol was approved by the local Research and Ethics Committee (Greater Glasgow Health Board, Eastern Unit). All patients gave their written and informed consent to the study conducted following their diagnostic procedure.

Procedure

All patients underwent routine diagnostic left ventriculography and coronary arteriography by the Judkins technique using non ionic contrast media (Iopamidol; Niopam 370, Merck U.K.) between 10.30 and 12.30 h. After completion of this procedure a Cournand catheter was placed in the central venous circulation also by a femoral approach. Right heart pressures through to the pulmonary capillary wedge pressure, and the on-line pressure from the descending aorta, were recorded at baseline and the right heart catheter was then withdrawn

Table 1 Demographic characteristics and concomitant therapy of the study population

Study (date)	Age (years)	Weight (kg)	Cardiac history	Medication	Ventri-culography	Angiography	Outcome
1 (6/3/89)	43	60	IHD Post infarct 1983	Atenolol 50 mg ISMN 20 mg bds	Inferior hypokinesia	RCA stenosis	Angioplasty → medical therapy
2 (14/3/89)	58	67	IHD Post infarct 1986	Atenolol 50 mg ISMN 20 mg bds Diltiazem 60 mg tds Aspirin 150 mg	Anterior hypokinesia	Distal LAD disease	Medical therapy
3 (23/3/89)	53	85	IHD Post infarct 1978	Atenolol 100 mg Diltiazem 60 mg tds Aspirin 300 mg	Normal	Diffuse CAD Occluded RCA Proximal and distal LCA stenoses	Coronary artery bypass grafting
4 (18/4/89)	42	66	IHD Post infarct 1986	Diltiazem 60 mg tds ISMN 20 mg bds	Anterior hypokinesia	Proximal LAD disease	Medical therapy after -ve thallium scintigraphy
5 (25/4/89)	63	86	IHD Post infarct 1982	Atenolol 50 mg Diltiazem 60 mg tds	Inferior hypokinesia	Occluded RCA LAD and 1st diagonal stenoses	Coronary artery bypass grafting
6 (2/5/89)	60	65	IHD ? infarct 1985	Atenolol 100 mg Nifedipine Retard 10 mg bds Betahistine 8 mg tds	Anterior hypokinesia	Severe proximal LAD and obtuse marginal stenoses	Angioplasty
7 (25/5/89)	39	105	Atypical chest pain	GTN prn	Normal	Normal	Further investigation
8 (30/5/89)	48	85	Atypical chest pain	"Franol" prn	Normal	Normal	Further investigation
9 (4/7/89)	42	85	Atypical chest pain	Cimetidine 400 mg nocte	Normal	Normal	? AV node dysfunction Further EP investigation
10 (11/7/89)	70	75	IHD Post infarct 1966	ISMN 20 mg bds Diltiazem 60 mg tds GTN prn Ranitidine 150 mg bds	Normal	Occluded LAD Filling from (R) 70-90% RCA lesion diffuse disease	Angioplasty

Key: IHD:
ISMN:
RCA:
LAD:
CAD:
LCA:
GTN:
EP:

ischaemic heart disease
isosorbide mononitrate
right coronary artery
left anterior descending artery
coronary artery disease
left coronary artery
glyceryl trinitrate
electrophysiological

to the main pulmonary artery for continuous pressure measurement and blood sampling. Aortic pressures were recorded throughout. A peripheral vein on the dorsum of the hand was cannulated for drug infusion. The surface electrocardiogram was monitored throughout the study. Immediately prior to infusion the infusate was prepared in sterile saline to contain 1 mg perindoprilat, 0.5 mg kg⁻¹ indocyanine green and 10 ml purified plasma protein solution in a total volume of 20 ml which was infused at a constant rate of 1000 µl min⁻¹ over 20 min. A Braun perfusor pump (Perfusor Secura E., Braun, Melsungen, F.R.G.) was used with the infusate primed and running for 2–5 min prior to connection to the patient. The co-infusion of indocyanine green was employed as a marker for the intravascular space and transpulmonary transit, a method analogous to that used in previous animal experiments employing multiple indicator dilution (Linehan *et al.*, 1985). Indocyanine green (0.5 mg kg⁻¹) is known to be eliminated in an exponential fashion (Meijer *et al.*, 1988). During the infusion (20 min) and for the first 40 min thereafter (i.e. total 1 h) simultaneous right and left heart blood samples (5 ml) were drawn from the appropriate catheters after removal of the dead space volume. Sampling was conducted at frequent intervals during the initial accumulation phase of the infusion (30 s intervals for 5 min) and during the early elimination phase after completion of the infusion (1 min intervals for 5 min). The infusion was stopped exactly at 20 min and the intravenous line disconnected but not flushed. Transpulmonary sampling was continued for a period of 1 h after which the central catheters and peripheral infusion cannula were removed. Later phases of drug elimination were monitored by samples withdrawn from a separate peripheral venous cannula up to 20 h post commencement of the infusion. All patients remained supine for the full duration of the study and were monitored overnight in the cardiac investigation unit according to local practice.

Biochemical methods

Drug concentrations and ACE activity were determined *in vitro* using the method of Cushman & Cheung (1971) as modified by Chiknas (1979). In our laboratory this assay has an intra- and inter-assay coefficient of variation of 2.3 and 6.1% respectively. Indocyanine green was measured, after dilution of the plasma with 0.1% w/v bovine serum albumin, by photometric assay at 805 nm (Bjornsson *et al.*, 1982; Svensson *et al.*, 1982). The intra- and inter-assay coefficients of variation were 2 and 6% respectively. Plasma renin activity was assayed according to Derkx *et al.* (1979) with an intra-assay coefficient of variation of 4% and inter-assay variation of 7%. The infusate was shown to be stable *in vitro* with respect to both drug and ICG at room temperature over 2 h. There was no analytical interference from the angiography dye.

The circulatory delay was calculated from comparison of the early, linear portions of the ICG accumulation data from the right heart samples with those from the left heart samples by applying linear regression of time upon ICG concentrations: the difference in y intercept was taken to represent circulatory delay. A similar

approach was used to assess apparent circulatory delay for the ACE inhibitor. Results are expressed as the mean of the individual circulatory delays for the nine subjects with complete ICG data.

A hierarchy of pharmacokinetic models was fitted to the plasma concentration-time data by least squares non-linear regression analysis with weighting of 1/concentration, using the biomedical statistics package BMD and the derivative-free option PAR (Ralston *et al.*, 1979) on an ICL 3980 series mainframe computer. The models tested included standard multiexponential models (1- to 3-compartment open, zero order input – Models A, B and C) and three modified 1-compartment open models which included non-linear tissue binding terms (Model D), non-linear plasma binding terms (Model E) or non-linear tissue and plasma binding parameters (Model F). The derivation and application of these models has been described previously (Lees *et al.*, 1989). The plasma concentration-time data included the right heart sampling concentrations during the transpulmonary catheterisation phase and the subsequent peripheral venous samples from 1–20 h. Where appropriate, model comparisons were based on the use of the general linear test (*F*-ratio test) (Neter & Wasserman, 1974) and the Schwarz criterion (Schwarz, 1978).

Results

There was no appreciable change in central or systemic cardiac pressures either during or up to one hour from the onset of the infusion (Figure 1). The mean profile of plasma drug accumulation during transpulmonary catheterisation is illustrated in Figure 2. The concentration of the ACE inhibitor did not approach steady state during the infusion. The small early gradient in perindoprilat concentrations between right and left heart samples is equivalent to a mean circulatory delay of 25 s. An identical circulatory delay was calculated for the control substance, ICG (Figure 3). Thereafter, ICG showed rapid accumulation towards steady state and then rapidly declined on cessation of the infusion, reflecting the known short half-life of this compound. The full pattern of drug elimination employing right heart samples followed by peripheral venous samples is shown

Figure 1 Mean blood pressure profiles ($n = 10$) in aorta (systolic, ▲; diastolic, ▼) and main pulmonary artery (systolic, △; diastolic, ▽) with mean heart rate (□) during transpulmonary catheterisation (1 h) and infusion of perindoprilat (1 mg, 20 min).

Figure 2 Accumulation concentration time profile (mean $\pm$ 1 s.d., $n = 10$) of perindoprilat in simultaneous plasma samples from right ($\circ$) and left ($\bullet$) heart catheters during constant rate infusion (1 mg, 20 min).

Figure 3 Mean concentration-time profile ($n = 10$) of indocyanine green in right ($\circ$) and left ($\bullet$) heart catheters expressed as absorbance during infusion (0.5 mg kg^{-1} , 20 min) and elimination.

Figure 4 Drug elimination profile (mean $\pm$ 1 s.d., $n = 10$) in right heart plasma ($\circ$) and peripheral venous plasma ($\blacksquare$).

in Figure 4. There was no evidence during the early post-infusion period of any relative increase in left heart concentrations compared with those from the right heart, to reflect dissociation of drug from pulmonary or post pulmonary (i.e. myocardial and initial aorta) binding sites. ACE activity in circulating plasma from both pre-pulmonary and post-pulmonary sampling showed virtually complete inhibition of circulating ACE

Figure 5 Mean ACE activity ($\pm$ 1 s.d., $n = 10$) in right ($\circ$) and left ($\bullet$) heart plasma and peripheral venous plasma ($\blacksquare$) during and following perindoprilat infusion.

Figure 6 Mean supine plasma renin activity ($\pm$ 1 s.d., $n = 10$) before and following perindoprilat infusion (1 mg, 20 min).

within 90 s of starting infusion (Figure 5). There was little evidence for either a transpulmonary gradient in ACE activity or reduction in post-pulmonary ACE after discontinuation of infusion. Marked and persistent ACE inhibition was noted during the later period of monitoring with substantial inhibition of ACE activity remaining at 20 h after the start of the infusion.

Plasma renin activity measured in samples from the post-pulmonary circulation during transpulmonary catheterisation and subsequent peripheral venous sampling showed low basal levels prior to infusion and a delayed rise with a wide variation between individual subjects (Figure 6).

Pharmacokinetics

The plasma concentration-time profiles of perindoprilat were fitted simultaneously for all data sets. It did not prove possible to fit simultaneously all data sets using a conventional three compartment linear model nor the non linear binding models which included terms for solely tissue binding or combined tissue and plasma binding. These were therefore rejected. Of the conventional models a two compartment zero order model provided a better fit for the observed data than one compartment as shown by the reduction in weighted

Table 2 Characterisation of concentration-time profiles of perindoprilat (right heart and peripheral venous plasma) by a hierarchy of pharmacokinetic models

Subject	Weighted residual sums of squares model			Schwarz criterion model		
	A	B	E	A	B	E
1	101.5	78.9	79.7	146.7	142.3	142.6
2	146.8	56.5	57.6	178.1	148.3	149.0
3	44.4	26.7	26.5	136.3	122.0	121.8
4	91.8	91.8	70.8	161.7	165.3	156.3
5	105.7	35.3	34.7	166.6	131.8	131.3
6	111.1	50.9	51.1	168.4	144.7	144.8
7	71.6	31.7	31.7	153.0	128.0	128.1
8	53.0	41.9	41.9	142.5	137.9	137.9
9	56.5	23.0	22.7	144.7	116.9	116.4
10	99.3	95.7	95.5	164.5	166.8	166.7
	Rank sum			27	18	15
	Friedman			NS		NS
	(ANOVA)			P = 0.05		

A = One compartment open, zero order input

B = Two compartment open, zero order input

E = One compartment open, zero order input, non-linear plasma binding.

residual sum of squares, *F* ratio and calculated Schwarz criterion (Table 2). The conventional two-compartment model (B) and the non-linear plasma binding model (E) described the data equally well. Both models have an equal number of parameters and therefore neither could be rejected on the grounds of being unjustifiably complex.

Discussion

The present study represents the first description of the transpulmonary pharmacokinetics of an ACE inhibitor in man.

Pulmonary ACE activity and the effects of ACE inhibitors on the enzyme at this site have been studied *in vitro*, *ex vivo* and in a limited number of *in vivo* animal experiments. The structure of the enzyme in pulmonary homogenates is suggested to be only marginally different from the circulating 'soluble' enzyme and it has been suggested that the pulmonary circulation may be the source of circulating ACE activity (Das & Soffer, 1988). The enzyme has been localised on the vascular endothelium of the lung and to vascular endothelium in a range of other organs (Ryan *et al.*, 1980; Ryan & Ryan, 1985). The enzyme has particular functions in the response to pulmonary hypoxia (Oliver *et al.*, 1989; Pitt *et al.*, 1987) or to chemical injury (Hollinger *et al.*, 1980; Kelley, 1988). It is known to be present in association with alveolar macrophages (Friedland *et al.*, 1977). In addition to its role as an exopeptidase contributing to localised AII generation, ACE has less clearly defined endopeptidase functions (Re & Rovigatti, 1988).

Studies in animals have shown temporal dissociation of plasma and pulmonary tissue ACE inhibition from the hypotensive response to ACE inhibitor drugs. It has been suggested that tissue ACE inhibition better describes the pattern of this latter response than the inhibition of

ACE in plasma (Cohen & Kurz, 1982; Chevillard *et al.*, 1989; Kamei *et al.*, 1989).

Previous animal work in rabbits had successfully defined pulmonary tissue ACE and its inhibition by captopril by employing single pass pharmacokinetic studies of the bolus injection of the synthetic ACE substrate, benzoylphenylalanyl-alanyl proline (BPAP) with the co-infusion of the intravascular marker dye ICG (Chen *et al.*, 1984; Howell *et al.*, 1984; Linehan *et al.*, 1985; Moalli *et al.*, 1985). These studies used the principle of multiple indicator dilution to define the temporal dissociation of plasma and tissue ACE inhibition *in vivo* following acute ACEI treatment.

As the structure and function of ACE is likely to differ between species (Ibarra-Rubio *et al.*, 1989; Takada *et al.*, 1982) there has been difficulty defining the qualitative or quantitative aspects of tissue ACE inhibition in man. The relative importance of tissue ACE in generating the pharmacodynamic responses to ACE inhibitors in normal volunteers or patients remains difficult to assess. We have previously suggested the use of a pharmacokinetic model to describe the concentration time profile of an ACE inhibitor. This model incorporates elements describing a saturable competitive binding process to both plasma and tissue ACE (Lees *et al.*, 1989). This may be one alternative means of describing tissue ACE inhibition in man. Along similar lines to the studies of Chen and colleagues (1984) in rabbits, this study was designed to use the pharmacokinetic profile of an ACE inhibitor in an attempt to mark tissue uptake, with the co-infusion of ICG to standardise for transpulmonary blood flow.

Our studies have been conducted in a patient population who required central cardiac catheterisation as part of their medical investigation. Both the dose and rate of infusion were chosen on the basis of previous studies in normal volunteers (Lees & Reid, 1987). We were confident that this regimen would have no adverse effects in a population with stable ischaemic heart disease and, as expected, the haemodynamic effects of infusion were minimal despite the presence of concomitant therapy in the population studied. Blood pressure data were collected primarily for reasons of safety and to exclude blood flow changes as a confounding factor.

Care was taken in this study to eliminate errors due to inadequate pump priming by ensuring infusion prior to attachment to the peripheral venous cannulae. Furthermore, as ICG is known to aggregate in concentrated solution (Bjornsson *et al.*, 1982) the infusate was prepared containing plasma protein solution to distribute the dye. This solution was prepared along with the drug 5–10 min prior to commencing the study.

Our previous studies using this dose (1 mg perindoprilat) and rate of infusion (3 mg h⁻¹) but with peripheral venous sampling had suggested that the early periods of accumulation were characterised by a sigmoid accumulation profile presumably due to the uptake of drug into the tissues during the process of arteriovenous passage (Lees *et al.*, 1989). Despite a marked increase in the frequency of sampling the present study failed to confirm such a sigmoid accumulation profile in mixed venous return samples taken from a main pulmonary artery. The inhibition of circulating ACE activity in the

initial samples was marked. Clearly the available drug within 30 s of commencing infusion was sufficient to inhibit the majority of circulating ACE with a residual component available for tissue uptake. Recirculation would be present beyond the first 2–3 min.

There may be several reasons for our failure to demonstrate either a sigmoidal pattern of accumulation or a significant early transpulmonary delay in drug concentration during this series of observations.

Although neither the total dose nor rate of perindoprilat infusion was different in the present study from previous observations (Lees *et al.*, 1989), the subtotal inhibition of plasma ACE which was present from 3 min onwards in the present study would be likely to be accompanied by subtotal binding to tissue ACE. A lower rate of infusion might have been more sensitive in detecting tissue binding, since binding saturation would not have occurred so early during the period of sampling. In addition although the lungs have been regarded as the dominant source of ACE activity for the circulation this view has had to be revised in the light of the widespread distribution of ACE activity at a variety of different tissue sites (Johnston *et al.*, 1987). The relative proportion of accessible whole body ACE which is situated in the pulmonary circulation remains unknown. Our previous studies involving peripheral venous sampling reflect exposure of the given dose to the 'whole body' ACE pool and therefore the present study might not reflect exposure of the drug to a large enough pool of enzyme. In addition as we had employed an infusate containing plasma protein solution for reasons described above this may have affected the distribution of very small quantities of drug. In effect a slowed equilibration of drug between plasma and tissue ACE might not have been reflected in *ex vivo* ACE inhibition as a substantial time period occurred *ex vivo* prior to assay.

An arteriovenous concentration gradient would be expected on theoretical grounds with any drug which interacts with a tissue in order to generate its pharmacodynamic effect. However, such arteriovenous concentration-time profiles are rarely defined (Chiou, 1989). The tissue extraction of a drug at a given site is a reflection of both the blood flow at that site and the blood:tissue partition coefficient for the drug (Bischoff, 1986). The flow rate across the lung is several hundred

times greater than that for the peripheral sampling site. Thus, although the peripheral site may show a small extraction ratio, uptake clearance may be higher there than in the lung. This element of site dependency in generation of a concentration time profile is probably the key element in our failure to describe a marked transpulmonary extraction of ACE inhibitors. It would seem unlikely that our patient population, predominantly with ischaemic heart disease, was associated with any particular reduction in pulmonary ACE sufficient to mask uptake.

Another important factor was the choice of a diacid ACE inhibitor in this study. These agents, although potent inhibitors and substrates for ACE, are poorly lipid soluble and require to be administered as ester prodrugs. Therefore this active metabolite may have inherently poor initial tissue uptake especially at low concentrations. The inter-relationship of prodrug ester and diacid metabolite ACE inhibitors in generating net tissue ACE inhibition is an important area which requires more investigation.

Since the non-linear saturable binding model could not be differentiated from the conventional two-compartment model, the present study has neither confirmed nor refuted our previous observations regarding the use of a 'physiological' pharmacokinetic model for ACE inhibitors (Lees *et al.*, 1989). As a sum of exponentials or polynomial functions can always be formed to fit a series of observed data, the main problem is that these have no physical meaning and give no information about the primary system (Rescigno & Beck, 1987). We would not suggest that the simple non linear models applied here are truly physiological but they may provide terms which are more descriptive of binding to ACE and consequently better related to pharmacodynamic effect. Our present observations would suggest that the most appropriate sampling site for such comparisons would be the traditional peripheral venous blood.

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