PHARMACOKINETICS, β -ADRENOCEPTOR BLOCKADE AND ANTI-HYPERTENSIVE ACTION OF LABETALOL DURING CHRONIC ORAL TREATMENT

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1 β -adrenoceptor blockade, plasma labetalol concentrations and anti-hypertensive actions were investigated at 2 hourly intervals during the interdose period of chronic oral therapy in six hypertensive patients.

2 β -adrenoceptor blockade varied during the inter-dose period and was maximal 2 and 4 h after the oral dose ($P < 0.05$).

3 Systolic pressure rose during the interdose period ($P < 0.05$). A significant correlation was found between the degree of β -adrenoceptor blockade and the change in systolic pressure at 2 h after the oral dose.

4 Efficacy of labetalol as a β -adrenoceptor antagonist and anti-hypertensive drug was assessed 2 h after an oral dose during chronic eight hourly dosage in sixteen hypertensive patients. Pharmacokinetics of labetalol were studied in the same patients.

5 Peak plasma labetalol concentration occurred 2 h after the oral dose and subsequently the plasma concentration declined monoexponentially.

6 The steady state concentration (C_{ss}) of labetalol was correlated significantly with the daily oral dose in mg kg^{-1} , the mid point labetalol concentration $\frac{(C_{\max} + C_{\min})}{2}$ and the isoprenaline dose ratio-1 at 2 h after the oral dose.

7 No correlation was found between the antihypertensive effect and the C_{ss} ng ml^{-1} labetalol or between the isoprenaline dose ratio-1 and the C_{ss} labetalol ng ml^{-1} .

Introduction

Labetalol is a combined α - and β -adrenoceptor antagonist, and an effective antihypertensive drug (Prichard, Thompson, Boakes, & Joeke, 1975; Sanders, Routledge, Rao, Gales, Davies & Rawlins, 1978). Considerable variation in plasma labetalol concentrations is likely to occur between individuals after oral administration because of extensive first-pass metabolism of the drug (Richards, Maconochie, Bland, Hopkins, Woodings & Martin, 1977) and to investigate this further, the pharmacokinetics and effects of labetalol were studied both between and within individual hypertensive patients.

Methods

Within patient study

Six male patients with essential hypertension (WHO Grade 1 aged 44.5 ± 4.1 years) who were receiving labetalol in an eight hourly dosage regime as their sole anti-hypertensive therapy, agreed to participate

in the study. Ethical approval for the study was given by the local hospital committee. All the patients had treated diastolic blood pressures of less than 90 mmHg and none had had any alteration in labetalol dosage during the previous 6 weeks. Immediately before the 09.00h labetalol dose, and every 2 h after throughout the 8 h interdose period, the following were carried out: measurement of systolic and diastolic (Korotkoff phase 4) arterial blood pressure with a random zero sphygmomanometer (Wright, & Dore, 1970) withdrawal of venous blood for estimation of labetalol concentration; and construction of isoprenaline dose-response curves with bolus intravenous injections (George, Connolly, Fenyesi, Briant & Dollery, 1972) and continuous electrocardiographic monitoring of heart rate.

Between patient study

Sixteen patients (nine women) whose mean age was 51.4 years, and who had essential hypertension

(WHO Grade 1), agreed to participate in this investigation which had also been approved by the local ethical committee. Since all patients had previously received other antihypertensive therapy, this was withdrawn for 3 weeks before the start of the investigation. Patients were randomly allocated to start treatment with either labetalol or placebo (both drugs being supplied in identical gelatin capsules). The patients were seen between 09.00h and 11.00h, at fortnightly intervals, and at each visit systolic and diastolic (Korotkoff phase 4) arterial blood pressures and radial pulse rates were measured after 15 min recumbency and 3 min standing. The arterial blood pressure was recorded from the mean of three measurements made with a random-zero sphygmomanometer by an observer who was unaware of the patient's drug treatment. The initial dose of labetalol was 100 mg eight hourly, but this was increased by a second observer (who was aware of the patient's blood pressure level but unaware of his drug treatment) until either the patient's diastolic blood pressure fell to less than 90 mmHg, or until a maximum dose of 3 g per day had been reached. This optimum dose was then maintained for 6 weeks. A placebo period of 6 weeks duration preceded or succeeded treatment with labetalol.

At the end of both labetalol and placebo treatment periods, intravenous dose-response curves to bolus injections of isoprenaline (George *et al.*, 1972) were constructed 2 h after the oral morning dose of the drug, as described above. Venous blood was withdrawn at 2, 4, 6 and 8 h after the morning dose of labetalol for determination of the mean steady-state labetalol concentration (C_{ss}). The plasma was stored at -20°C prior to fluorimetric analysis (Martin, Hopkins & Bland, 1976) which was performed on duplicate samples. Detection limit for the drug assay was 25 ng/ml.

Calculations

Isoprenaline dose-response lines were calculated by least squares regression analysis of the change in heart rate ($\text{beats}/\text{min}^{-1}$) upon the logarithm of the isoprenaline dose (pmol kg^{-1} bodyweight). For the within-patient study, the dose of isoprenaline which increased the heart rate by 20 beats min^{-1} (the chronotropic dose or CD_{20}) was calculated for each isoprenaline dose response curve. In the between-patient study, β -adrenoceptor blockade was quantitated by the isoprenaline dose ratio (DR) which was calculated from the dose-response curves measured during labetalol treatment and placebo therapy (Finney, 1964).

Mean labetalol C_{ss} ng ml^{-1} for each patient was determined from the area under the plasma

concentration-time curve between two successive doses (AUC):-

$$C_{ss} = \frac{\text{AUC}}{T}$$

where T is the dosage interval (Wagner, Northam, Alway & Carpenter, 1965). The C_{ss} was used to calculate the affinity constant (K_A) for the binding of labetalol to its receptor (Arunlakshana & Schild, 1959):

$$K_A = \frac{\text{DR}-1}{0.5 C_{ss}}$$

assuming that the free fraction of labetalol in human plasma is 0.5 (Martin *et al.*, 1976). Since this free fraction was not measured in this study, the affinity constant is referred to as the apparent affinity constant $K_{A(a)}$.

Results are expressed as mean $\pm$ 1 s.e.mean. In the within-patient study statistical comparisons of labetalol concentrations, isoprenaline CD_{20} and changes in arterial blood pressure were made with the Wilcoxon matched pairs signed rank test and Spearman rank correlation. In the between-patient study, comparisons of mean arterial blood pressures and pulse rates were made using a paired Student's *t*-test, and for the data on β -adrenoceptor blockade and labetalol concentrations, Wilcoxon signed rank test or Spearman rank correlation were used. Mann Whitney U Test was used to compare apparent affinity constants.

Results

Within-patient study

Plasma labetalol concentrations in these six patients reached a peak of 307 ± 132 ng ml^{-1} at 2 h, and a trough concentration of 64 ± 26 ng ml^{-1} at 8 h. Mean C_{ss} ranged from 48–464 ng ml^{-1} (mean 153 ± 66 ng ml^{-1}) and was positively correlated with CD_{20} at 2 and 4 h ($P < 0.05$). The changes in CD_{20} at 2, 4, 6 and 8 h paralleled the changes in plasma labetalol concentrations (Figure 1) and both were significantly greater at 2 and 4 h after the morning dose than at 6 and 8 h ($P < 0.05$). The CD_{20} at 2 and 4 h was correlated significantly with the labetalol concentration of these times. Systolic, but not diastolic blood pressure rose significantly 4 h after the morning oral dose ($P < 0.05$). There was a positive correlation between the change in systolic blood pressure at 2 h (i.e. time of peak labetalol concentration) and CD_{20} at this time, but otherwise

Figure 1 Plasma labetalol concentrations ($\circ$), CD_{20} ($\triangle$) and changes in arterial pressure mmHg (systolic $\bullet$, diastolic $\blacklozenge$) $\pm$ 1 s.e. mean are shown against time. ($n=6$). * denotes $P < 0.05$ compared with time 0 h.

there were no correlations between changes in β -adrenoceptor blockade and arterial blood pressure.

Between-patient study

Labetalol was tolerated well by all the patients, and no adverse effects occurred which necessitated its withdrawal. Optimum blood pressure control was achieved in 6.4 ± 1.1 weeks. The effects on blood pressure and heart rate are shown in Table 1. Mean falls in arterial blood pressure (systolic/diastolic) during labetalol treatment were $24 \pm 5/14 \pm 2$ mmHg and $31 \pm 5/16 \pm 2$ mmHg for lying and standing positions respectively.

A mean peak plasma labetalol concentration (C_{max}) of 298 ± 65 ng/ml was observed 2 h after the oral dose. The labetalol concentration declined monoexponentially to trough concentration (C_{min}) of 114 ± 27 ng/ml at 8 h with a half-life of 3.4 ± 0.3 h (Figure 2). Steady state concentrations ranged from 37 ng ml $^{-1}$ to 510 ng ml $^{-1}$ (mean 165 ± 37 ng ml $^{-1}$) and correlated significantly with the midpoint concentration $\frac{(C_{max} + C_{min})}{2}$ (Figure 5). Plasma

labetalol concentrations correlated significantly with oral daily dose in mg/kg $^{-1}$ (Figure 4).

When the DR-1 was plotted against the labetalol C_{ss} ng ml $^{-1}$ (Figure 3), the 16 patients appeared to comprise two groups; in most (13) of the patients, there appeared to be a close relationship between DR-1 and labetalol C_{ss} ($r_s = 0.72$, ($P < 0.05$), but in three patients there was a greater degree of β -adrenoceptor blockade at equivalent plasma labetalol

Figure 2 Between patient study. Plasma labetalol concentration $\pm$ 1 s.e. mean plotted against time during the dosage interval.

Table 1 Between patient study ($n=16$)

	Placebo	Labetalol
Radial artery pulse rate (beats/min)		
Lying	79 ± 2	$71 \pm 2^{**}$
Standing	81 ± 2	$74 \pm 2^{**}$
Systolic blood pressure (mmHg)		
Lying	178 ± 7	$155 \pm 6^{**}$
Standing	171 ± 7	$141 \pm 5^{**}$
Diastolic blood pressure (mmHg)		
Lying	106 ± 4	$91 \pm 3^{**}$
Standing	105 ± 4	$88 \pm 3^{**}$
Postural fall blood pressure (mmHg)		
Systolic	-7 ± 3	$-13 \pm 3^*$
Diastolic	-2 ± 1	-4 ± 1

* $P < 0.01$, ** $P < 0.001$

Figure 3 Estimation of β -adrenoceptor blockade (DR-1) is plotted against the C_{SS} labetalol ($n=13$). Three patients denoted $\triangle$ were excluded from the correlation (r_s is Spearman rank correlation coefficient)

Figure 4 Labetalol C_{SS} for plotted against total oral daily dose. $\bullet$ and $\triangle$ symbols as for Figure 3 (r_s is Spearman rank correlation coefficient).

C_{SS} . The affinity constants, $K_{A(a)}$, were higher in the latter three patients ($K_{A(a)} 32.3 \pm 5.1 \times 10^7$) than those for the remaining thirteen patients ($K_{A(a)} 9.8 \pm 1.8 \times 10^7$) ($P < 0.005$).

No correlation was found between DR-1 and the antihypertensive effect.

Discussion

These studies show that plasma labetalol concentrations vary widely both within and between patients during prolonged treatment. Since the calculated plasma labetalol concentration midway between trough and peak levels (midpoint concentration) correlated significantly with plasma

Figure 5 Shows mean labetalol concentration $\frac{C_{max} + C_{min}}{2}$ plotted against the C_{SS} labetalol. $\bullet$ symbol

represents thirteen patients and $\triangle$ symbol the remaining three patients who differed in relationship of DR-1: C_{SS} labetalol (r_s is Spearman Rank correlation coefficient).

labetalol C_{SS} (Figure 5) this may provide a reliable estimate of the latter. Plasma labetalol C_{SS} correlated with the daily dose despite the extensive first-pass elimination which has been described following oral administration (Richards *et al.*, 1977).

The within-patient study was designed to investigate whether the fluctuations in plasma labetalol concentration during a dose interval were accompanied by changes in the drugs effectiveness as indicated by the degree of β -adrenoceptor blockade as assessed by changes in CD_{20} and the antihypertensive response. Many β -adrenoceptor blocking drugs have been shown to have a prolonged antihypertensive effect despite often rapid disappearance of the drug from the plasma. β -adrenoceptor blockade (CD_{20}) was observed to vary during the 8 h interdose period, and was correlated significantly with the plasma labetalol concentration at 2 and 4 h after oral dose (Figure 1). Changes in CD_{20} could either be due to changes in the degree of β -adrenoceptor blockade or to the circadian variation in the sensitivity of the β -adrenoceptors. The relationship between changes in CD_{20} and plasma labetalol concentration support the former explanation. Systolic arterial blood pressure rose significantly towards the end of this study (4, 6, and 8 h after the oral dose) but was not accompanied by any consistent change in diastolic pressure. The mechanisms of the antihypertensive effect of β -adrenoceptor blocking drugs is unknown, and the significance of the correlation between β -

adrenoceptor blockade and change in systolic pressure 2 h after an oral dose of labetalol is unclear. Studies with propranolol which also shows a very wide-interindividual variation in plasma concentration (Shand, Nuckolls & Oates, 1970) due to first-pass metabolism have failed to find a correlation between serum drug concentrations and arterial blood pressures during interdose periods (Vervloet, Pluym, Cilissen, Kohlen, & Merkus, 1977).

In the between patient study, the pharmacokinetics of labetalol were more carefully examined in a larger patient population. Labetalol was shown to be an effective antihypertensive drug. The postural fall in systolic blood pressure was small and although statistically significant was not accompanied by symptoms in any of the patients. However, mean oral daily dose was only 919 ± 198 mg and symptomatic postural hypotension attributable to the drug is reputed to occur more frequently at doses which exceed 2000 mg daily (Prichard & Boakes, 1977) though this statement was based on data from only four patients.

The positive correlation between β -adrenoceptor blockade (DR-1) at 2 h after oral labetalol and labetalol C_{ss} ng ml⁻¹ as predicted in the within-patient study ($n=6$) was confirmed for 13 out of 16 patients in the second study. Since this occurred despite wide variation in labetalol C_{ss} ng ml⁻¹, the labetalol concentrations at the β -adrenoceptor in all patients must have exceeded the minimum necessary to inhibit function of the receptor. This blocking effect at the β -adrenoceptor was investigated further by calculating the apparent affinity constant. Estimations for $K_{A(a)}$ of labetalol at the β -adrenoceptor assumed that the drug in plasma water (total plasma concentration corrected for protein binding) was at equilibrium with drug in the biophase. The $K_{A(a)}$ calculated for the 16 patients were comparable to those reported for guinea pig atria *in vitro* (6.6×10^7 – 33.9×10^7 , Brittain & Levy, 1976). For the majority of patients, ($n=13$) they varied six-fold (3.4×10^7 – 20.4×10^7 , mean 9.8×10^7). However, three

of the 16 patients had significantly higher apparent affinity constants (mean 32.3×10^7) and required a much lower plasma labetalol C_{ss} to produce a given degree of β -adrenoceptor blockade. Since only total plasma concentrations of labetalol were measured, it is possible that these three patients had exceptionally high degrees of protein binding but plasma water concentrations comparable to the remaining patients. It is an unlikely explanation that the higher association constant in these three patients was due to formation of a biologically active drug metabolite since such a metabolite has not been found in man (Hopkins, Martin & Bland, 1976), and the fluorimetric assay used to measure labetalol concentrations is specific for the parent drug (Martin *et al.*, 1976). It is necessary to study a larger population to see whether these three patients represent a distinct group. A similar phenomenon was observed by Zacest & Koch-Weser (1972) for propranolol and for alprenolol by Collste, Haglund, Frisk-Holmberg & Rawlins (1976) but in neither instance were the affinity constants calculated. Furthermore, in the previous studies those patients with a relatively high degree of β -adrenoceptor blockade for a given plasma drug concentration may have had active hydroxylated metabolites present in their blood which was not detected by the analytical techniques used. However, since labetalol has no active metabolites in man, our group of three patients must differ in some other way but could not be separated from the other subjects on the basis of total plasma concentrations or daily dosage requirements.

In these studies no significant correlation was found between lowering blood pressure and plasma labetalol C_{ss} . Another study which examined the plasma concentration of labetalol drug chronic treatment with labetalol have reported similar findings (Lunde-Johansen, & Bakke, 1979).

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