PLASMA LEVELS AND β -ADRENOCEPTOR BLOCKADE WITH ACEBUTOLOL, PRACTOLOL AND PROPRANOLOL IN MAN

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1 The degree of β -adrenoceptor blockade of isoprenaline-induced tachycardia has been determined in three healthy volunteers after the administration of single oral doses of acebutolol, practolol or propranolol.

2 Plasma levels of these drugs were determined either colorimetrically (acebutolol and practolol) or fluorimetrically (propranolol). The colorimetric assay of acebutolol in plasma is fully described but the other drugs were assayed by published methods.

3 The degree of β -adrenoceptor blockade and the plasma level for acebutolol and practolol were well correlated, whereas in the case of propranolol correlation was poor, due in part to the presence in plasma of active metabolites not detected by the fluorimetric assay. The plasma levels of practolol and propranolol are in agreement with those previously reported.

4 The maximum cardiac β -adrenoceptor blockade achieved in this study with the respective single oral doses of acebutolol (300 mg), practolol (400 mg) or propranolol (40 mg) were similar in each of the three subjects. Therefore the β -adrenoceptor blocking potencies of these drugs against isoprenaline-induced tachycardia are inversely related to these doses; indicating that propranolol is 7-8 times more potent than acebutolol and the latter slightly more potent than practolol.

Introduction

Acebutolol ('Sectral') is a β -adrenoceptor blocking agent which shows evidence of cardioselectivity in laboratory animal experiments (Basil, Jordan, Loveless & Maxwell, 1973, 1974) and is currently under clinical trial for the treatment of angina pectoris, arterial hypertension and cardiac arrhythmias. There has been much discussion concerning the methods employed to measure the degree of β -adrenoceptor blockade in man in relation to drug plasma levels, but inhibition of isoprenaline-induced tachycardia probably provides the best method for comparative drug studies (George, Fenyvesi & Dollery, 1973).

Initial clinical pharmacological studies with acebutolol (Cuthbert & Owusu-Ankomah, 1971) compared the effects of single oral doses of acebutolol and propranolol on the tachycardia produced by 80° head-up tilt in healthy volunteers and it was suggested that the degrees of β -adrenoceptor blockade produced by single oral doses of acebutolol (100 mg and 300 mg) were similar to those given by propranolol (10 mg or 40 mg). These investigators also showed that a

single oral dose of acebutolol (300 mg) inhibited the tachycardia produced by the intravenous infusion of isoprenaline and that the degree and duration of β -adrenoceptor blockade were similar to those from a single oral dose of propranolol (40 mg).

In the present study, the degrees of reduction of isoprenaline-induced tachycardia in three healthy volunteers, each of whom received single oral doses of acebutolol (300 mg), practolol (400 mg) or propranolol (40 mg) on a random double-blind basis has been determined and compared with the plasma levels of these drugs. A preliminary account of this study has been previously reported (Basil, Collins & Cuthbert, 1973).

Methods

Subjects

The experiments were performed in three healthy male volunteers aged between 26-31 years. None

had any history of cardiac or respiratory disease and none was concurrently receiving any other drug. The subjects were persons who by intelligence and training were able to appreciate the nature of the experiments.

The degree and duration of β -adrenoceptor blockade produced by the three drugs were determined in each of the volunteers on a random basis with an interval of at least one week between each experiment to ensure complete independence for the individual investigations, as far as possible.

Isoprenaline-induced tachycardia

The subject rested supine on a couch with a comfortable back rest. A butterfly needle, gauge 19 or 21 (Abbott) was introduced into a suitable forearm vein and isotonic saline (0.9% w/v NaCl) containing 1000 units of heparin sulphate/500 ml was infused under hydrostatic pressure. The rate of the infusion was kept constant at 15 drops/min during the observation and discontinued only during the infusion of isoprenaline. A motor-driven infusion pump containing isoprenaline hydrochloride in isotonic saline was connected to the system through a three-way tap. The duration of each infusion of isoprenaline was 30 s and the different doses were achieved by varying the infusion volume from 0.1125–0.45 ml and by changing the concentration of isoprenaline solution (range 10–100 $\mu\text{g/ml}$ of base). Ascorbic acid (200 $\mu\text{g/ml}$) was present in all solutions of isoprenaline. Under these conditions increases in the heart rate of 10–45 beats/min were achieved with isoprenaline in the range 1.125–9 μg before the administration of the β -adrenoceptor blocking drugs.

The increase in heart rate due to each isoprenaline infusion was calculated from a rate meter triggered by the R-R interval of the electrocardiogram which was displayed continuously.

β -adrenoceptor blockade

To investigate the duration and extent of the β -adrenoceptor blocking actions of acebutolol, practolol and propranolol, two types of experiment were required. Firstly, the log dose response curves were determined for the effect of intravenous isoprenaline on the heart rate before the drug was given. Acebutolol (300 mg), practolol (400 mg) or propranolol (40 mg) were administered orally on separate occasions and the dose response curves for isoprenaline tachycardia (three concentrations for each curve) were redetermined at specific intervals. These doses were determined from a pilot study designed to

establish doses which gave approximately comparable degrees of β -adrenoceptor blockade. The responses were reasonably parallel at the different times for all three drugs and it was possible to determine from the dose-response lines the quantity of isoprenaline required to produce an increase in heart rate. The degree of β -adrenoceptor blockade was calculated from the quantity of isoprenaline required to give the same increase in heart rate after administration of the β -adrenergic receptor blocking drug compared with the quantity of isoprenaline required before administration of the drug. β -adrenoceptor blockade was thus expressed as the isoprenaline ratio (Cuthbert & Owusu-Ankomah, 1971).

Plasma levels of drug

Blood samples (10 ml) were taken at each interval of time and the plasma was isolated fairly rapidly. Plasma samples were deep frozen (-20°C) and transferred in this condition to May & Baker, Dagenham for acebutolol assays (see below for method) or to I.C.I. Pharmaceuticals, Macclesfield, for plasma practolol and propranolol assays which were determined by the methods of Fitzgerald & Scales (1968) and Shand, Nuckolls & Oates (1970) respectively.

Colorimetric assay of acebutolol

Acebutolol was assayed by a colorimetric method based upon acid hydrolysis to the corresponding aromatic amine which can be assayed down to about 0.1 $\mu\text{g/ml}$ plasma by a modification of the Bratton & Marshall (1939) method for diazotisable aromatic amino compounds. This method is not entirely specific and would detect 1-(4-acetamido-2-acetylphenoxy)-2-hydroxy-3-isopropylamino-propane which is a known metabolite of acebutolol.

The method employed for plasma was as follows. To plasma (2 ml) was added glycine buffer (0.5 ml, pH 10; containing 10% w/v glycine in solution as sodium salt, saturated with sodium chloride) and the mixture was extracted with redistilled ethyl acetate (6 ml) by shaking the stoppered tube vigorously for 2 minutes. After the slow centrifuging, the lower layer was removed completely by careful suction through a drawn out disposable ('Pasteur') pipette, and it was discarded. The ethyl acetate (5.5 ml) was extracted with 3 M sulphuric acid (0.7 ml) by shaking thoroughly for 2 minutes. The acid layer was carefully separated and the ethyl acetate extracted once more with 3 M sulphuric acid (0.3 ml). The combined acid extract (1 ml) was heated at 99°C for 2 h to hydrolyse the butyramide group. Water (1.3 ml)

was added and the solution was cooled to 4°C. When it was cold, a solution (1% w/v) of sodium nitrite (0.2 ml) was added and the mixture was left at 4°C for 5 minutes. Ammonium sulphamate (5% w/v, 0.5 ml) was then added and the mixture was well shaken at intervals for 15 minutes. Finally a colourless solution (1% w/v) of naphthylethylenediamine dihydrochloride (0.5 ml) was added and the mixture stood aside at room temperature for 2 h until full colour development had occurred. The absorbance at 565 nm was determined on a Unicam S.P. 800 spectrophotometer scanning from 400-700 nm. A scale expansion accessory was employed to increase the accuracy of the optical density measurements. For these low concentrations a longer light path (4 cm) micro-cell was employed and the optical density was obtained by division of the instrument reading by a factor of 4.

A calibration graph was prepared from control human plasma samples (2 ml) to which had been added known quantities (1, 2, 4 or 10 μ l) of an aqueous solution of acebutolol hydrochloride (1.11 mg/ml of hydrochloride salt, which corresponds to a solution containing 1 mg/ml of acebutolol base) to give plasma concentrations equivalent to 0.5, 1.0, 2.0 or 5.0 μ g/ml acebutolol, respectively. After a sufficient number of analyses had been completed at each level, (usually 6) a calibration graph was prepared for use in the colorimetric assay of plasma samples. The overall recovery of acebutolol was about 70% at 0.5 μ g/ml from human plasma under these conditions of assay. The graph was linear approximately to 2 μ g/ml. Higher levels require dilution with control plasma before assay.

Unknown sample assays Samples of plasma (2 ml) containing unknown quantities of acebutolol may be assayed, as above, by direct reference to a calibration graph. The blank values from different subjects were fairly constant but pre-treatment samples of plasma should always be included since some other drugs may also be detectable, particularly if they are bases and should partition like acebutolol and if they also possess an aromatic amino group (or an acylated aromatic amino group that would be hydrolysed to an aromatic amino group under the stated conditions).

Drugs

Gelatin capsules of identical appearance containing acebutolol (100 mg, in the form of hydrochloride) (Lot No. TT 1597) or lactic acid were supplied by May & Baker Ltd. Gelatin capsules containing propranolol (40 mg) or practolol (200 mg) were

supplied by I.C.I. Pharmaceuticals Division and were similar to the capsules containing acebutolol. Solutions for intravenous infusion were freshly prepared from isoprenaline hydrochloride (1 mg/ml) (Sustcardia, Pharmax Ltd); heparin sulphate (1000 units/ml) (Evans Medical Ltd) and ascorbic acid (50 mg/ml) (Redoxon, Roche Products Ltd).

Results

The peak plasma levels of acebutolol and practolol occurred at about the same time, 3-4 h, after the single oral doses of 300 mg and 400 mg respectively (Table 1), whereas the peak plasma level after a single oral dose of propranolol (40 mg) occurred at 2-3 h; this is shown in Fig. 1 and the individual results given in Table 1. The maximum degree of β -adrenoceptor blockade (isoprenaline ratio) also occurred at approximately the same time as the peak plasma level for each of the three compounds.

Although both the doses of acebutolol and practolol were similar and the maximum degree of β -adrenoceptor blockade obtained with each drug was comparable, maximal plasma levels of practolol were about three times greater than those of acebutolol. The degree of β -adrenoceptor blockade (isoprenaline ratio) and the log plasma levels can be fitted to a linear correlation graph for both acebutolol and practolol (Fig. 2) as would be expected for a competitive β -adrenoceptor blocking agent (Coltart & Shand, 1970). It can be seen (Fig. 2) that for β -adrenoceptor blockade corresponding to an isoprenaline ratio of 7, the mean plasma level of acebutolol is 0.2 μ g/ml and for practolol it is 1.2 μ g/ml (Basil *et al.*, 1973). The plasma levels recorded for practolol in this study after the 400 mg dose (mean peak plasma levels of 3.5 ± 0.6 μ g/ml, determined by Dr D.M. Foulkes, I.C.I. Pharmaceuticals Ltd, Macclesfield) are comparable with other published data at this dose (3.1 ± 0.5 μ g/ml; Traub, Shaver, McDonald & Shapiro, 1973 and 3.7 ± 0.5 μ g/ml; Carruthers, Kelly, McDevitt & Shanks, 1974).

These plasma levels of acebutolol (Table 1) are in accord with other studies in healthy volunteers at this dose (300 mg) and also with plasma levels in patients determined by an alternative fluorimetric method being developed in the laboratories of May & Baker Ltd and can be regarded as normal plasma levels after oral administration of acebutolol (300 mg).

With propranolol, the plasma levels were much more variable and it was not possible to fit the data to an acceptable correlation curve, possibly due to the formation of active metabolites,

Figure 1 Degree of β -adrenoceptor blockade (continuous line) and plasma levels (dotted line) measured in three healthy volunteers receiving single oral doses of (a) acebutolol (300 mg), (b) practolol (400 mg) and (c) propranolol (40 mg). The degree of β -adrenoceptor blockade is expressed in terms of the isoprenaline dose-ratio determined from the antagonism of isoprenaline-induced tachycardia.

4-hydroxypropranolol (Paterson, Conolly, Dollery, Hayes & Cooper, 1970; Fitzgerald & O'Donnell, 1971) which is not measured by the method employed, and other unidentified metabolites. In this respect Brain, Card, Kane, Lyonnais & Dollery (1974) have recorded a tenfold variation in the plasma level at 2 h in four healthy volunteers.

A small degree of β -adrenoceptor blockade was detected with both acebutolol and practolol at 23-24 h after administration in some subjects (Figure 1). It was not possible to calculate the late

Figure 2 Relationship between the dose ratio for isoprenaline and the plasma acebutolol (●) and practolol (▲) concentrations. The straight lines represent the computed least squared error for each population of results expressed as a linear equation of the abscissa variable.

Table 1 Inhibition of isoprenaline-induced tachycardia (Iso-P ratio) and plasma levels of three β-adrenoceptor blocking drugs in three healthy volunteers

Time after dose (h)	Acebutolol				Practolol				Propranolol			
	R.G.		A.C.		R.G.		A.C.		R.G.		O.L.	
	Plasma level (μg/ml)	Iso-P ratio	Plasma level (μg/ml)	Iso-P ratio	Plasma level (μg/ml)	Iso-P ratio	Plasma level (μg/ml)	Iso-P ratio	Plasma level (μg/ml)	Iso-P ratio	Plasma level (μg/ml)	Iso-P ratio
1	<0.1	5.0	0.44	8.4	<0.1	1.3	<0.1	1.3	0.02	18.6	0.028	11.2
2	<0.1	7.9	0.46	9.2	1.1	6.9	1.5	4.06	0.03	20	0.044	13.0
3	0.41	14.8	0.96	19.0	2.75	11.2	2.7	3.88	0.06	14.0	0.02	10.3
4	1.18	17.3	0.62	10.1	3.6	14.4	2.9	3.29	0.03	10.2	0.028	8.1
6	0.3	12.3	0.30	6.53	2.55	6.3	2.0	2.88	<0.005	7.4	0.028	6.2
10	<0.1	7.9	<0.1	4.4	2.0	3.7	1.5	1.0	<0.005	5.0	0.025	4.1
24	<0.1	2.9	<0.1	1.3	0.9	1.1	0.54	—	—	1.4	—	1.6

plasma half-life of acebutolol from the limited plasma level data obtained in the present study because the level of acebutolol fell below the limit of sensitivity (0.1 μg/ml) of the colorimetric assay within 6-10 h after administration.

Discussion

Isoprenaline-induced tachycardia is considered to be the most satisfactory method for comparing the activities of different β-adrenoceptor blockers (George *et al.*, 1973). The choice of an arbitrary increase in heart rate (25 beats/min) for the selection of isoprenaline dose ratios was similar to that made by Coltart & Shand (1970) and gives a reliable experimental basis for comparison since it was roughly at the mid-point of the observed dose/response curves to isoprenaline in the present study.

In a previous study, Cuthbert & Owusu-Ankomah (1971) compared acebutolol (300 mg) and propranolol (40 mg) for their inhibition of isoprenaline-induced tachycardia in two healthy male subjects. The maximum degree of β-adrenoceptor blockade for acebutolol in the present study occurred between 3-4 h (2-4 h in the previous study) and at the same time for practolol, whereas for propranolol the maximum blockade occurred earlier, at 2-3 h (2 h in the previous study). The maximum degree of β-adrenoceptor blockade for these β-adrenergic receptor blocking drugs in the present study showed a range of isoprenaline ratios of 14-19 for acebutolol, a range of 6-16 for practolol and a range of 14-20 for propranolol (Table 1), which are not appreciably different. This study was considered valid for comparative purposes since, as far as is known, the metabolic status (food intake with respect to time of dose etc.) of the subjects remained the same throughout the investigation.

It has recently been claimed for practolol that the half-life for the decline of the reduction of exercise tachycardia is much greater than the biochemical half-life of 11 h for the period 7-24 h after dosing (Carruthers, Kelly, McDevitt & Shanks, 1973). For practolol the formation of active metabolites is unlikely to provide an explanation for this difference since approximately 85% of the compound is excreted unchanged in urine (Fitzgerald & Scales, 1968).

A limited number of studies have been published in which the plasma levels of practolol have been compared in man with its capacity to inhibit isoprenaline-induced tachycardia. An earlier study (Brick, Hutchinson, McDevitt, Roddie & Shanks, 1968), suggested that practolol had less activity against isoprenaline-induced

tachycardia than against exercise-induced tachycardia. However, it has been shown more recently (Schneck, Aoki, Kroetz & Wilson, 1972) that practolol produces a serum concentration-dependent reduction in heart rate and systolic and diastolic blood pressure during isoprenaline infusion. However, almost maximum β -adrenoceptor blockade occurred with 400 mg orally in that study at serum practolol levels of 2 $\mu\text{g/ml}$. In the present study one subject achieved a relatively low level of β -adrenoceptor blockade (isoprenaline ratio 6.2) despite a practolol plasma level of 3.9 $\mu\text{g/ml}$.

The results obtained with orally administered propranolol showed that, for an isoprenaline dose ratio of about 10, the propranolol plasma level was 15-30 ng/ml, a slightly higher value than that obtained by Coltart & Shand (1970), i.e. 10-20 ng/ml, for the same isoprenaline dose ratio after intravenous propranolol. The results for isoprenaline-induced tachycardia were different from those for exercise-induced tachycardia where, for the same degree of β -adrenoceptor blockade, plasma levels of propranolol (also determined fluorimetrically by the method of Shand *et al.*, 1970) after a single oral dose were about one third of the plasma level required after an intravenous dose. The differences observed by these authors for exercise-induced tachycardia was explained, in part, by the presence of 4-hydroxypropranolol (not detectable by the

fluorescence assay) which has comparable β -adrenoceptor blocking activity to propranolol and is present in plasma after an oral dose but not after an intravenous dose of the drug. The metabolism of acebutolol is much less extensive than propranolol, and it more closely resembles practolol, so that metabolites are likely to contribute less to the β -adrenoceptor blocking activity of acebutolol after oral administration than for propranolol.

Since the degree of maximum adrenoceptor blockade was approximately the same for each of the three drugs in the same subjects, the relative potencies of acebutolol (300 mg), practolol (400 mg) and propranolol (40 mg) can be reasonably assumed to be inversely related to these doses, indicating that propranolol is 7-8 times more potent than acebutolol and the latter slightly more potent than practolol.

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