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A STUDY OF THE PHARMACOKINETICS AND PHARMACODYNAMICS OF NIFEDIPINE IN COMBINATION WITH ATENOLOL

T. J. FITZSIMONS*, S. C. NORRIS*, H. K. ADAM‡, J. RYAN‡ AND J. McAINSH†

Medical Research, †Medical Affairs and ‡Safety of Medicines Departments, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG, England

ABSTRACT

This double-blind randomized, crossover study was undertaken to determine the pharmacokinetic properties of nifedipine retard and atenolol when given separately, as a free or a fixed combination, compared with placebo in 15 healthy male volunteers. There was no difference between the three atenolol formulations in time to maximum blood concentration or elimination half-life. The fixed combination showed significant differences in both maximum observed blood concentrations (+16 per cent) and total area under the curve (+16 per cent) compared to atenolol alone. Urinary recovery of unchanged drug from the fixed combination was also slightly increased but the difference was not statistically significant. Furthermore, statistical evaluation of the plasma pharmacokinetics of nifedipine retard and urinary recovery of nifedipine metabolite showed that all three formulations were indistinguishable. Thus, it is concluded that the fixed combination of nifedipine and atenolol is bioequivalent to the free combination and that the bioavailability of both drugs in the fixed combination is equivalent to that of the single entities.

KEY WORDS Atenolol Nifedipine Pharmacokinetics Pharmacodynamics Combination Human

INTRODUCTION

Simplified antihypertensive drug regimens have been shown to benefit patient compliance and have helped gain increasing acceptance of low-dose fixed combination therapy in selected patients.[1–4]

A fixed combination of the $beta_1$-selective adrenergic receptor blocker atenolol and the dihydropyridine calcium antagonist nifedipine (retard formulation) is now available for the treatment of hypertensives who fail to respond to monotherapy.

The present study was designed to examine the comparative pharmacokinetic and pharmacodynamic properties of the 'fixed' and 'free' combination of atenolol and nifedipine and to compare these findings with these agents alone in normal volunteers.

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METHODS

Study Design

The study was a double-blind, randomized five-way crossover design comparing the pharmacokinetic and pharmacodynamic properties of nifedipine (20 mg) and atenolol (50 mg) given separately, given as a free combination, and as a fixed combination compared with placebo. Each volunteer was given a complete explanation of the details of the study and allowed to read the protocol, which was subject to the written approval of an independent Ethical Committee.

Selection Criteria

Fifteen male volunteers were identified and examined to ensure that they were healthy and not on any current therapy. A pre-study examination which included medical history, physical examination, 12-lead electrocardiogram (at rest and on exercise), and laboratory tests (haematology, urine analysis, and blood chemistry) was undertaken on all subjects. Subjects were excluded from the study if there was any evidence of heart disease, liver disease, renal disease, or obstructive airways disease. Smokers or those requiring medication for any other condition were also excluded.

Drug Presentation

Atenolol was presented as a white film coated sales tablet containing 50 mg drug. The slow release sales tablet contained 20 mg nifedipine and the combination capsule contained 50 mg atenolol (granules as used in the sales tablet) and 20 mg nifedipine retard.

Each of the drug treatments was given as a single oral dose and only one treatment was allowed in a 1-week period, i.e. a minimum of 7 days between doses.

The treatment was given according to a predetermined randomization procedure. A double dummy technique was employed to preserve blindness. Each treatment consisted of two tablets plus one capsule, each being taken with 100 ml of water.

Samples

Blood samples for plasma levels of nifedipine and whole blood levels of atenolol were undertaken at the following times after dosing: 0, 30, 60, and 120 min and 3, 4, 5, 6, 8, 12, 24, and 30 h. Clinical measurements were taken immediately prior to venepuncture.

Urine was collected for the 12-h period prior to each dose. Two consecutive 24-h urine collections were made after each dose (i.e. urine collected for 60 h continuously). The volume of each collection was measured and recorded and an aliquot (25 ml) stored frozen at $-20°$ pending analysis for atenolol and nifedipine metabolite.

All samples were analysed blind and the code broken only after analyses were completed. The analyses of atenolol in blood and urine was by gas liquid chromatography previously published.[5]

Since nifedipine is light sensitive all stages of the assay, including separation of the plasma, were carried out under artificial lighting from gold fluorescent tubes. The analysis of nifedipine in plasma was by gas liquid chromatography as follows: to 1 or 2 ml of plasma were added an appropriate amount of diazepam (internal standard), 1 ml of tris buffer, and 5 ml of toluene. The mixture was shaken for 10 min and centrifuged. Then 4·5 ml of the organic layer was transferred to a clean tube and evaporated to dryness at 50° under a stream of nitrogen. The residue was redissolved in an appropriate volume of tetradecane/toluene (2/1) and analysis was by capillary gas–liquid chromatography (30 M × 0·32 mm i.d. column of SE 30 0·25 μm film) with electron capture detection.

It is well established that the major route of metabolism of nifedipine is oxidation of the dihydropyridine ring, followed by hydrolysis of one of the methyl ester moieties.[6] The product of this process, the pyridine carboxylic acid, is described in the paper as 'nifedipine metabolite'. The analysis of nifedipine metabolite in the urine was by gas–liquid chromatography as follows. To 1 ml of urine were added 1 ml of 3 N hydrochloric acid and 5 ml of ethyl acetate. The mixture was shaken for 20 min and centrifuged. Then 4 ml of the ethyl acetate was transferred to a clean tube and evaporated to dryness at 50° under a stream of nitrogen. To the dry residue were added 5 ml dichloromethane, 150 μl of tetrabutyl ammonium hydroxide (25 per cent solution in methanol) and 250 μl of methyl iodide and the mixture heated in a water bath for 90 min at 50°. Distilled water (2 ml) was added and the mixture shaken for a further 20 min. After centrifuging, 4 ml of the organic layer was taken and evaporated to dryness at 50° under a stream of nitrogen. The residue was dissolved in 2 ml of ethyl acetate, transferred to a 'Pye' auto-sampler vial and 1 μl injected onto a GC column. Analyses were by gas–liquid chromatography on a packed column of 5 per cent SE 30 at 250° with electron capture detection.

Unknown concentrations were determined by comparison of peak height ratios (plasma) or peak height (metabolite) with samples of control biological fluid to which known amounts of nifedipine or metabolite had been added and treated in an identical manner to that described above. The limit of detection was assessed by a confidence interval approach. The mean limits of quantification were 1 ng ml^{-1} and 0·4 μg ml^{-1} for plasma and urine, respectively.

Blood Pressure and Heart Rate

Blood pressure and heart rate were measured immediately prior to each blood sample in the supine and erect positions following 5- and 2-min intervals, respectively. A standard sphygmomanometer and cuff were employed using

the Korotkoff Sound 5 for the diastolic blood pressure reading. Heart rate was measured by palpation of the radial pulse.

Exercise Test

Before the study commenced each subject was exercised on an electrically braked 'Elema-Schonander' bicycle ergometer to determine the load required to give a heart rate of 140 to 150 beats min^{-1} during the last 30 s of a 4-min period of exercise. The load required differed from volunteer to volunteer. For any one volunteer the load (and bicycle saddle height) remained constant throughout the study.

A 'Cambridge Camtrace' electrocardiogram (ECG) was used to monitor heart rhythm throughout the exercise period. With a volunteer seated on the bicycle, the heart rate was measured immediately before exercise began and then during the last 30 s of each 4-min exercise period from the ECG recording.

Data handling

Pharmacokinetic data. From the log blood or plasma/time profile the half-life was calculated for each individual data set by linear regression from the point at which the profile was judged to be linear (generally 8 to 12 h for both atenolol and nifedipine. Area under the curve to infinite time (AUC) was calculated by trapezoidal rule up to the first point on the regression and by integration thereafter.

Statistical evaluation of pharmacokinetic parameters was by analysis of variance (ANOVA) on log transformed data, taking into account variation between subjects, between visits, and between treatments. The data was further examined by a *t*-test comparing the fixed and free combinations with the results from the single drug administration. In addition, the 95 per cent confidence interval (95 per cent C.I.) around the percentage differences observed was also calculated.

Clinical observations. Statistical evaluation of the maximal changes in heart rates and blood pressures from the pre-dose values, and the 24-h value difference from pre-dose values, was undertaken using ANOVA. Differences between heart rates and systolic blood pressures during the pre-dose exercise test and exercise 3 h and 24 h after dosing were also analysed using ANOVA.

RESULTS

The mean age of the subjects was 32 ± 2 years; their mean weight 72 ± 2 kg.

Pharmacokinetics

Atenolol whole blood concentrations. The mean blood concentrations are summarized in Table 1 and illustrated in Figure 1. There was no difference between the three formulations in mean time to peak blood concentration or elimination

Table 1. The mean pharmacokinetics of atenolol (± SE) after the oral administration of monotherapy and combination therapy with nifedipine retard

Parameter	Atenolol	Fixed combination	Free combination
C_{max} (ng ml^{-1})	292 ± 26	326 ± 30	284 ± 32
C24 (ng ml^{-1})	24 ± 2·3	27 ± 1·7	25 ± 1·5
AUC (ng ml^{-1}h)	2833 ± 182	3249 ± 158	2854 ± 214
$t_{½}$ (h)	7·8 ± 0·5	7·6 ± 0·4	7·8 ± 0·4
t_{max} (h)	3·0 ± 0·2	2·8 ± 0·3	3·0 ± 0·2
U (mg)	16·9 ± 1·4	19·2 ± 1·3	17·2 ± 1·6

C_{max}: mean peak concentration.
C_{24}: mean concentration 24 h after dosing.
AUC: mean area under curve.
$t_{½}$: mean elimination half-life.
t_{max}: mean time to peak concentration.
U (mg): mean urinary recovery of atenolol.
Combinations: 50 mg atenolol plus 20 mg nifedipine retard.

half-life. The elimination half-life was not significantly different for the free (+1 per cent, 95 per cent C.I. −10 per cent to +12 per cent), or fixed (−2 per cent, 95 per cent C.I. −12 to 10 per cent) combinations. The fixed combination was significantly different in both peak blood concentration ($p < 0·05$, +16 per cent, 95 per cent C.I. +1 per cent to +33 per cent) and total area under the curve ($p < 0·01$, +16 per cent; 95 per cent C.I. +5 per cent to +29 per cent) compared to atenolol alone.

Atenolol urinary recovery. There was no significant difference in urinary recovery of atenolol from either combination when compared to atenolol alone (Table 2).

Nifedipine plasma concentrations. The mean plasma concentrations are summarized in Table 2 and illustrated in Figure 2. Comparison of the free and fixed combinations against nifedipine retard alone confirmed that none of the parameters, viz peak plasma concentration, area under the curve, and half-life were significantly different.

Nifedipine metabolite. There was no significant difference in the urinary recovery of the nifedipine metabolite, compared to nifedipine retard alone, with either the free combination or the fixed combination.

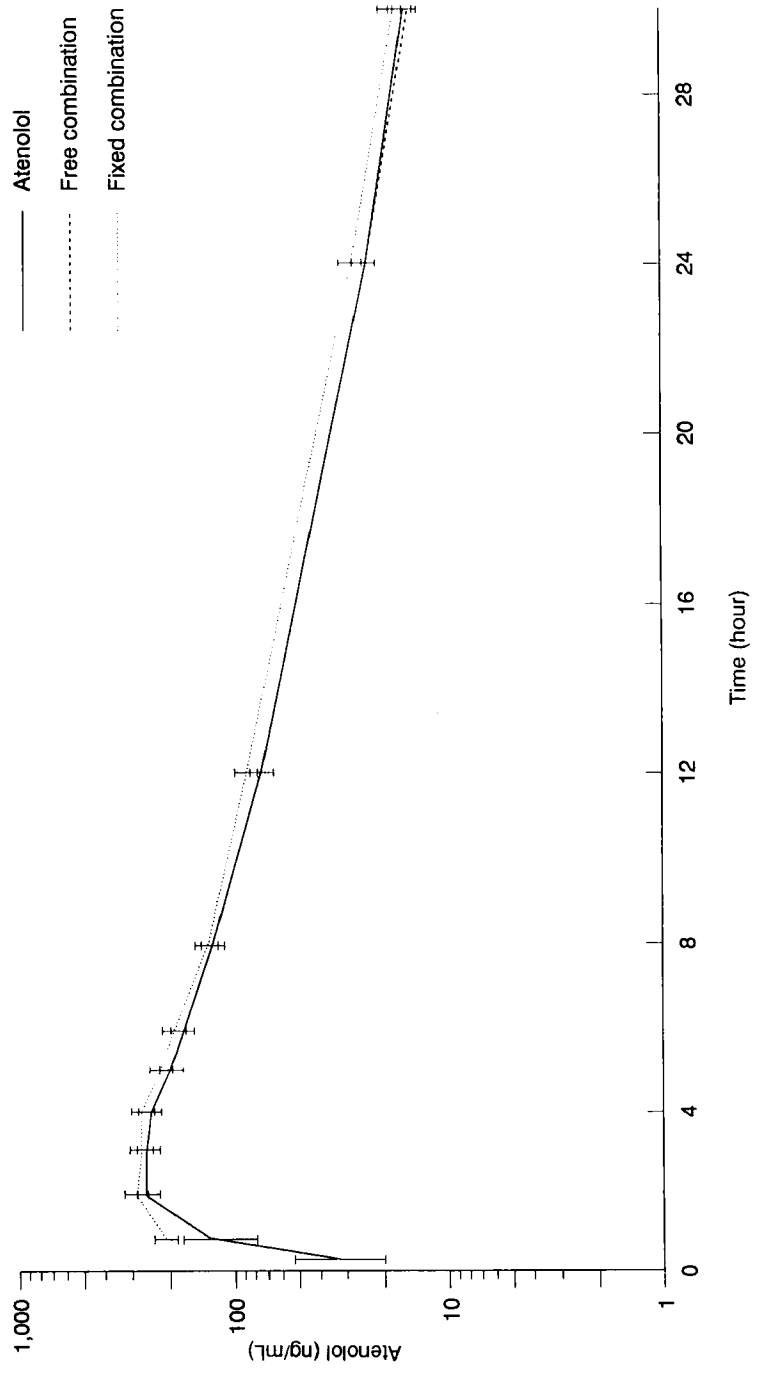

Figure 1. Mean whole blood concentrations of atenolol ± SE

Table 2. The mean pharmacokinetics of nifedipine (±SE) after the oral administration of monotherapy and combination therapy with atenolol

Parameter	Nifedipine	Fixed combination	Free combination
C_{max} (ng ml^{-1})	34 ± 4·7	41 ± 5·5	35 ± 3·0
C_{24} (ng ml^{-1})	2·0 ± 0·5	1·3 ± 0·3	1·9 ± 0·3
AUC (ng ml^{-1} h)	255 ± 34	252 ± 34	243 ± 19
$t_{½}$ (h) 5·3 ± 0·4	5·6 ± 0·5	5·0 ± 0·5	
t_{max} (h)	2·5 ± 0·2	2·1 ± 0·2	2·3 ± 0·2
U (mg)	10·2 ± 0·5	9·7 ± 0·5	9·3 ± 0·4

C_{max}: mean peak concentration.
C_{24}: mean concentration 24 h after dosing.
AUC: mean area under curve.
$t_{½}$: mean elimination half-life.
t_{max}: mean time to peak concentration.
U (mg): mean urinary recovery of nifedipine metabolite.
Combination: 50 mg atenolol plus 20 mg nifedipine retard.

Heart rate and blood pressure

The fixed and free combinations significantly reduced standing systolic blood pressure (Figure 3) to a greater extent than either atenolol or nifedipine alone with the maximum effect being between 3 and 4 h post-dosing. This effect was not sustained over a 24-h period. There was no demonstrable difference between the fixed and free combinations. Both atenolol and the fixed and free combination reduced exercise systolic blood pressure at 3 h, but not 24 h, after dosing in these normotensive subjects (Figure 4).

Atenolol, the free combination and the fixed combination, all reduced heart rate in the lying and standing position (Figure 5) to a similar extent, and this effect was sustained throughout the 24-h period. Nifedipine retard alone and placebo did not reduce heart rate. Reductions in heart rate were also demonstrable for the treatments containing beta blockers under conditions of moderate exercise over a 24-h period (Figure 6).

Side-Effects

Headache was experienced by 8 subjects following nifedipine treatment, but appeared to occur less commonly when nifedipine was co-administered with atenolol, i.e. in the fixed ($n = 2$) and free combination ($n = 3$). Fixed combination was generally well tolerated by all subjects.

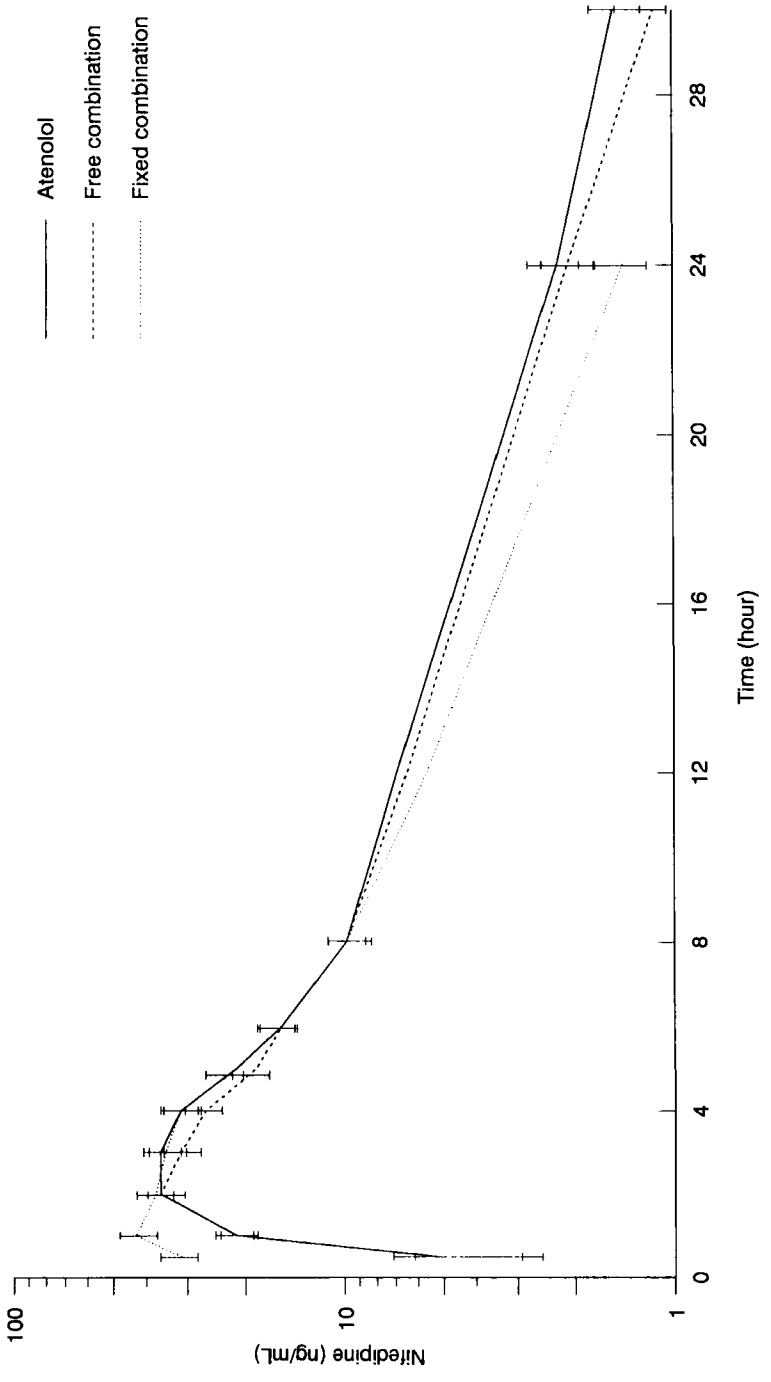

Figure 2. Mean plasma concentrations of nifedipine ± SE

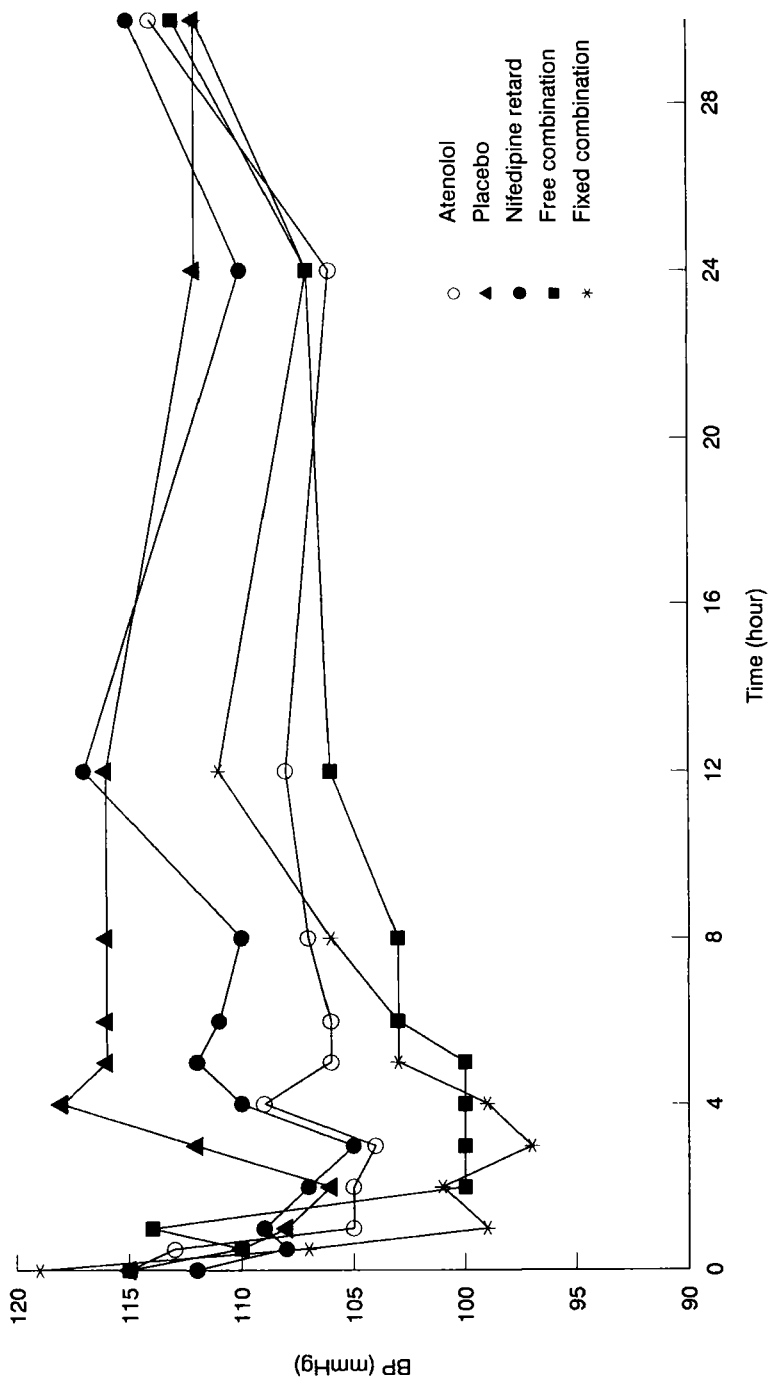

Figure 3. Mean standing systolic blood pressure

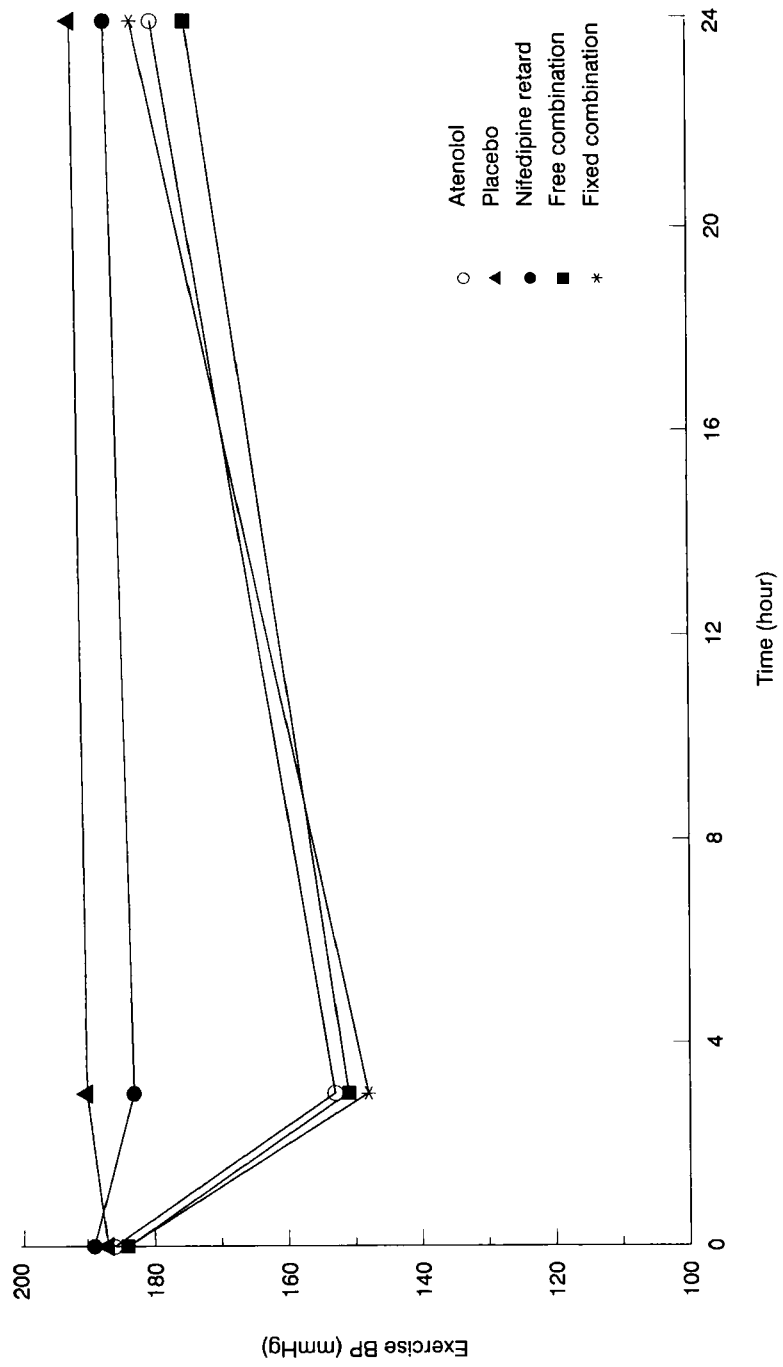

Figure 4. Mean exercise systolic blood pressure

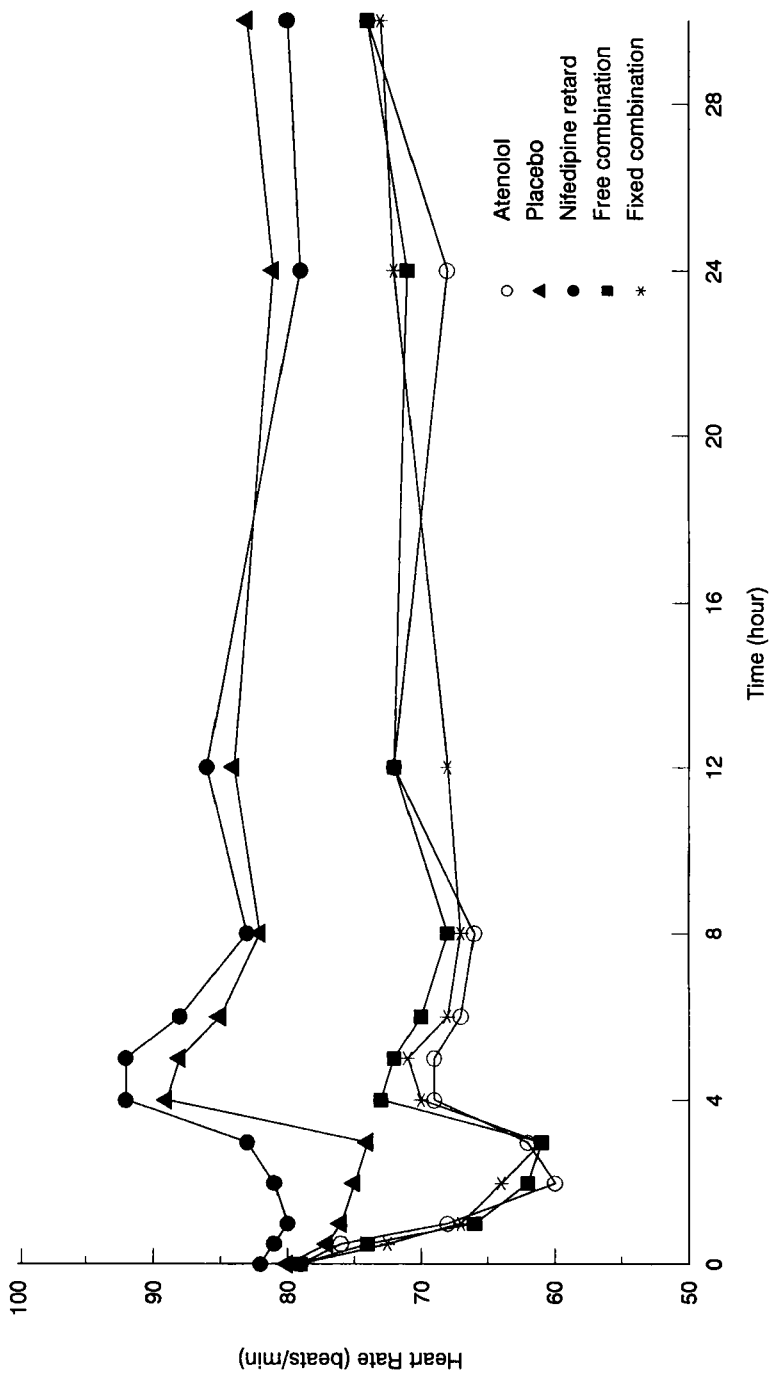

Figure 5. Mean standing heart rate

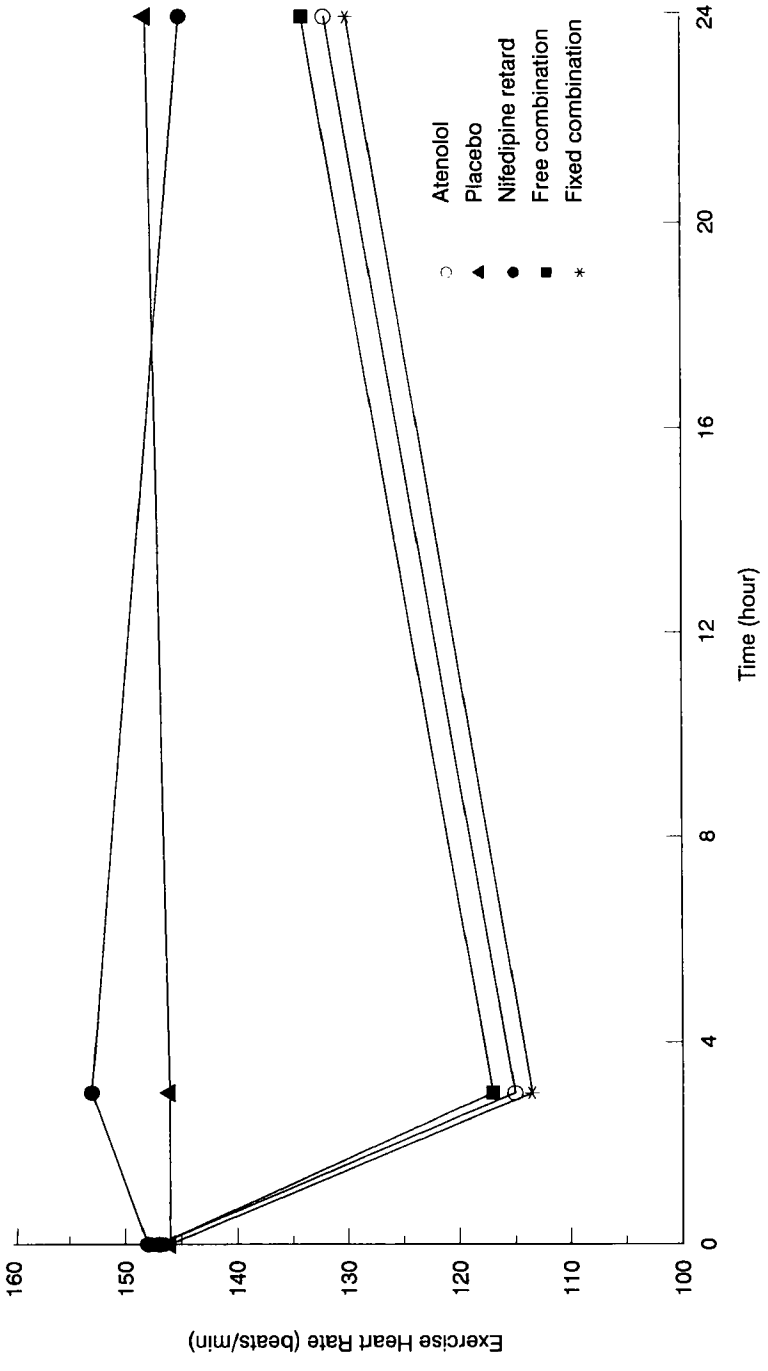

Figure 6. Mean exercise heart rate

DISCUSSION

The pharmacokinetic data generated in this study are in agreement with literature values for nifedipine retard tablets.[7] The present study has demonstrated that, both in terms of plasma pharmacokinetics of nifedipine and urinary recovery of the nifedipine metabolite, all three formulations of nifedipine examined were indistinguishable.

The pharmacokinetic data from the present study on atenolol is also in agreement with literature values.[8] The statistical evaluation of the present study has shown that, in terms of urinary recovery of unchanged drug and blood pharmacokinetics, the free combination is indistinguishable from the single entity. Thus it has been shown that no significant biochemical interaction occurs between nifedipine and atenolol. Comparison of the fixed combination with the single entity, however, did show statistically significant increases in maximum blood concentration and area under the blood concentration curve (about 16 per cent) but not in elimination half-life. Urinary recovery of atenolol from the fixed combination was also slightly higher but the difference was not statistically significant. These data suggest that the absorption of atenolol is slightly increased when the fixed combination is administered.

Heart rate, particularly under conditions of exercise, is a reproducible measure of the degree of $beta_1$-blockade. No significant differences in heart rate at rest or under exercise conditions were shown between any two treatments containing the $beta_1$-blocker atenolol. Nifedipine retard had little or no effect upon heart rate in this study. Thus it is concluded that the degree of $beta_1$-blockade achieved was similar for all treatments containing atenolol, and consequently the higher maximum observed blood concentrations and area under the curve of atenolol in the fixed combination are unlikely to be of clinical significance.

Combination therapy significantly reduced blood pressure to a greater extent than monotherapy with a maximum effect occurring 3–4 h post-dosing. This effect, however, was not sustained over 24 h in these subjects.

CONCLUSIONS

Co-administration of atenolol with nifedipine, both in free combination and in the fixed combination had no effect on nifedipine pharmacokinetics. Equally, co-administration of nifedipine with atenolol in a free combination had no effect on atenolol pharmacokinetics.

Administration of the two drugs in fixed combination caused a slight increase in atenolol absorption. However the pharmacodynamic measurements confirmed that this small difference was not clinically significant.

Thus it may be concluded from the study that the fixed combination of nifedipine and atenolol is bioequivalent to the free combination and that the

bioavailability of both drugs in the fixed combination is equivalent to that of each single entity.

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