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BIOAVAILABILITY IN MAN OF ATENOLOL AND CHLORTHALIDONE FROM A COMBINATION FORMULATION

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ABSTRACT

In this comparative bioavailability study in 12 healthy volunteers the blood level profiles and urinary recoveries of both atenolol and chlorthalidone were studied following the administration of the drug as a fixed combination ('Tenoretic'), as a free combination, and individually, at doses of 100 mg atenolol, and 25 mg chlorthalidone.

There were no statistically significant differences between the three formulations of atenolol in terms of individual blood levels, half-life, area-under-the-curve, and urinary excretion. The half-lives were between 5 and 6 h in agreement with other published data. Thus the bioavailability of atenolol from the fixed combination is equivalent to that from the free combination and from the atenolol tablet.

The chlorthalidone blood levels were slightly higher following the administration of the fixed combination when compared with the free combination or the chlorthalidone tablet. This observation was reflected in estimates of the area under the curves and the urinary recoveries. The half-lives of all three formulations were similar at about 60 h.

It is concluded that combining chlorthalidone and atenolol in a single tablet does not reduce the systemic bioavailability of either component.

KEY WORDS Atenolol Chlorthalidone Comparative bioavailability Pharmacokinetics Human 'Tenoretic'

INTRODUCTION

β-blockers and diuretics are frequently used together in the treatment of hypertension. Diuretics are usually administered once daily and the β-adrenoceptor blocking drug atenolol ('Tenormin') is effective in treating hypertension with 100 mg once/day administration.[1] Chlorthalidone is a widely used diuretic which has been administered together with atenolol clinically, for hypertension as a free combination without problem. The comparative pharmacokinetic profiles of both atenolol and chlorthalidone have been studied under

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these circumstances.[2] 'Tenoretic' is a fixed combination of these two drugs comprising 100 mg of atenolol and 25 mg of chlorthalidone.

The purpose of this study was to determine from whole blood and urine concentrations of atenolol and chlorthalidone in normal subjects, the bioavailability of 'Tenoretic' relative to its constituents when administered separately and together.

MATERIALS AND METHODS

Volunteer selection

The study was carried out under medical supervision in the Clinical Pharmacology Unit of Imperial Chemical Industries Limited, Pharmaceuticals Division, and was approved by the ethical committee of the Division.

Twelve healthy adult males were selected from those who responded to a request for volunteers. The volunteers were employees of the Division. Selection was based on the subject's willingness to participate, his availability for the study dates, and the absence of any significant abnormality on a pre-study examination which included a clinical history, complete physical examination, 12-lead electrocardiogram (ECG), and laboratory tests (haematology, urinanalysis, and blood chemistry).

Volunteers were excluded if the pre-study examination indicated a history or evidence of: asthma, hay fever, allergy, eczema, peptic ulcer, jaundice, or heart, respiratory, gastro intestinal, genitourinary, central nervous, locomotor, skin, or psychiatric disease. No subject was receiving or had recently received any form of continuous drug therapy. Each subject agreed not to take concomitant medication, ingest alcohol or take excessive fluids for the duration of the study.

The 12 male subjects ranged in age from 24 to 47 years (mean, 33 years) and in weight from 59 to 94 kg (mean, 73 kg).

Drug presentation and doses

The four dosage regimes for oral administration were supplied as follows:

Regimen A—'Tenoretic', white, film-coated sales tablet containing 100 mg atenolol and 25 mg chlorthalidone;

Regimen B—(i) 100 mg atenolol white, film-coated calcium phosphate clinical trials tablet, equivalent in bioavailability to the sales tablet of 'Tenormin';[9] (ii) 25 mg chlorthalidone white, uncoated tablet.

Regimen C—100 mg atenolol white, film-coated calcium phosphate clinical trials tablet equivalent in bioavailability to the sales tablet of 'Tenormin';[9]

Regimen D—25 mg chlorthalidone white, uncoated tablet.

Procedures

Each volunteer was given a complete explanation of the details of the study and gave his consent in writing prior to participation. The 12 subjects then received single doses of the 4 oral regimens in a randomized crossover manner,

with at least 2 weeks between doses for the chlorthalidone regimens. The subjects were aware of the regimen received each time. Each dose was administered with 100 ml water at 9.00 a.m. after a light breakfast (tea or coffee and toast). Normal meals were allowed during the 48 h evaluation period. The subjects were ambulatory in and around the Clinical Pharmacology Unit for the first 8 h and then allowed to go home. The 12 h blood samples were taken by the medical staff visiting the subjects in their homes. After 24 h the subjects resumed their normal working duties.

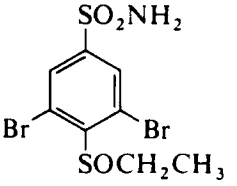
Blood samples for measurement of whole blood concentrations of atenolol and chlorthalidone were taken from an antecubital vein just before and at 1, 3, 5, 7, 12, 24, 48, 72, and 96 h after drug administration. The samples were collected in tubes containing a suitable anticoagulant. Approximately 20 ml was taken before dosing and aliquots were removed for drug analysis; the remaining aliquots were then pooled for generating calibration curves. The samples taken after dosing were approximately 4 ml. Samples were stored at $-20°$ prior to analysis.

Urine samples were collected before drug administration and the time periods 0–24 h, 24–48 h, and 48–72 h after drug administration. The total volume was measured and recorded. The urine was stored at $-20°$ prior to analysis.

Drug analysis

The analysis of atenolol in the blood and urine were performed by a modification of the gas-liquid chromatography method of Scales and Copsey.[3] The analyses of chlorthalidone were done by high pressure liquid chromatography and since this method is not published a brief outline follows.

One millilitre of oxalated whole blood or urine was mixed with 80 µl of a 0·1 mg ml^{-1} solution of the sulfoxide with the following structure which acts as an internal standard:



One millilitre of a pH5 citrate/phosphate buffer and 10 ml of diethyl ether were added to the biological sample. After shaking for 15 min and centrifuging to separate the phases, 9 ml of the diethyl ether was transferred to a tube containing 1 ml of 0·1 M sodium hydroxide. The mixture was shaken for 10 min and centrifuged to separate the phases. The diethyl ether was removed and 1 ml of 5·0 M potassium dihydrogen orthophosphate and 5 ml ethyl acetate were added to the sodium hydroxide layer. After shaking for 10 min and centrifuging for approximately 3 min, 4·8 ml of the ethyl acetate layer was blown to dryness under oxygen free nitrogen. The residue was redissolved in 400 µl of 50 per cent methanol in water and 20 µl of this solution was injected onto a 20 cm × 1/4" o.d.

column packed with 10 μ spherisorb ODS. A Pye Unicam LC20 separator constant volume pump and Cecil CE212 variable wavelength ultraviolet detector were used. The mobile phase comprised a 50/50 methanol water mixture and the flow rate was 1 ml min^{-1}. After the chromatographic separation the column was monitored at a wavelength of 204 nm.

In a preliminary experiment it was shown that atenolol did not interfere with the assay of chlorthalidone and vice versa.

The urine samples were analysed with slightly different chromatographic conditions to the above to better accomodate the variation in background contaminants. The same column was used but the packing material was 5 μ Hypersil ODS; the mobile phase was a 30/70 mixture of acetonitrile in water. The column eluent was monitored at a wavelength of 220 nm.

MATHEMATICAL CONSIDERATIONS

Determination of pharmacokinetic parameters

Elimination rate constants (β) were estimated by linear regression from the slope of the terminal linear segment of the curve of the logarithm of the blood concentration ($c(t)$) versus time (t). The half-life was calculated by the expression:

$$t_{\frac{1}{2}} = \frac{0{\cdot}693}{\beta}$$

and the area under the blood level curve to infinity (AUC (∞)) by the expression:

$$\text{AUC}(\infty) = [\text{AUC}_{(0-t)}]\ \text{trapezoidal} + \frac{c(t)}{\beta}$$

Statistics

Paired t-tests were carried out with standard methodology on the concentrations of drug in the blood at each time point and on the half-lives, areas under the blood level curve, and total urinary excretion. These parameters were also analysed by a confidence limit approach based on percentage difference.[10]

RESULTS

No untoward effects occurred during this investigation and each volunteer completed each section of the study.

Levels in blood

Atenolol. The mean concentrations of atenolol in the blood ($\pm$S.E.M.) are illustrated in Figure 1. Atenolol was not detected in the predose aliquots or in the samples taken after administration of regimen D (chlorthalidone only).

The mean peak concentrations of atenolol in the blood occurring 3 h after administration were $0{\cdot}5 \pm 0{\cdot}05$ (SE), $0{\cdot}58 \pm 0{\cdot}07$, and $0.49 \pm 0{\cdot}07$ μg ml^{-1} for the

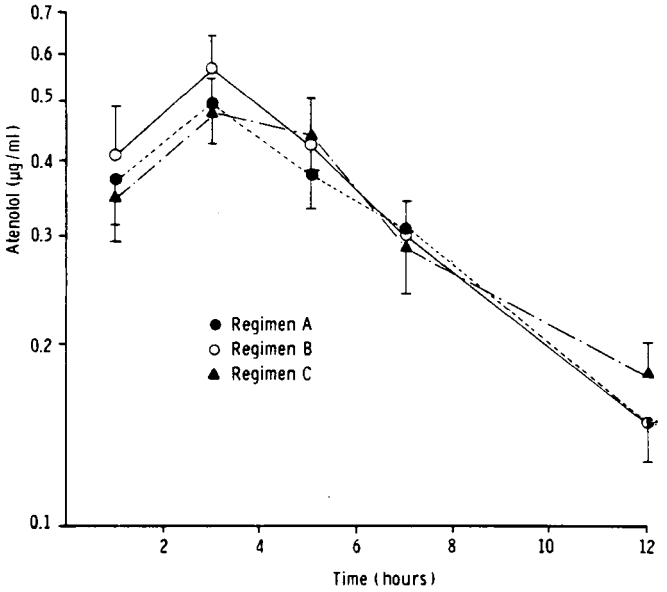


Figure 1. Mean blood levels of atenol ($\mu g\ ml^{-1}$) $\pm$SE in whole blood of volunteers.

fixed and free combinations and the atenolol dose respectively. The final elimination phase was associated with a mean half-life of 5–6 h for all regimens. The mean areas under the blood level curve to infinity for regimens A, B, and C were $4{\cdot}93 \pm 0{\cdot}58$ (S.E.), $5{\cdot}41 \pm 0{\cdot}77$, and $5{\cdot}03 \pm 0{\cdot}78$ $\mu g\ ml^{-1}\ h$ respectively. There were no statistically significant differences between the three atenolol regimens A, B, and C in terms of blood level, half-life, area under the curve or urinary excretion of atenolol (Table 4).

Chlorthalidone

The mean concentrations of chlorthalidone ($\pm$S.E.M.) are illustrated in Figure 2. Chlorthalidone was not detected in the predose aliquots or in the samples taken after the administration of regimen C (atenolol only).

The mean peak concentrations of chlorthalidone in the blood occurring 12 h after administration were $1{\cdot}80 \pm 0{\cdot}09$ (S.E.), $1{\cdot}30 \pm 0{\cdot}10$, and $1{\cdot}46 \pm 0{\cdot}07$ $\mu g\ ml^{-1}$ for the fixed and free combinations and the chlorthalidone tablet respectively. The mean half-life was $52{\cdot}4 \pm 3{\cdot}3$ (S.E.), $63{\cdot}6 \pm 3{\cdot}5$, and $62{\cdot}0 \pm 4{\cdot}8$ h for the fixed and free combinations and the chlorthalidone dose respectively. The mean areas under the blood level curve to infinity for these regimens were $161{\cdot}7 \pm 10{\cdot}5$, $132{\cdot}1 \pm 12{\cdot}2$, and $146{\cdot}4 \pm 14{\cdot}9$ $\mu g\ ml^{-1}\ h$ respectively.

The statistical analysis of the data (Table 4) shows that the chlorthalidone blood levels are higher after administration of 'Tenoretic' when compared with the chlorthalidone dose ($p < 0{\cdot}05$). There are no statistically significant differences between regimens B and D.

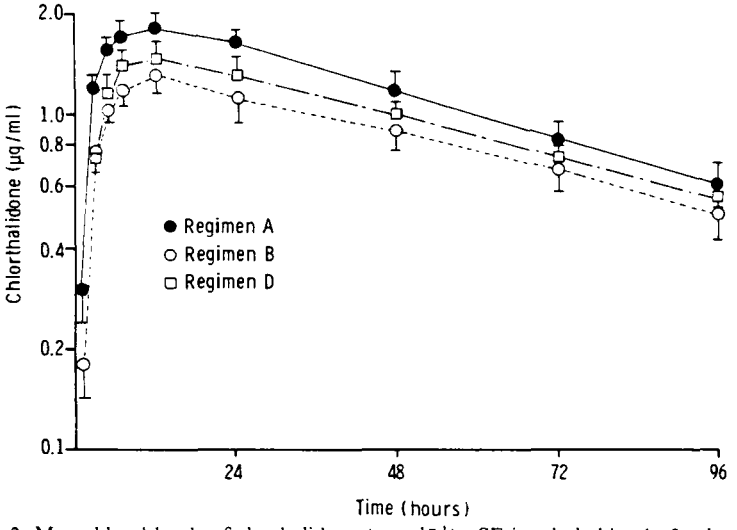


Figure 2. Mean blood levels of clorthalidone (µg ml^{-1}) ± SE in whole blood of volunteers.

Levels in urine

The mean volumes of urine collected following administration of regimen A, B, C, and D are shown in Table 1. The three regimens containing the diuretic chlorthalidone, i.e. A, B, and D all show higher urinary output over the first 24 h after dosing than the atenolol only formulation (C). However, only the comparisons of regimen A ('Tenoretic' versus C, and regimen B (chlorthalidone tablet)) show a statistically significant enchancement of urine output ($p = 0{\cdot}05$).

Atenolol recovery

The mean urinary recoveries of unchanged atenolol for the 72 h period after

Table 1. Mean volumes of urine excreted (ml)

Regimen	Parameter	Time period (h) 0–24	24–48	48–72
A	Mean	1634	1305	1278
	S.E.	142	157	161
B	Mean	1783	1187	1520
	S.E.	184	227	186
C	Mean	1290	1442	1295
	S.E.	115	228	152
D	Mean	1677	1205	1329
	S.E.	272	131	170

A = 'Tenoretic' white, film-coated tablet containing 100 mg atenolol and 25 mg chlorthalidone.
B = (i) 100 mg atenolol white, film-coated tablet. (ii) 25 mg chlorthalidone white, film-coated tablet.
C = 100 mg atenolol white, film-coated tablet.
D = 25 mg chlorthalidone white, film-coated tablet.

drug administration were 32·0 ± 4·9 (S.E.), 32·1 ± 3·6, and 34·1 ± 4·3 mg, i.e. 32, 32, and 34 per cent of the dose, for the fixed and free combinations and the atenolol tablet respectively (Table 2). There were no statistically significant differences between regimens in these results (Table 4) and the majority of the urinary elimination of atenolol occurred within 24 h of dosing.

Table 2. Mean urinary recovery of atenolol (mg)

Regimen	Parameter	Time period (h) 0–24	24–48	48–72	Total (mg)	% of dose
A	Mean	27·9	4·5	ND	32·0	32
	S.E.	4·5	0·9	ND	4·9	5
B	Mean	29·5	3.1	ND	32·1	32
	S.E.	3·2	0·7	ND	3·6	4
C	Mean	30·4	4·5	ND	34·1	34
	S.E.	3·9	0·8	ND	4·3	4
D	Mean	ND	ND	ND	ND	ND
	S.E.	ND	ND	ND	ND	ND

A = 'Tenoretic' white, film-coated tablet containing 100 mg atenolol and 25 mg chlorthalidone.
B = (i) 100 mg atenolol white, film-coated tablet. (ii) 25 mg chlorthalidone white, film-coated tablet.
C = 100 mg atenolol white, film-coated tablet.
D = 25 mg chlorthalidone white, film-coated tablet.
ND = Non-detectable, limit of detection about 0·1 μg ml^{-1}.

Table 3. Mean urinary recovery of chlorthalidone (mg)

Regimen	Parameter	Time period (h) 0–24	24–48	48–72	Total (mg)	% of dose
A	Mean	4·90	2·10	1·72	8·72	35
	S.E.	0·47	0·19	0·20	0·68	3
B	Mean	3·41	1·65	1·38	6·44	26
	S.E.	0·21	0·14	0·15	0·42	2
C	Mean	ND	ND	ND	ND	ND
	S.E.	ND	ND	ND	ND	ND
D	Mean	3·59	1·66	1·40	6·64	27
	S.E.	0·31	0·22	0·12	0·59	2

A = 'Tenoretic' white, film-coated tablet containing 100 mg atenolol and 25 mg chlorthalidone.
B = (i) 100 mg atenolol white, film-coated tablet. (ii) 25 mg chlorthalidone white, film-coated tablet.
C = 100 mg atenolol white, film-coated tablet.
D = 25 mg chlorthalidone white, film-coated tablet.
ND = Non-detectable, limit of detection about 0·1 μg ml^{-1}.

Table 4. Mean ± 95 per cent confidence limits for percentage difference in the blood and urine data

Drug and formulation	Time after dose (h)									$T_{1/2}(\beta)$ (hr)	AUC ($\propto$) ($\mu g\ ml^{-1}h$)	U (mg)
	1	3	5	7	12	24	48	72	96			
Atenolol												
[(B − A)/A] × 100	14 ± 62	15 ± 36	15 ± 29	−2 ± 25	4 ± 24	ID	ID	ID	ID	−10 ± 35	2 ± 29	1 ± 49
[(C − A)/A] × 100	−6 ± 42	−4 ± 32	17 ± 41	−5 ± 41	19 ± 36	ID	ID	ID	ID	2 ± 33	−3 ± 32	7 ± 38
[(C − B)/B] × 100	−18 ± 31	−16 ± 27	2 ± 36	−3 ± 45	14 ± 30	ID	ID	ID	ID	−7 ± 36	2 ± 17	6 ± 34
Chlorthalidone												
[B − A)/A] × 100	−38 ± 33	−37 ± 17*	−34 ± 8*	−31 ± 6*	−28 ± 9*	−31 ± 8*	−24 ± 11*	−19 ± 13*	−17 ± 13*	21 ± 19*	−18 ± 12*	−26 ± 22*
[D − A)/A] × 100	−42 ± 37*	−43 ± 21*	−31 ± 17*	−23 ± 20*	−25 ± 17*	−27 ± 14*	−21 ± 15*	−18 ± 17*	−14 ± 19	18 ± 20	−9 ± 17	−22 ± 24
[(D − B)/B] × 100	−6 ± 43	−9 ± 24	4 ± 24	11 ± 26	4 ± 21	5 ± 19	4 ± 20	1 ± 22	5 ± 21	−5 ± 19	7 ± 23	3 ± 24

* = Statistically significant difference ($p = 0.05$).
A = 'Tenoretic' white, film-coated tablet containing 100 mg atenolol and 25 mg chlorthalidone.
B = (i) 100 mg atenolol white, film-coated tablet. (ii) 25 mg chlorthalidone white, film-coated tablet.
C = 100 mg atenolol white, film-coated tablet.
D = 25 mg chlorthalidone white, film-coated tablet.
ID = Indeterminant.

Chlorthalidone recovery

The mean urinary recoveries of unchanged chlorthalidone for the 72 h period after drug administration were $8{\cdot}72 \pm 0{\cdot}68$ (S.E.), $6{\cdot}44 \pm 0.42$, and 6.64 ± 0.59 mg, i.e. 35, 26, and 27 per cent of the dose, for the fixed and free combinations and the chlorthalidone tablet respectively (Table 3). It was noted however that urinary elimination of chlorthalidone was still occurring between 48 and 72 h after dosing, and may continue beyond this time, thus increasing the reported urinary recoveries. As expected no urine levels of chlorthalidone were observed after dosing with regimen C (atenolol only). Only a comparison of regimen A ('Tenoretic') and B (chlorthalidone tablet and atenolol tablet) show a statistically significant difference ($p = 0{\cdot}05$) with the 'Tenoretic' tablet giving the higher recovery.

DISCUSSION

The results of this investigation show that there were no statistically significant differences between the peak blood levels, half-lives, areas-under-the-curve, and urine excretion of atenolol in the three regimes in which it was present. Individual peak values ranged from 0·16 to 0·96 μg ml^{-1}, a six-fold variation. The variation in peak systemic levels is less than that observed for a number of other β-blocking drugs.[4-8] and is of the same order as seen in other investigations involving atenolol. Further for half-life measurements, AUC and urinary elimination, results are in good agreement with previously published data.[9] Thus the bioavailability of atenolol from the fixed ('Tenoretic') combination is equivalent to that from the free combination and that from the tablet containing atenolol alone. It can be concluded therefore that co-administration of chlorthalidone does not affect the absorption and elimination of atenolol in volunteers.

The mean peak blood concentrations of chlorthalidone were 1·80, 1·30, and 1·46 μg ml^{-1} for the fixed ('Tenoretic') and free combinations and the chlorthalidone tablet respectively. The blood levels at other time points were slightly higher following administration of the fixed combination and this was reflected in the mean area under the curve being higher, i.e. 161·7 μg ml^{-1} h compared with 132·1 and 146·4 μg ml^{-1} h respectively. The mean half-lives were little different for the three chlorthalidone containing formulations being 60 h. The urinary excretion of chlorthalidone was higher following administration of the fixed combination compared with the other chlorthalidone containing regimens. Thus the bioavailability of chlorthalidone from the fixed ('Tenoretic') combination is slightly enhanced relative to that from the free combination or from the tablet containing chlorthalidone alone. This may be related to the observed slightly increased dissolution rate of the chlorthalidone fraction of the fixed combination. Thus co-administration with atenolol does not reduce the bioavailability of chlorthalidone in volunteers.

It is concluded therefore that by combining chlorthalidone and atenolol in a tablet does not reduce the systemic bioavailability of either component.

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