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Pharmacokinetics of Bendroflumethiazide After Low Oral Doses

Lars Borgström,[1,2] Carl-Gustav Johansson,[1] Hans Larsson,[1] and Rune Lenander[1]

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The pharmacokinetics of bendroflumethiazide after oral administration of 1.25, 2.5, and 5.0 mg were studied in nine healthy male volunteers. Bendroflumethiazide was analyzed by GLC after extractive alkylation. After the lowest dose, the plasma concentration could be followed to 14 hr, and the data were adequately fitted by a one-compartment model; the half-life was 3.1 hr. After the 2.5 and 5.0 mg doses, the plasma concentration was followed for 24 hr, and the data were fitted by a two-compartment model with half-lives of 8.9 hr. The urinary sodium concentration was doubled after bendroflumethiazide intake, but the urinary potassium concentration remained almost constant. The renal clearance of bendroflumethiazide was around 30 ml · min^{-1}.

KEY WORDS: bendroflumethiazide; pharmacokinetics; male volunteers; sodium and potassium excretion.

INTRODUCTION

Bendroflumethiazide is a potent diuretic that has been extensively used for over 10 years in the treatment of hypertension and edema, but little work has been published on the pharmacokinetics of this drug in man. ^{35}S-labeled bendroflumethiazide has been given intravenously and orally to humans. All the radioactivity was recovered in the urine within 24 hr, indicating complete absorption of the administered dose (1). In dogs, a terminal half-life of $2\frac{1}{2}$ hr after intravenous and 4 hr after oral administration has been reported by Piala *et al.* (2), using a spectrophotometric method for the determination of bendroflumethiazide. The urinary excretion pattern of bendroflumethiazide after oral administration has been studied by

[1]Department of Research and Development, Biochemical Laboratory, AB Ferrosan, Box 839, S-201 80 Malmö, Sweden.
[2]Author to whom reprint requests should be addressed.

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Ford and Nickell (3). They reported that sodium excretion is proportional to dose between 1.25 and 5.0 mg.

Recently, using a newly developed analytical method (4, 5), the pharmacokinetics of bendroflumethiazide in man has been reported after both single and repeated oral doses of 10 mg (6). A terminal half-life of 3 hr was observed after the oral dose. The present study is an investigation of pharmacokinetics of bendroflumethiazide after clinically relevant (1.25–5 mg) single and repeated oral doses.

MATERIAL AND METHODS

Subjects

Nine healthy male volunteers (mean ± SD: age 39.0 ± 8.7 years, weight 79.4 ± 9.2 kg, height 180.2 ± 7.7 cm), each of whom gave their written consent, were included in the study. They had no history of heart, liver, or kidney malfunction, or of alcoholism, drug dependence, blood dyscrasia or bleeding diathesis. A physical examination, including ECG, blood pressure, and spirometry, showed no evidence of clinically significant disease. No abnormalities were found in a laboratory screening by hematology, urine analysis, and blood biochemical analyses.

No subjects received any other medication, whether prescribed or self-administered, during two weeks preceding admission to, and during, the study. It was also agreed that alcohol consumption be kept to a minimum during the treatment period. The investigation was approved by the local ethical committee.

Preexperimental Studies

The amount of voided urine and excreted electrolytes were used as measures of the pharmacological response to bendroflumethiazide. Care was taken to ensure that fluid intake (including tea and coffee) was kept constant during each experimental day (200 ml/hr with additional intake of 330 ml at lunch and dinner). The intake of sodium was not strictly controlled; the average daily intake was considered approximately the same as similar food was served on all experimental days. This study on the pharmacokinetics and pharmacologic effects of bendroflumethiazide was part of a larger study, involving a combination with propranolol. Accordingly, to minimize the variable parameters in the overall study, the exercise routine, used to assess the degree of β-blockade achieved with propranolol, was also performed when the subjects received only bendroflumethiazide.

Studies

Bendroflumethiazide (Salures®, Ferrosan) or placebo tablets were ingested together with a standardized meal, consisting of 1 egg, 2 pieces of crisp bread, margarine (5 g), orange marmalade (20 g), cheese (20 g), orange juice (100 ml), low-fat milk (150 ml), and nonsweetened coffee or tea without cream or milk (100 ml). This meal gave 20 g protein, 17 g fat, 50 g carbohydrate, and a total energy of 1.840 kJ(= 440 kcal). The experiments were performed at weekly intervals.

Single Dose Studies

The experiments began in the morning after the subjects had fasted overnight and rested in the laboratory for about 20 min. The volunteers received a placebo or either a 1.25, 2.5, or 5 mg tablet of bendroflumethiazide. The exercise test was carried out 30 min before drug administration (pretreatment test) and was repeated at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 hours. The 24-hour test was omitted after giving the placebo. A light lunch was served 4 hr after drug ingestion and dinner 6-hr later. Urine was voided (and discarded) immediately after drug ingestion. Thereafter urine was collected every hour for 14 hr.

Multiple Dose Study

In the multiple dose study, bendroflumethiazide (2.5 mg) was given at 8.30 a.m. every day for 8 days. An exercise test was performed 30 min before drug ingestion. On the eighth day, the effect of the drug was followed for 24 hr according to the single dose protocol. No urine was collected in this study.

Parameters

Urine was collected every hour for 14 hr. The volume of each sample was measured, and 40 ml was kept for analysis of sodium, potassium, chloride, and of excreted bendroflumethiazide. Sodium and potassium were determined with a flame photometer and chloride by automatic amperometric titration. In the single dose studies, venous blood samples were taken before (0 hr) and at 20 and 40 min, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, and 24 hr after administration.

In the multiple dose study, blood samples were taken before the morning dose every day. On the eighth day, the single dose experiment protocol was followed. Plasma was separated by centrifugation, frozen, and stored at −20°C until assayed for its concentration of bendroflumethiazide, according to the method of Beerman *et al.* (4) and Fagerlund *et al.* (7).

Calculations

Pharmacokinetic parameters were calculated with the aid of a multiexponential curve stripping program, AUTOAN-NONLIN (8, 9). The program uses the squares of the deviations to evaluate the goodness of fit of the estimates. The input data were not weighted. Student's *t*-test was used for evaluating statistical significance.

RESULTS AND DISCUSSION

Single Dose Study

The individual experimental plasma concentration values were entered directly into the AUTOAN-NONLIN computer program. The means of the pharmacokinetic parameters are given in Table I, together with estimates of their standard deviations. The figures in parentheses indicate the number of values used to estimate each mean. In 4 out of 27 data sets, the computer program did not carry out any pharmacokinetic analysis. When the function chosen by AUTOAN gave negative values for k_{12}, k_{21}, or k_{10}, no analysis was performed by NONLIN, and in some cases, AUTOAN could not fit any *oral* model to the experimental values. In one case, an estimate of the total AUC value was obtained, but AUTOAN performed no further analysis on this data set. This AUC value, however, was included in calculating the mean following the 5.0 mg dose. The absorption rate constant was used for calculating the mean only when a model with one first order input was given. No significant difference in absorption rate after the different doses was found when tested by Student's *t*-test. The AUC values were estimated by integrating the equation used by the AUTOAN-NONLIN program to fit the data; the AUC values up to 14 hr were calculated manually by the trapezoidal rule. The lines of best fit and the arithmetic mean values of the plasma concentrations are shown in Fig. 1. The vertical bars on the plasma concentrations after the 2.5 mg dose refer to the standard error of the mean. Similar relative SEM values were obtained after the 1.25 and 5.0 mg dose and multiple dose studies.

The mean AUC value after the 5 mg dose is higher than would be expected from the correspondinmg values after the 1.25 and 2.5 mg doses, and the standard deviation is large. This is mainly due to two high values (1643 and 1479 nmol · hr · liter^{-1}). When these two high values are excluded, the mean AUC value is 527.0 ± 260.0 (mean $\pm$ SD, $n = 6$). Figure 2 is a plot of the logarithm of the AUC against the log dose. The value at 10 mg, taken from the literature (5), is given for comparison and was not used in any analysis. A linearity test was performed by log transformation of the data prior to regression analysis and testing whether the

Table I. Pharmacokinetic Parameters After Single Oral Doses of 1.25, 2.5, and 5.0 mg bendroflumethiazide and After 2.5 mg Given Once a Day for Eight Days.[a]

Dose (mg)	Experimental peak plasma concentration (nmol · liter^{-1})	k_a (hr^{-1})	V/F (liters)	AUC(14)[b] (nmol·hr·liter^{-1})	AUC(24)[c] (nmol·hr·liter^{-1})	AUC[d] (nmol·hr·liter^{-1})	$t_{1/2}$ (hr)	Best "mean" compartment model[e]
1.25	22.0±4.7 ($n=9$)	0.79±0.67 ($n=7$)	85±33 ($n=8$)	144±43 ($n=9$)		144±50 ($n=8$)	3.1	One
2.5	38.0±8.6 ($n=9$)	1.09±0.42 ($n=7$)	123±70 ($n=8$)	221±54 ($n=9$)		269±127 ($n=7$)	8.9	Two
5.0	79.9±12.5 ($n=9$)	0.74±0.28 ($n=4$)	80±13 ($n=5$)	483±82 ($n=9$)		786±529 ($n=8$)	8.9	Two
2.5 daily through 8th day	56.6±14.7 ($n=9$)	1.18±0.52 ($n=4$)	84±40 ($n=8$)		232±56 ($n=8$)		3.6	One

[a] The values are means ± SD, except for the $t_{1/2}$ values, which are the $t_{1/2}$ of the means. For discussion, see text.
[b] AUC between 0 and 14 hr after administration of single dose.
[c] Estimated AUC within the eighth day after repeated dosing.
[d] Estimated total AUC after single dose.
[e] Minimally acceptable model as given by AUTOAN.

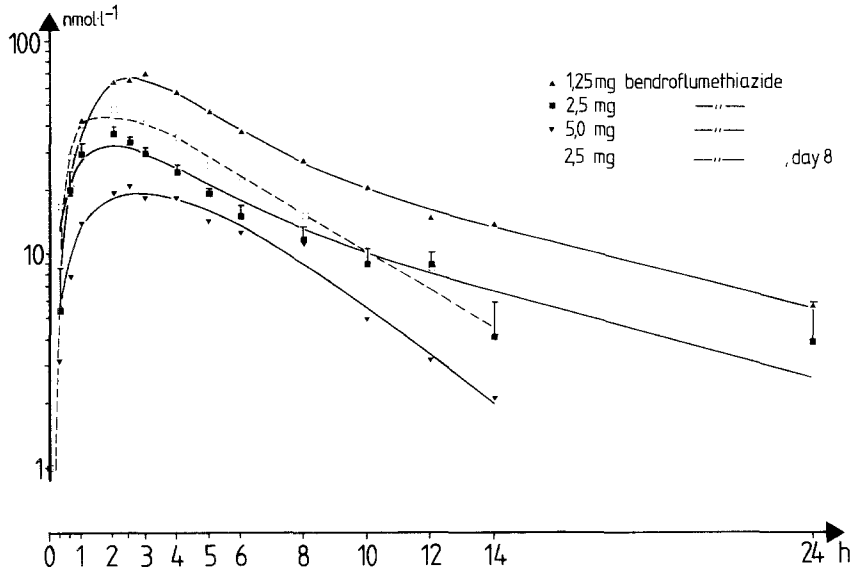

Fig. 1. Plasma concentration curves of bendroflumethiazide after oral doses. The curves were generated by the AUTOAN-NONLIN program (9, 10). The bars on the 2.5 mg values are the SEM. The SEM for the other doses were of the same relative order of magnitude.

slope was significantly different from unity. A failure to find a slope significantly different from unity indicated direct proportionality between AUC and dose. These observations indicate that availability and clearance are independent of dose, in the range studied. When the experimental peak plasma concentrations were compared, no significant difference could be found between the dose normalized values.

The estimated value of the terminal rate constant is markedly influenced by which compartment model the AUTOAN-NONLIN program selects

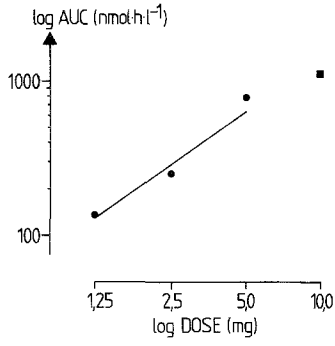

Fig. 2. Plot of the logarithm of the total AUC against the logarithm of the dose, after single oral doses of bendroflumethiazide. The 10 mg value is from ref. (5), and was not included in the analysis.

as the best fit to the data. In the case of the 1.25 mg dose study, a one-compartment model adequately fitted the data for all nine subjects; the resulting mean of the individual $t_{1/2}$-values was 3.2 ± 1.8 (SD). In the case of the 2.5 and 5.0 mg dose studies, some data sets were adequately fitted by a one-compartment model, while for others, a two-compartment model was required. Instead of presenting the individual terminal $t_{1/2}$-values, we present only those that were obtained upon fitting the arithmetic mean of the experimental points.

Considering the mean data, the results following the 1.25 mg dose were adequately fitted by a one-compartment model with a terminal half-life of 3.1 hr. After the 2.5 and 5.0 mg doses, a two-compartment model was required to fit both data sets; the terminal half-life was 8.9 hr. The difference in half-life is due to insufficient analytical sensitivity of the method (detection limit 3 nmol · Liter^{-1}). The method obviously could not pick up the second exponential phase after the 1.25 mg dose, and the half-life of 3.1 hr probably reflects both continued distribution and elimination.

Beerman *et al.* (5, 6) were unable to detect any plasma concentration of bendroflumethiazide 24 hr after oral administration of 2.5, 5.0, or 10.0 mg. They concluded that the distribution and elimination of this drug follows one-compartment kinetics, and they reported a half-life of 3.0 hr. Their detection limit was 24 nmol · liter^{-1} (5). The second terminal half-life of 9 hr, reported here, is longer than reported earlier, but is in good accord with the satisfactory clinical results obtained from once daily administration of bendroflumethiazide.

A relevant volume of distribution can only be calculated from concomitant i.v. and p.o. experiments (10). However, we can calculate the volume of distribution divided by the availability (V/F). The following equations were used:

$$\frac{V}{F} = \frac{\text{dose}}{k \cdot \text{AUC}} \qquad \text{(one-compartment model)}$$

$$\frac{V}{F} = \frac{\text{dose}}{\lambda_2 \cdot \text{AUC}} \qquad \text{(two-compartment model)}$$

where k, λ_2 are the exponential coefficients associated with the one- and two-compartment models, respectively.

After 1.25 mg, where all the individual sets of values were most adequately fitted to a one-compartment model, V/F was 85 ± 33 liters. After 2.5 mg and 5.0 mg, some of the sets of values were best fitted to a one-compartment model and some to a two-compartment model. V/F for the central compartment after 2.5 mg bendroflumethiazide was 123 ± 70 liters ($n = 8$), and after the 5.0 mg dose, it was 80 ± 13 liters ($n = 5$). The

Table II. Renal Clearance and 14-hr Urinary Excretion of Bendroflumethiazide After Single Oral Dose.[a]

Dose (mg)	14-hr recovery (nmol)	Renal clearance (0–14 hr) (ml · min^{-1})
1.25	213 ± 107	27.1 ± 16.7
2.5	409 ± 130	32.6 ± 12.5
5.0	931 ± 402	32.7 ± 16.4

[a]The values are means ± SD from nine volunteers.

mean of the two-compartment values after 2.5 and 5.0 mg was 466 ± 355 liters ($n = 4$). This indicates the existence of a peripheral compartment with high affinity for bendroflumethiazide and a smaller central compartment. As was discussed above, the elimination phase in the one-compartment model could reflect both continued distribution and elimination. The value of V/F for the central compartment thus possibly is an overestimation of the true value. The value of V/F for the central compartment when a two-compartment model was given was 21.8 ± 5.4 liter ($n = 4$), a value much lower than the above-mentioned ones.

The excretion of bendroflumethiazide during the first 14 hr is given in Table II. The recovery of around 7% of the dose is lower than previously reported (5). We are unable to explain this discrepancy. The renal clearance, calculated by dividing the amount of drug recovered in the urine during the first 14 hr by the AUC (14), was independent of dose; the mean value was 30 ml/min.

The increase in urine volume and electrolyte excretion during the first 14 hr after drug administration is given in Fig. 3. It can be seen that the excretion patterns after different doses are essentially the same. All the values were significantly ($p < 0.001$) different from the placebo administration. The mean concentrations of sodium and potassium in the urine over the 14 hr collection interval are given in Table III. The potassium concentra-

Table III. Mean Urinary Concentrations of Sodium and Potassium During the 14 hr Collecting Interval After Administration of Different Doses of Bendroflumethiazide.[a]

Dose (mg)	Na^+ (mmol) · liter^{-1})		K^+ (mmol · liter^{-1})
Placebo	0.10 ± 0.04	←	0.07 ± 0.03
1.25	0.18 ± 0.03,	$p < 0.001$	0.07 ± 0.03
2.5	0.16 ± 0.04,	$p < 0.01$	0.07 ± 0.03
5.0	0.14 ± 0.04,	$p < 0.05$	0.06 ± 0.03

[a]The values are means ± SD from nine volunteers.

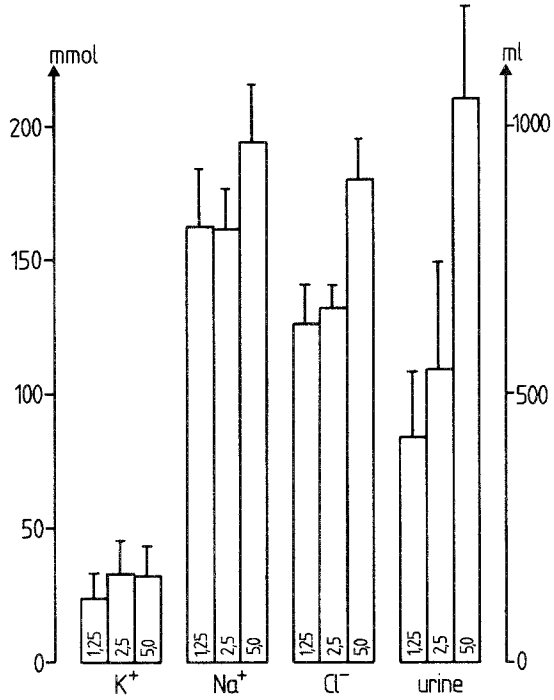

Fig. 3. The *increase* in urine volume and electrolyte excretion for the first 14 hr after administration of different doses of bendroflumethiazide is shown. Values are means ± SEM. All values are highly significantly different from the placebo administration.

tion does not increase after bendroflumethiazide administration, but the sodium concentration increase significantly after all three doses. Thus bendroflumethiazide seems primarily to block sodium reabsorption in the tubules. The concomitant increase in potassium excretion is explained by the increase in urine volume. The changes in the excreted electrolytes after the 2.5 mg dose are shown in Fig. 4. It is noted that the potassium excretion is low and relatively constant with time compared to sodium and chloride excretion. Of interest is the observation that bendroflumethiazide has some pharmacological effect even 14 hr after administration. Similar profiles were observed after the 1.25 and 5.0 mg doses.

Repeated Dose Study

This study was designed to mimic the common clinical situation where bendroflumethiazide is administered once a day. The experimental values

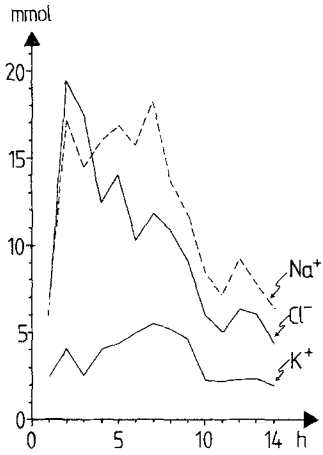

Fig. 4. Rate of excretion of some electrolytes after oral administration of 2.5 mg bendroflumethiazide. The urine was collected hourly.

on the eighth day of the repeated dose study were treated in the same way as the single dose values. As with the 2.5 mg single dose study, the repeated dose data was adequately fitted by a one-compartment model.

None of the parameters were significantly different from the values after a single dose of 2.5 mg (Table I) using *t*-test statistics. The experimental peak plasma concentration was higher, although not significantly, on the eighth day. This could be due to a slight accumulation of bendroflumethiazide in the body. The equivalence of the AUC(24) value, in the repeated administration study, with the total AUC value from the single 2.5 mg dose experiment, indicates that steady-state is reached at least within eight days of dosing, and that the ratio of clearance to availability is unchanged during this period. It is also likely that neither of these parameters changed.

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