Journal of Pharmaceutical & Biomedical Analysis
Vol. 1, No. 3, pp. 311–320, 1983
Printed in Great Britain

0731–7085/83 $3.00 + 0.00

The plasma levels of chlormethiazole and two of its metabolites in elderly subjects after single and multiple dosing

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Abstract: Plasma levels of chlormethiazole and two of its metabolites have been estimated in elderly subjects both after single and repeated oral dosage and on withdrawal. A sensitive analytical method was developed to permit the quantitation of chlormethiazole and its two major metabolites in plasma. The two major metabolites were positively identified against authentic standards as 5-acetyl-4-methylthiazole and 5-(1-hydroxyethyl)-4-methylthiazole. Chlormethiazole and its metabolites were shown to have accumulated to varying extents after seven nightly doses. On withdrawal of the drug, the plasma was free of chlormethiazole within 44 h and free of all traces of its metabolites within 84 h. The accumulation was not accompanied by any undue clinical effect and was not in itself considered to be detrimental to the normal use of chlormethiazole in the treatment of sleep disturbances in the elderly.

Keywords: *Chlormethiazole; elderly subjects; metabolites; therapeutic drug monitoring; gas–liquid chromatography.*

Introduction

The ideal hypnotic should have an intermittent activity restricted to the duration of the night and devoid of any effect the following day. This action may be complicated by the conversion of the parent compound into one or more metabolites, which may also show some degree of activity and whose kinetic profile may differ from that of the parent compound. It is therefore desirable that there should be no accumulation of either the parent drug or its metabolites, especially since hypnotic medication may be continued over a long period of time and disturbed sleep patterns in the elderly are often long-lasting.

Chlormethiazole (CTZ) has been shown to be well tolerated by the elderly and lacking in cumulative side effects and 'hangover' effects, which can be a major disadvantage of hypnotics which are more slowly eliminated [1–4]. A number of papers have been published on the pharmacokinetics of chlormethiazole or on the identification of its

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metabolites in urine ([5] and references therein). In earlier studies chlormethiazole in plasma was quantified by gas chromatography (GC) using a flame ionization detector (FID). However, this system has been found to be insufficiently sensitive to detect chlormethiazole in plasma after about 2 h. Using more sensitive detection systems the single dose pharmacokinetics of chlormethiazole have been documented in the young and the elderly [6–9]. However, only two methods — the GC–mass spectrometric method of Nation *et al.* [10] and a more recent nitrogen-specific detection system from the same group [11] — allow the simultaneous estimation of the acetyl and hydroxyethyl metabolites of chlormethiazole. Of these two papers, only the first reported the presence of metabolites in the aged, based on only three subjects studied after a single dose of CTZ.

In view of the lack of published information on this matter, the present work was carried out to establish the disposition of chlormethiazole and its metabolites during prolonged therapy in the elderly.

Experimental

Subjects and sampling

As indicated in Table 1, 7 male and 4 female elderly hospital in-patients, aged 67–92 years (mean age 76 years), were each given two capsules of chlormethiazole (384 mg base; 600 mg edisylate salt) as a single oral dose at approximately 9 p.m. on seven consecutive nights. Each subject had given informed consent to take part in the pharmacokinetics section of a clinical trial of chlormethiazole, for which ethical permission had been granted.

The methodology and results of the clinical section of this trial have been described in an earlier report [12]. At the first and seventh doses, blood samples (5–10 ml) were taken at 0, 0.5, 1.0, 1.5, 2, 12, 13, 14 and 16 h post-dose, before and after, but not during, the drug-induced sleep. It was clear in designing the clinical trial, that the enforced absence of blood sampling during the sleep period (2–12 h post-dose) would preclude a detailed pharmacokinetic analysis of the disposition of the metabolites. It was therefore intended to study only the extent of the accumulation of metabolites during prolonged therapy and the rapidity of their elimination on withdrawal. This was important, since chlormethiazole is claimed to have a short half-life, with no reference made to its metabolites. For this reason, in a further study chlormethiazole was replaced by Temazepam (10 mg) as the sedative in 3 male patients who had been on nightly chlormethiazole for at least two weeks previously. Blood was taken 12 and 20 h after the final dose and at 9 a.m. and 5 p.m. for the subsequent three days to follow the elimination of chlormethiazole and its metabolites.

Differences between groups were tested by Student's *t*-test. All kinetic data was calculated by CSTRIP [13]. The Area Under the Curve (AUC) data were computed by the trapezoidal rule. Data are given as the mean ± standard error (SE). The individual concentrations of CTZ and its metabolites in plasma are available on request from the authors.

Reagents and materials

Chlormethiazole (5-(2 chloroethyl)-4-methylthiazole) was generously donated by Astra (U.K.) Pharmaceuticals Ltd., St. Albans, Herts, UK. 5-Acetyl-4-methylthiazole (AMT) and 5-(1-hydroxyethyl)-4-methylthiazole (HEMT) were kindly donated by Dr R.

Nation, Department of Pharmacy, University of Sydney, Australia. The molecular formulae are illustrated in Fig. 1. Quinaldine (2-methylquinoline) and all other chemicals were obtained from British Drug Houses Ltd, Poole, Dorset, UK. All reagents and solvents were of analytical reagent grade, except for the diethyl ether which was of anaesthetic grade.

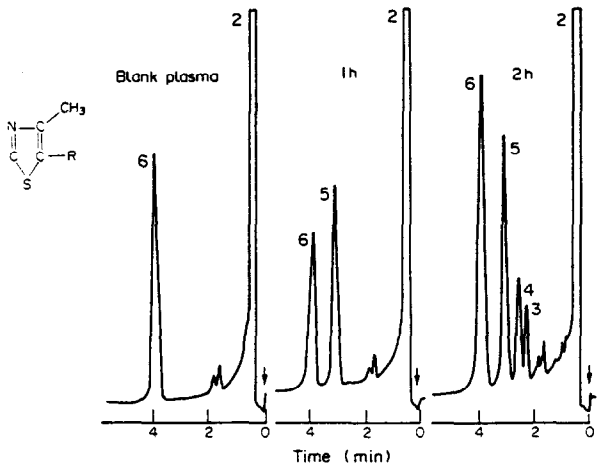

Figure 1
The structure and chromatographic separation of chlormethiazole and metabolites. Typical chromatograms of blank plasma and of plasma taken 1 h and 2 h after administration of chlormethiazole (attenuation 128). 2, Solvent peak. 3, 5-Acetyl-4-methylthiazole (R = $COCH_3$). 4, 5-(1-Hydroxyethyl)-4-methylthiazole (R = $CH(OH)CH_3$). 5, Chlormethiazole (R = CH_2CH_2Cl). 6, Internal Standard.

Sample preparation

To a sample of plasma or serum (0.2–1.0 ml) was added 1.0 ml of the aqueous internal standard solution (quinaldine, 0.1 or 1.0 μg, dependent on the sample, in 0.1 M hydrochloric acid) in a 15 ml glass-stoppered centrifuge tube. To this was added 0.5 ml of 5M sodium hydroxide and 5 ml diethyl ether. The contents of the tube were mixed on a rotary shaker for 5 min and centrifuged at 2000 rpm for 5 min in order to separate the phases. The aqueous layer was removed with a Pasteur pipette and discarded. A 2 ml portion of 1M hydrochloric acid was added and the contents of the tube were mixed and centrifuged as before. The ether layer was removed and discarded. A 1 ml portion of 5M sodium hydroxide and a 5 ml portion of diethyl ether were added and the tube was shaken and centrifuged as before. The aqueous layer was carefully removed and discarded. The remaining ether layer was concentrated to approximately 100 μl on a hot plate under nitrogen and stored below 5°C until analysed. A 5 μl portion was injected onto the gas chromatograph and samples were analysed in duplicate.

Chromatography

A silanized glass column (1 m × 3 mm i.d.) was filled with 8% OV 17 on GasChrom Q (80–100 mesh) and preconditioned at 200°C overnight. A Perkin-Elmer F33 gas chromatograph with a nitrogen-specific detector was used. The temperatures of the injector, detector and oven were set at 250, 250 and 170°C respectively. The nitrogen carrier gas flow rate was 30 ml/min.

The detector was adjusted according to the manufacturer's instructions. Under these conditions 5-acetyl-4-methylthiazole (AMT), 5-(1-hydroxyethyl)-4-methylthiazole (HEMT), chlormethiazole (CTZ) and quinaldine eluted at approximately 3, 4, 5 and 7 min respectively (Fig. 1). The relative detector responses (peak height) for CTZ, AMT and HEMT were 1, 1.58 and 0.93 respectively. The response was linear up to 10 μg/ml for each compound. Replicate analyses ($n = 6$) indicated that the reproducibility of the method was good (relative standard deviation, RSD, less than 8%). The sensitivity for accurate detection was less than 10 ng/ml for each compound and could be further reduced if a sample size greater than 1 ml plasma was used.

It was found not to be necessary to alter the sensitivity of the detector for the later, low concentration samples. However, as the attenuation was reduced the amount of the internal standard was also reduced ten-fold, from 1 μg to 100 ng, to obtain a comparable peak height.

The slope, intercept and correlation coefficient for CTZ, AMT and HEMT were: 0.0117, 0.04, 0.97; 0.0185, 0.02, 0.99; and 0.0109, 0.01, 0.96, respectively. However the absolute values of the slope varied slightly from day to day due to small changes in detector sensitivity.

Results

Details of subject age, weight and sex are given in Table 1. The females were significantly older ($p < 0.01$) but not heavier than the males. No other differences between male and female subjects were statistically significant for any parameter measured or calculated in this study, either for the parent drug or for its metabolites ($p > 0.1$). For this reason, the statistical analyses are presented as combined data from subjects of both sexes, although means (±SE) are given for both sexes independently.

Plasma levels of chlormethiazole (CTZ), 5-acetyl-4-methylthiazole (AMT) and 5-(1-hydroxyethyl)-4-methylthiazole (HEMT) were estimated up to 16 h after the first and seventh nightly doses of chlormethiazole. The profiles of mean plasma concentration (±SE) *versus* time for all subjects on both days are shown in Fig. 2. The large standard errors indicate the extent of the inter-individual variation.

After the seventh dose of chlormethiazole, the mean plasma levels of chlormethiazole itself were just significantly higher at 2, 12, 13, 14 and 16 h ($p < 0.05$ in each case). Mean plasma levels of AMT were significantly higher at 0, 13, 14 and 16 h ($p < 0.01$ in each case), whereas mean plasma levels of HEMT were significantly elevated at all times ($p < 0.01$ except at 14 h, $p < 0.05$).

Table 2 gives the half lives of absorption and the data for alpha and beta elimination for CTZ, calculated from the original data by the CSTRIP programme. These parameters did not alter significantly in either males or females over the seven nightly doses ($p > 0.3$ in each case). As discussed below, these data could not be calculated for either of the metabolites.

Table 3 gives the mean values (±SE) for the observed maximum plasma levels, the time of their occurrence, areas under the curve and residual (12 h post-dose) levels for CTZ, AMT and HEMT for grouped data. Statistical differences for CTZ, AMT and HEMT *between* days 1 and 7 and also between CTZ, AMT and HEMT *on* days 1 and 7 are also given.

It was not ethically justifiable to take blood samples while the subjects were sleeping (2–12 h post dose). Moreover, this would have interfered with sleep-study measure-

Table 1
Details of subjects involved in chlormethiazole study

Subject	Age (years)	Weight (kg)
Males		
GM	68	55
JH	73	70
RL	67	43
SW	74	72
MG	78	55
ED	67	69
RM	80	67
Mean	72	62
±SE	2	4
Females		
CB	86	59
EL	77	56
ES	82	73
MQ	87	40
Mean	83*	57
±	2	7
Combined data		
Mean	76	60
±SE	2	3

* Significantly older ($p = 0.004$) than male subjects.

Table 2
Derived parameters for chlormethiazole on days 1 and 7 of treatment

	Calculated half life (h)					
	Absorption		Alpha-elimination		Beta-elimination	
Day	1	7	1	7	1	7
Males						
Mean	0.18	0.30	0.60	0.41	4.8	3.7
±SE	0.04	0.14	0.13	0.03	0.8	0.6
Females						
Mean	0.10	0.19	0.24	0.73	3.1	4.5
±SE	0.0	0.05	0.01	0.27	0.5	0.6
Combined data						
Mean	0.16	0.25	0.49	0.57	4.2	4.1
±SE	0.03	0.08	0.11	0.14	0.6	0.4

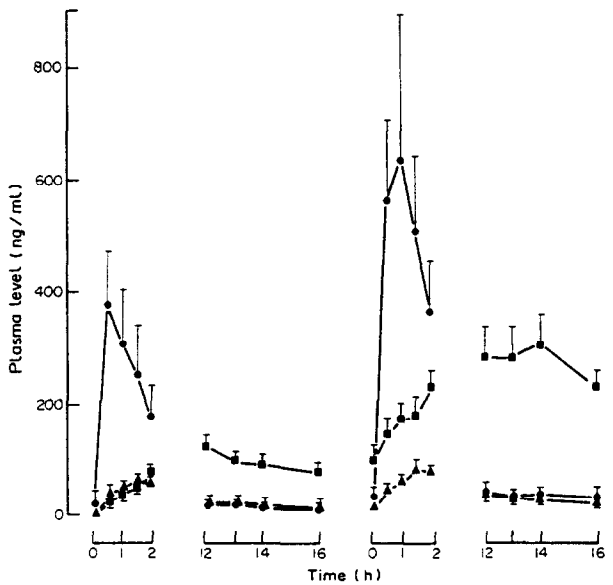

Figure 2
Plasma levels of chlormethiazole and its metabolites on days one and seven of treatment. Chlormethiazole (●), 5-acetyl-4-methylthiazole (▲) and 5(1-hydroxyethyl) 4-methylthiazole (■). Differences between days one and seven for combined data for both sexes (±SE).

ments. For these reasons the clinical trial had to be designed such that there was no plasma level data during the sleep period. Moreover, in many cases the plasma levels of the metabolites before or after the sleep period were not approaching zero. Hence it was not possible to calculate meaningful individual kinetic parameters on the absorption, distribution and elimination of either AMT or HEMT. In the absence of these data, it was not possible to investigate more fully the rates of formation of these metabolites or to present areas under the curve beyond the 0–16 h time period. It is, therefore, only possible to comment on the observed peak concentrations of AMT or HEMT, the times at which they occurred, the computed areas under the curve and the 12 h post-dose 'residual' levels.

Chlormethiazole absorption was seen to be rapid. In all cases the observed peak plasma levels occurred within 1 h after the dose and were just significantly elevated over the seven days ($p < 0.05$).

In the majority of cases peak AMT levels occurred within 2 h post-dose and were not significantly increased over the seven days. Peak HEMT levels occurred between 2 and 12 h post-dose. The increase in peak HEMT levels over the seven days was significant ($p < 0.005$).

Areas under the curve (AUC) for 0–2 h and 12–16 h were calculated from the raw data. AUC for 0–16 h was calculated by the trapezoidal rule. By this measurement accumulation of chlormethiazole was found to be just significant over 12–16 h ($p < 0.05$). AMT was shown to have accumulated significantly at 12–16 h ($p < 0.002$) and 0–16 h ($p < 0.001$). HEMT was shown to have accumulated significantly 0–2 h ($p < 0.001$), 12–16 h ($p < 0.001$) and 0–16 h ($p < 0.002$) after the seventh dose.

The average amount of chlormethiazole remaining in the plasma 12 h after the dose

Table 3
Observed and derived parameters for CTZ, AMT and HEMT on days 1 and 7 of treatment

	Maximum observed plasma level Concentration		Time		Area under the curve ((ng/ml).h)						Residual levels 12 h post-dose			
	(ng/ml)		(h)		0–2 h		12–16 h		0–16 h		(ng/ml)		(% peak)	
Day	1	7	1	7	1	7	1	7	1	7	1	7	1	7
Chlormethiazole														
Mean	433	812	0.65	0.94	520	917	105	471	1573	2891	20	46	5	9
±SE	108	190	0.11	0.20	132	232	22	264	447	676	4	12	1	3
p	a		ns		ns		ns		a		a		ns	
5-Acetyl-4-methylthiazole														
Mean	78	91	1.5	3.7	91	112	72	141	613	888	23	42	37	61
±SE	10	15	0.2	1.3	15	24	11	19	61	98	3	9	7	12
p	ns		ns		ns		c		c		a		a	
5-(1-Hydroxyethyl)-4-methylthiazole														
Mean	147	289	11.5	10.6	71	345	432	1117	1469	3418	125	277	84	88
±SE	17	48	1.1	1.9	11	55	67	150	179	644	22	35	9	5
p	b		ns		c		c		b		c		ns	
Statistical differences on days 1 and 7														
CTZ versus AMT	b	c	c	a	c	c	ns	ns	a	b	ns	ns	c	c
CTZ versus HEMT	b	b	c	c	c	b	c	a	ns	ns	c	c	c	c
AMT versus HEMT	c	c	c	c	ns	c	c	c	c	c	c	c	c	a

p values: a = < 0.02; b = < 0.01; c = < 0.001, ns = not significant. Combined data for both sexes.

doubled over the seven days. This increase was only just significant, owing to the large interindividual variation. The 12 h plasma level of AMT and HEMT had risen significantly over the week ($p < 0.02$; $p < 0.001$ respectively).

As has already been pointed out, in the main study the 16-h time period did not allow an accurate estimation of the elimination of the two metabolites in individual subjects, nor was it thought justifiable to compute figures based on pooled data. Neither metabolite was available as a pure substance in sufficient quantity for administration to humans, nor indeed would this have been ethical. Therefore the elimination of chlormethiazole and its metabolites was followed for 92 h after cessation of therapy in a further three male subjects who had been given chlormethiazole nightly for at least two weeks (Fig. 3).

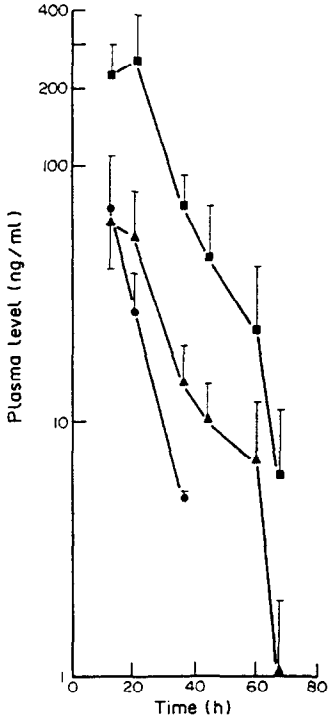

Figure 3
Decrease in plasma levels of chlormethiazole and its metabolites after withdrawal of drug. Chlormethiazole (●), 5-acetyl-4-methylthiazole (▲) and 5-(1-hydroxyethyl) 4-methylthiazole (■). Combined data for three subjects (± SE).

Plasma levels of CTZ, AMT and HEMT 12 h after the final dose were not statistically different from those found after seven days' treatment in the eleven subjects in the main study ($p > 0.3$). Once the drug had been withdrawn the plasma level of chlormethiazole and its metabolites fell rapidly. The plasma contained no detectable chlormethiazole after 44 h and at less than 84 h no detectable amount of either of the two metabolites could be found. The average half-lives of CTZ, AMT and HEMT (7.7±1.2; 9.1±0.3; 8.3±0.7 h respectively) were parallel and not statistically different from each other, although the half-life of chlormethiazole in these three subjects (7.7±1.2 h) was significantly longer than that observed in the main study (4.1±0.4 h: $p < 0.01$).

Discussion

This work was undertaken because of concern about the apparent lack of published information on the disposition of chlormethiazole and its metabolites in the elderly

during prolonged therapy. The analytical system described in this paper using nitrogen-specific detection is 10–50 times more sensitive than the flame ionization detector and, although not as sensitive as GC–MS, it is more readily available and was able to detect CTZ throughout the study period at levels below 10 ng/ml. The reproducibility was acceptable.

No endogenous substances were found to interfere with the assay method, as indicated by the analysis of analytical standards and 'blank' plasma samples (Fig. 1). In particular, the hypnotic Temazepam (used when chlormethiazole was withdrawn) and its metabolites did not interfere with the assay. All peaks on the gas chromatogram were positively identified by comparison with authentic pure standards and by GC–MS.

The metabolism of chlormethiazole has been well studied [5] and the presence of AMT and HEMT in urine and plasma has been reported. Of these reports, only Nation *et al.* [10] have measured AMT and HEMT in the plasma of aged subjects after a single oral dose of chlormethiazole. In this study the beta-elimination half-lives of CTZ in three aged subjects were reported as 3.2, 9.3 and 6.5 h; the beta-elimination half-lives of AMT were found to be 6.7 and 4.3 h in two of the three subjects. It was, however, not possible to calculate an elimination half-life for HEMT from this data and other data on the disposition of the metabolites was not given. These data underline the large individual differences in the disposition of CTZ and its metabolites.

By comparison, the beta-elimination half-lives reported here were 4.1±0.05 h for CTZ in the main study, and 7.7±1.2 h for CTZ, 9.1±0.3 for AMT and 8.3±0.7 for HEMT in the withdrawal study. The half-lives in the present work are not statistically different from the results reported by Nation. An interesting point of difference between the two studies is that although the peak levels of CTZ reported by Nation occurred at the same time as in the present study, they were much elevated (1859–4697 ng/ml) compared with the values 108–1093 ng/ml in the present work, even though the dose was the same. This greater systemic bioavailability in the Nation study implies a reduced functional liver capacity in his subjects compared with those here.

Chlormethiazole accumulation was shown to be just significant over the study period, by reference to the increase in some of the mean plasma levels, peak levels, 12-h residual levels and the AUC.

Chlormethiazole undergoes extensive first-pass metabolism in the liver [6], with an average systemic bioavailability of about 20%. It is probable that this large removal of CTZ in the liver after an oral dose is predominantly responsible for the large individual differences observed in these and in other studies. It is also possible that a decrease in the amount of chlormethiazole removed during the first pass results in the increase observed in chlormethiazole plasma levels over the 7 days of treatment. However, an induced change in the volume of distribution over the study period cannot be discounted.

Whereas maximum levels of AMT were attained within 2–3 h, maximum HEMT levels were not attained until much later. It is not possible for peak levels of a metabolite to be observed in the plasma after the parent has been eliminated, unless its own elimination half-life is longer than that of the parent. From the results of Nation's group [11] and the present withdrawal study, this does not appear to be the case. Both AMT and HEMT have elimination half-lives comparable to that of CTZ itself. The observed peak HEMT plasma levels may therefore be attributable to some other mechanism. The most probable mechanism would be hydroxylation and conjugation (probably with glucuronic acid) during first-pass metabolism, followed by rapid clearance from the plasma and enterohepatic circulation. It is also probable that the observed HEMT is in

fact a mixture of free and conjugated HEMT, since the 1 M hydrochloric acid used in the back-extraction for sample preparation would readily hydrolyse any conjugate back to free HEMT.

AMT accumulation was observed by the increase in mean plasma levels, AUC and residual levels. However, the actual plasma levels of this metabolite 12–16 h post-dose were in the same range as those for chlormethiazole. HEMT was found to accumulate significantly according to all parameters measured. The accumulation of HEMT lead to plasma levels 12–16 h post-dose which were, on average, 6-fold higher than plasma chlormethiazole levels.

The activity of these metabolites is a matter of some controversy. They have been shown to possess approximately 20% of the anticonvulsant activity of chlormethiazole itself in animal tests [14]. However, they have shown no hypnotic or sedative properties in animal tests and no such effect has yet been attributed to them in clinical trials of chlormethiazole. As yet, neither AMT nor HEMT have been given to humans as pure substances. The clinical efficacy of chlormethiazole has been reported previously [12]. The clinical effect did not change over the seven-day treatment period, suggesting that although the metabolites accumulate, they do not appear to exert any significant hypnotic effect.

In conclusion, these data show that 5-(1-hydroxyethyl)-4-methylthiazole and to a lesser extent 5-acetyl-4-methylthiazole accumulate in the elderly after nightly therapy over one week. This accumulation was not found to be detrimental to the hypnotic efficacy of chlormethiazole, which did not appear to accumulate over this interval of time.

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[*First received for review 3 August 1982; revised manuscript received 25 February 1983*]