Journal of Chromatography, 276 (1983) 471—477
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 1757

Note

Determination of chlormethiazole in plasma by high-performance liquid chromatography

R. HARTLEY*, M. BECKER and S.F. LEACH

Department of Paediatrics and Child Health, University of Leeds, Leeds, West Yorks, LS1 3ET (Great Britain)

(First received December 15th, 1982; revised manuscript received April 7th, 1983)

Chlormethiazole, 5-(2-chloroethyl)-4-methylthiazole, a drug with sedative, hypnotic and anticonvulsant properties [1, 2] is becoming increasingly preferred by obstetricians for the treatment of pre-eclamptic toxaemia because, compared to alternative medications, associated side-effects are less severe [3]. Furthermore, these side-effects, which include muscular relaxation, hypothermia, respiratory depression and muscular hypotonia, are more easily managed since the short half-life of the drug and intravenous route of administration allow good dose control.

Chlormethiazole crosses the placenta easily [4, 5] and neonatal blood levels measured by gas—liquid chromatography (GLC) [6] indicate that the drug remains in the newborn much longer than in adults [5]. Cases of prolonged respiratory depression of the newborn have been reported following administration of chlormethiazole during labour [7, 8]. The possibility exists, therefore, that the differential diagnosis of neonatal respiratory depression together with muscular hypotonia should include excessive plasma chlormethiazole levels.

The frequent use of chlormethiazole in maternity hospitals specialising in the management of high risk pregnancies and the increasing sophistication of neonatal intensive care justify the availability of a simple method for monitoring the drug. In the absence of an established high-performance liquid chromatographic (HPLC) procedure we have developed such a technique to measure maternal, cord and neonatal drug levels following chlormethiazole therapy during labour.

0378-4347/83/$03.00

EXPERIMENTAL

Reagents

Methanol (Analar quality) was purchased from BDH Chemicals (Poole, Great Britain). Carbamazepine was generously donated by Geigy Pharmaceuticals (Horsham, Great Britain) and chlormethiazole edisylate was a gift from Astra Pharmaceuticals (St. Albans, Great Britain). Bovine serum albumin (30% solution) was supplied by Armour Pharmaceutical (Eastbourne, Great Britain).

Equipment

The Waters Assoc. high-performance liquid chromatograph consisted of a Model 6000A constant volume pump together with a U6K universal loop injector and a Model 440 UV detector set at 254 nm with an attenuation of 0.005 a.u.f.s. The detector output was connected to a 10-mV Linseis Model LS24/80/80 two-pen recorder operating with a chart speed of 200 mm/h.

Chromatography

Separation of chlormethiazole and the internal standard, carbamazepine (5-carbamyl-5H-dibenz[*b,f*]azepine), was achieved using methanol—water (45 : 55, v/v) in conjunction with a Waters Assoc. 30 cm × 3.9 mm I.D. μBondapak C_{18} analytical column (10-μm reversed-phase packing) protected by a guard column containing Bondapak C_{18}/Corasil. The mobile phase was prepared fresh daily, filtered through a 0.45-μm Millipore Filter (type HA), and used at a flow-rate of 1.8 ml/min.

Sample preparation

Heparinised plasma (500 μl) was diluted with an equal volume of methanol containing the internal standard, carbamazepine, at a concentration of 10 μg/ml. The sample was mixed for 30 sec on a Vortex mixer and centrifuged for 5 min at 2500 rpm (approximately 750 *g*). A 25-μl aliquot of the supernate was injected into the chromatograph.

Preparation of calibration standards

Two series of standards were compared. These were prepared in either heparinised plasma or 3% bovine serum albumin by making additions of aqueous chlormethiazole edisylate to give concentrations of 1, 2, 6, 10, 14 and 20 μg/ml. These samples were then subjected to the previously described sample preparation procedure and injected onto the chromatograph.

RESULTS

Fig. 1a shows the chromatogram obtained following injection of authentic chlormethiazole edisylate and the internal standard, carbamazepine. A chromatogram of extracted blank plasma is illustrated in Fig. 1b whilst Fig. 1c represents extracted plasma from a patient following intravenous administration of chlormethiazole. Comparison of Figs. 1b and 1c clearly indicates blank plasma to be free from any interfering compounds which may have coextracted along with chlormethiazole and the internal standard.

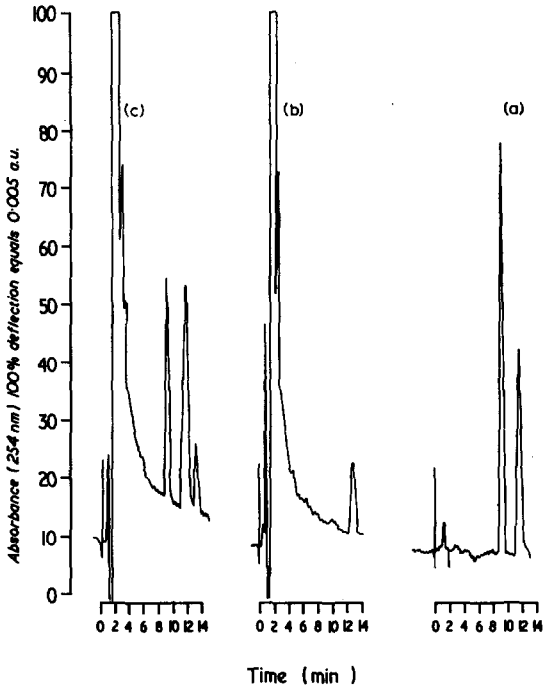

Fig. 1. (a) Chromatogram of authentic chlormethiazole edisylate and the internal standard, carbamazepine. The retention times are 9 min and 11.5 min, respectively, under normal assay conditions. Column, μBondapak C_{18} 30 cm × 3.9 mm I.D.; mobile phase, methanol—water (45:55); flow-rate, 1.8 ml/min; detection at 254 nm. (b) Chromatogram of extracted blank plasma showing absence of interference in the region of interest (8—12 min). (c) Chromatogram of extracted plasma from the same patient following intravenous administration of chlormethiazole. Peaks corresponding to carbamazepine (internal standard) and chlormethiazole are clearly visible with retention times of 11.5 min and 9 min, respectively.

TABLE I

THE REPRODUCIBILITY OF CHLORMETHIAZOLE DETERMINATIONS IN PLASMA AND 3% BOVINE SERUM ALBUMIN SHOWING THE EFFECTS OF STORAGE AT −20°C FOR 3 MONTHS

Sample	Concn. of chlormethiazole added (μg/ml)	Concn. of chlormethiazole determined at zero time (μg/ml)	Concn. of chlormethiazole determined after storage at −20°C for			Mean recovery (%) (n = 19)	Intra-batch coefficient of variation (n = 5)	Inter-batch coefficient of variation (n = 14)
			4 weeks	8 weeks	12 weeks			
Plasma	2	2.1	2.1	2.0	2.0	102.5	2.61	6.03
	14	14.1	13.3	14.0	13.7	98.4	3.45	3.19
3% BSA	2	2.2	1.8	1.8	2.0	97.5	2.03	5.8
	14	14.1	13.7	13.4	13.3	97.3	2.12	3.76

Calibration curves were obtained by comparing the peak height ratio (chlormethiazole/internal standard) with the actual concentration of chlormethiazole in spiked aliquots of plasma or 3% bovine serum albumin. In both cases the relationship was linear over the concentration range 0—20 μg/ml. Slope values are 0.09 and 0.088 with correlation coefficients (r) of 0.992 and 0.988 for plasma and 3% bovine serum albumin, respectively.

Storage trials were also carried out using both plasma and 3% bovine serum albumin. Samples, spiked with chlormethiazole to give concentrations of 2 and 14 μg/ml, were kept at −20°C and assayed at monthly intervals for up to three months. The results are presented in Table I. Five replicate samples measured at zero time gave values of 2.1 and 14.1 μg/ml with intra-batch coefficients of variation of 2.61 and 3.45 for plasma concentrations of 2 and 14 μg/ml, respectively. The same samples determined at monthly intervals over the 3-month storage period gave mean values of 2.03 and 13.67 μg/ml with inter-batch coefficients of variation of 6.03 and 3.19. Similar results (see Table I) were obtained for equivalent samples prepared in 3% bovine serum albumin.

The application of the method in clinical practice can be appreciated from the results listed in Table II which were obtained from patients treated with chlormethiazole during the later stages of labour. The plasma levels of chlormethiazole measured using this technique can be compared with those established using the alternative GLC and GLC—mass fragmentographic (MF) methods [4, 5, 7, 9—11] shown in Table III.

TABLE II

THE DETERMINATION OF CHLORMETHIAZOLE IN MATERNAL, CORD AND NEONATAL PLASMA USING HPLC

Sample	Mean plasma chlormethiazole concn.* (μg/ml)	Range of concn. (μg/ml)	S.D.	Number of determinations (*n*)
Maternal plasma	4.57	0.7 — 8.9	3.04	5
Cord plasma	5.37	0.6 —12.6	3.96	16
Neonatal plasma				
1 h	3.01	0.8 — 5.8	1.66	6
6 h	1.51	0.25— 2.9	1.09	4
12 h	0.975	0.6 — 1.3	0.29	4

*Mean plasma concentration of chlormethiazole edisylate (0.63 g of chlormethiazole base = 1 g of chlormethiazole edisylate).

TABLE III

CLINICAL RESULTS OBTAINED USING ALTERNATIVE METHODS SHOWING MEAN CHLORMETHIAZOLE PLASMA LEVELS

Literature source	Patient group	Analytical technique	Mean plasma concentration and range* (μg/ml)	Mean umbilical vein concentration and range (μg/ml)	No. of patients
Jostell et al. [9]	Healthy adults	GLC and GLC—MF	0.746 (0.370—1.350)	— —	10
Jostell et al. [11]	Alcohol withdrawal	GLC MF	5.37 (3.12 —6.82)	— —	3
			5.81 —	— —	1
Tischler [5]	Maternity	GLC	11.5 (4.9 —20.4)	9.9 (7.2—15.3)	4
Duffus et al. [4]	Maternity	GLC	14.6 (4.7 —22.4)	10.8 (4.5—16.9)	11
Tunstall et al. [10]	Maternity	GLC—MF	1.59 —	1.30 —	1**
Young and Rasheed [7]	Maternity	GLC	3.1*** —	— —	1

*Mean plasma concentration of chlormethiazole base (0.63 g of base = 1 g of chlormethiazole edisylate).
**Single patient receiving intravenous chlormethiazole only.
***Neonatal level after 34 h.

DISCUSSION

The method described has proved useful for measuring drug levels in maternal, cord and neonatal plasma during studies of respiratory problems of the newborn following chlormethiazole therapy of pre-eclamptic toxaemia in labour. During twelve months of periodic use there has been no suggestion of interference from endogenous components which might invalidate the technique, Fig. 1b and c being typical of the many blank and patient plasma samples analyzed.

The advantage of this HPLC approach compared with the GLC methods of Jostell et al. [9] and Frisch and Ortengren [6] is the appreciably smaller sample requirement whilst maintaining a similar overall sensitivity. This is particularly important in paediatric applications. The detection limit of approximately 200 ng/ml may not facilitate pharmacokinetic studies but sensitivity is sufficient to enable the determination of chlormethiazole in neonates 12 h after delivery.

The possibility of improving overall sensitivity of the assay by alternative sample preparation exists since the method we have adopted, using methanol as a protein precipitant, causes dilution of the plasma due to mutual solubility of the two components. Solvent extraction into chloroform, followed by concentration of the drug, proved unsuccessful because although chlormethiazole is quantitatively extracted into the organic phase, recovery was poor due to simultaneous loss of sample during solvent evaporation under nitrogen at 55° C. Even when carried out at room temperature the recovery was only in the region of 20%. This phenomenon has also been observed in the case of ethchlorvynol [12] which is considered to be too volatile to permit concentration by solvent evaporation without significant loss.

Improvements in sample preparation, however, are unlikely to achieve the sensitivity of the GLC—MF method of Jostell et al. [11] which is capable of measuring plasma concentrations of 1 ng/ml but for those laboratories without access to such facilities, requiring only to monitor plasma chlormethiazole levels in excess of 200 ng/ml, the HPLC method is rapid and simple to operate.

Two pharmacologically active metabolites of chlormethiazole, 5-acetyl-4-methylthiazole (AMT) and 5-(1-hydroxyethyl)-4-methylthiazole (HEMT) [13], which have been measured in plasma using GLC—mass spectrometry [14] and more recently by GLC [15] do not interfere with the quantitation of the drug by this method. HMET, the major metabolite observed by Tsuei et al. [15], is not significantly retained on the column under the described conditions and elutes with the front material. According to the same authors, the maximum plasma level of the secondary metabolite only approaches 200 ng/ml. Since the lowest detectable concentration using our method exceeds this, any contribution from AMT would be expected to be minimal.

Although the GLC method of Tsuei et al. [15] is very sensitive and may be used in pharmacokinetic studies to estimate chlormethiazole and its two metabolites simultaneously, the HPLC approach for measuring chlormethiazole offers the advantage of a significant reduction in sample preparation time. Only a single addition of methanol is required in order to precipitate plasma proteins

compared with the relatively tedious three-stage extraction procedure employed by the GLC method.

Clinical results obtained using this technique (Table II) compare favourably with those gathered using alternative methods shown in Table III. Comparisons may be difficult to interpret since dosages are not standardised but based on individual clinical requirements and administered accordingly. However, at delivery the mean maternal plasma level (4.57 μg/ml) lies within the range of published values and the mean cord plasma level (5.37 μg/ml) is in close agreement with the maternal plasma concentration of chlormethiazole. In the neonate, mean plasma concentrations of the drug range from 3.01 μg/ml at 1 h after delivery decreasing to 1.51 μg/ml after 6 h and 0.975 μg/ml after 12 h.

For calibration purposes samples were prepared in both plasma and 3% bovine serum albumin. On comparison the results were so similar that, if preferred, the latter may be used for preparation of calibration standards.

The effects of storage on extraction efficiency and reproducibility were also examined using both plasma and 3% bovine serum albumin spiked with chlormethiazole. Again both series of samples behaved in a similar manner, the results (see Table I) clearly indicate that storage for three months at −20°C has no serious effect on recovery or reproducibility. The recovery of chlormethiazole using this method is predictably in the region of 100% since sample preparation involves dilution rather than extraction.

This technique was developed to permit quantitation of circulating chlormethiazole levels in mother and baby following treatment of pre-eclampsia during labour. It is being used in ongoing studies of neonates subsequent to the use of chlormethiazole during labour in order to examine relationships between circulating drug levels and symptoms of respiratory depression which may be encountered. The results of these studies will be published upon completion.

ACKNOWLEDGEMENT

The technical assistance of Mr. J.R. Cookman is gratefully acknowledged by the authors.

REFERENCES

1 R. Charonnat, P. Lechat and J. Chareton, Therapie, 12 (1957) 68.
2 P. Lechat, Acta Psychiat. Scand., 42, Suppl. 192 (1966) 15.
3 C. Berger, Schweiz. Rundschau Med. Prax., 68 (1979) 971.
4 G.M. Duffus, M.E. Tunstall and I. MacGillivray, Lancet, i (1968) 335.
5 E. Tischler, Aust. N.Z. J. Obstet. Gynaecol., 13 (1973) 137.
6 E.P. Frisch and B. Ortengren, Acta Psychiat. Scand., 42, Suppl. 192 (1966) 35.
7 J.A. Young and S. Rasheed, Arch. Dis. Child., 44 (1969) 509.
8 G.M. Duffus, M.E. Tunstall, R.G. Condie and I. MacGillivray, J. Obstet. Gynaecol. Br. Commonw., 76 (1969) 645.
9 K.-G. Jostell, S. Agurell, L.-G. Allgen, B. Kuylenstierna, J.-E. Lindgren, G. Aberg and G. Osterlof, Acta Pharmacol. Toxicol., 43 (1978) 180.
10 M.E. Tunstall, D.M. Campbell, B.M. Dawson and K.-G. Jostell, Brit. J. Obstet. Gynaecol., 86 (1979) 793.

11 K.-G. Jostell, S. Agurell, L.E. Hollister and B. Wermuth, Clin. Pharmacol. Ther., 23 (1978) 181.
12 R.J. Flanagan and T.D. Lee, J. Chromatogr., 137 (1977) 119.
13 U.H. Lindberg, Acta Pharm. Suecica, 8 (1971) 39.
14 R.L. Nation, J. Vine, E.J. Triggs and B. Learoyd, Eur. J. Clin. Pharmacol., 12 (1977) 137.
15 S.E. Tsuei, J. Thomas and R.L. Nation, J. Chromatogr., 182 (1980) 55.