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Determination of chloral hydrate metabolism in adult and neonate biological fluids after single-dose administration

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ABSTRACT

A simple, rapid and sensitive electron-capture gas chromatographic method has been developed for the simultaneous determination of chloral hydrate, trichloroethanol and trichloroacetic acid in biological fluids. The described method is applicable to single-dose pharmacokinetic studies of chloral hydrate in the adult. The method also meets the important requirement of using very small sample volumes and is sufficiently sensitive and reliable for disposition studies in the neonate.

INTRODUCTION

Chloral hydrate (CH) was first synthesized more than 150 years ago and is the oldest sedative hypnotic prescribed today. CH continues to be widely used in infants, children and the elderly [1–3].

Major advances in neonatal medicine has resulted in marked improvements in the survival rate of neonates. In Neonatal Intensive Care Units (NICU) mechanical ventilation using intermittent positive pressure has become routine. While undergoing mechanical ventilation infants who become agitated often breath out-of-phase with the ventilator resulting in profound hypoxemia and hypercarbia. As an alternative to pancuronium-induced muscle paralysis,

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sedatives can be used to decrease agitation and thus improve pulmonary gas exchange with less hazards to infants. CH is frequently used as the agent of choice in these situations. A significant decrease in spontaneous extubation in chronically ventilated infants is evident when sedated with chloral hydrate [4].

The metabolic pathway for CH, in the adult, is well known [5]. CH is rapidly reduced to trichloroethanol (TCE). TCE is then excreted unchanged, conjugated with glucoronic acid or oxidized to trichloroacetic acid (TCA).

Pharmacokinetic studies of CH in the human adult have been reported [6–8]. Although the drug is widely used in infants and children very little information is available on its metabolism and excretion in this age group. Because of this paucity of information and because of the frequent use of the drug and some implicated toxicities [9] the present study was designed to establish a viable method so that metabolism and disposition studies in the neonate would be possible.

Various methods have been reported for measuring CH and metabolites in biological fluids including standard colorimetric procedures [10–12] and gas chromatography (GC) employing either flame ionization [9,13,14] or electron-capture detection (ECD) [13,15–18]. Of the published procedures, few permit concurrent measurement of unchanged CH and its metabolites and none offer the required sensitivity for neonatal studies.

This report describes a simple, rapid and sensitive GC method which allows for the simultaneous determination of CH and its metabolites in biological fluids. The described method is applicable to single-dose pharmacokinetic studies of CH in the adult and the neonate.

EXPERIMENTAL

Materials and reagents

CH was supplied by Squibb Canada (Montreal, Canada). TCE, N-methyl-N-nitroso-*p*-toluene sulfonamide (Diazald) and β-glucuronidase (from *Helix pomatia*, 420 000 U/g) were obtained commercially from Sigma (St. Louis, MO, U.S.A.). Analytical-grade diethyl ether and TCA were obtained commercially from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Methyl trichloroacetate (MTCA) and the internal standard, 1,2,3-trichloropropane (TCP), were obtained from Aldrich (Milwaukee, WI, U.S.A.). All other reagents and solvents were analytical grade and used without further purification.

Preparation of diazomethane

Diazomethane was prepared using a commercial generator apparatus (Diazald Kit, Aldrich). All the glass fittings were smooth and the procedure was carried out behind safety shields in a well ventilated fumehood. A solution of Diazald (4.3 g in 45 ml of diethyl ether) was added to a heated (65 °C) mixture

containing 5 ml of 95% ethanol, 1 g of potassium hydroxide and 1.6 ml of water. The resulting ethereal alcohol solution of diazomethane was stored at −20°C in tightly corked flasks, in an explosion proof freezer, and used as required.

Stock solutions

Stock solutions of CH, TCA and TCE were prepared fresh daily.

Extraction of plasma

CH, TCA and free TCE. Plasma (50 μl) was added to a PTFE-lined screw-capped test tube (120×16 mm) along with distilled water (0.25 ml), sulfuric acid (0.25 ml, 3*M*) and diethyl ether (5.0 ml) containing the internal standard, TCP (5 μg/ml). The tube was tightly capped and vigorously shaken for 1 min, centrifuged (Dynac centrifuge, Clay Adams, Parsippany, NJ, U.S.A.) for 10 min at 500 *g*. An aliquot (1.0 ml) of the ether layer was transferred to another tube, ethereal diazomethane solution (50 μl) added and the tube capped, shaken and allowed to stand (5 min) at room temperature. Aliquots (1 μl) were injected into the gas chromatograph.

Total TCE. TCE conjugated as the glucuronide (TCE-G or urochloralic acid) was hydrolyzed by β-glucuronidase using a method similar to that described by Sellers et al. [7]. Plasma (50 μl) was added to a screw-capped vial containing 5 mg of β-glucuronidase and 1.5 ml of acetate buffer (0.05 *M*, pH 4.5). The vial was tightly capped, the contents mixed by vortex-mixing (1 min) and then incubated (37°C) for 18 h on a gyrotary water bath shaker (New Brunswick Scientific, New Brunswick, NJ, U.S.A.). Extraction and quantitation of TCE was accomplished using the procedure described above. The amount of TCE measured represents the total TCE present in the sample. The difference between free TCE and total TCE, determined after hydrolysis, is equal to the amount bound as the glucuronide.

Extraction of urine

CH, TCA and free TCE. Urine samples were analyzed similar to the method described for plasma. Aliquots of urine (0.5–1.0 ml) were treated with sulfuric acid (2.5 ml, 3 *M*) and subsequently extracted with diethyl ether (10.0 ml) containing the internal standard (5 μg/ml).

Total TCE. Aliquots of urine (0.5 or 1.0 ml) were treated with 0.05 *M* ammonium acetate buffer (1.5 or 3.0 ml, respectively) and incubated (37°C) for 18 h in the presence of β-glucuronidase (5 mg). The mixture was then extracted with diethyl ether (10.0 ml) and the total and conjugated TCE determined as described for plasma.

Apparatus

Chromatography was performed on a Varian Model 3700 gas chromatograph (Varian Assoc., Palo Alto, CA, U.S.A.) equipped with a ^{63}Ni electron-capture detector and a coiled-glass column (2.44 m × 2 mm I.D.) packed with Supelco GP 10% SP-1200/1% H_3PO_4 on 80–100 mesh Chromosorb W AW (Supelco, Bellefonte, PA, U.S.A.). The column was conditioned (175°C) for 24 h prior to use. The analysis was carried out isothermally with injection port, column oven and detector temperatures of 160, 110 and 300°C, respectively. Argon–methane (95:5) was used as the carrier gas at a flow-rate of 12.0 ml/min. A Shimadzu Model C-R3A Chromatopac integrator–recorder (Shimadzu, Kyoto, Japan) was used for peak-height determination and quantitation.

Standard curve determination

Standard curve data were generated by spiking a series of drug-free plasma samples with CH, TCA and TCE to produce concentration ranges of 3.91–62.5 μg/ml for CH and 1.56–25 μg/ml for TCA and TCE. The samples were taken through the extraction procedure and analyzed as described above. Calibration curves were constructed by plotting the ratios of peak height of the respective compound to that of the internal standard against concentration and analyzed by linear regression analysis. Similar standard curve data were generated for spiked urine samples.

Extraction efficiency

Analytical recoveries of CH and metabolites from plasma and urine were determined by comparing peak-height ratios of standard to internal standard obtained from extracted spiked plasma and urine samples to those obtained from direct injection of standards containing equivalent amounts of the compounds. Recoveries were established for CH, TCA and TCE individually and for combinations of TCA–TCE and CH–TCA–TCE in plasma as well as for the CH–TCA–TCE combination in urine. MTCA was used to determine the absolute recovery of TCA after extraction and derivatization.

Patient studies

Adult. A healthy 91-kg man received 1000 mg of CH orally after an overnight fast. At various times after drug administration (0, 15, 30, 45, 60 and 90 min and 2, 3, 4, 6, 8, 12, 24, 36, 72, 96, 120, 144, 168 and 192 h), venous blood samples (5 ml) were collected in 10-ml Vacutainer tubes (Becton and Dickinson, Mississauga, Canada). The samples were immediately centrifuged and the separated plasma stored in plastic snap-top tubes (Falcon Plastics, Oxnard, CA, U.S.A.) at −20°C until analysis. Total urine was collected over 12-h periods for two days and then for 24-h periods for eight days. Sample aliquots of the urine samples were stored at −20°C until analyzed.

Neonate. A premature male infant (27 weeks, 1830 g) received an oral dose

of 80 mg of CH. Blood samples (400 μl) were obtained from an arterial line at 1, 3, 6, 54, 77, 99 and 165 h. The samples were immediately centrifuged, and the separated plasma was stored in plastic snap-top tubes at −20°C until analysed.

RESULTS

Fig. 1A shows the chromatogram of a blank plasma sample spiked with CH, TCA, internal standard and TCE extracted and derivatized to yield MTCA. The peaks were well separated from each other with retention times of 1.40, 4.09, 6.09 and 9.22 min for CH, MTCA, internal standard and TCE, respectively. Fig. 1B is the chromatogram of a plasma extract from the volunteer after having received the oral dose of CH. Recoveries of CH, TCA an TCE from spiked plasma and urine samples were 95% or greater (Table I). The method developed is linear (over the range of 3.91–25 μg/ml for CH, TCE and MTCA) and reproducible (coefficient of variation <5%).

DISCUSSION

An important prerequisite for undertaking drug disposition studies in the neonatal and pediatric age group is analytical methodology. It must be more sensitive than that developed for investigations in adults. Microassay techniques are often required, particularly when multiple blood samples are drawn over a short period of time. As there are limitations to sample size, it is advantageous to simultaneously quantitate unchanged drug and circulating metabolites in a single determination. In this study, in order to optimize the assay procedures, samples (plasma and urine) from an adult volunteer who had received CH (1000 mg orally) were initially analyzed. ECD was chosen because of its sensitivity to halogenated compounds and with this method the metabolites were easily detected after extraction of only 50 μl of plasma and 0.5 ml of urine. This is sensitive enough so that kinetic studies are possible in the neonate without compromising them in any way.

Fig. 2 shows the plasma concentration–time profiles of TCE, TCE-G and TCA after oral administration of CH to a healthy male volunteer. The pharmacokinetics are in good agreement with those previously reported [6,7]. TCA is still detectable after 165 h. TCE and TCE-G were detectable for 36 h after dosing. CH was not detected in the plasma of either the adult (Fig. 1) or neonate participants. This is consistent with previous reports in which no CH was detected in the blood following normal doses [5].

Fig. 3 shows the urine excretion–time profile obtained from the same adult volunteer. TCE was detectable for up to four days after dosing, whereas the glucuronide, TCE-G, and TCA were still being detected after eight days.

The analytical procedure was extended to plasma analysis in the neonate.

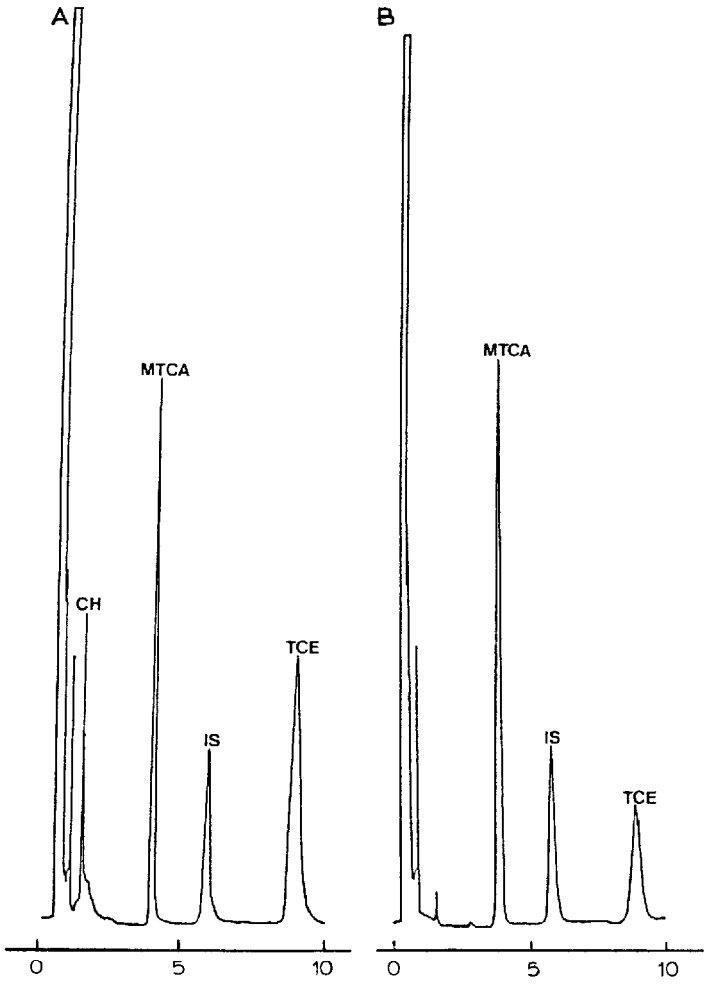

Fig. 1. Chromatograms of (A) extracted blank plasma sample spiked with CH (18.0 μg/ml), MTCA (25 0 μg/ml), internal standard (5.0 μg/ml) and TCE (23.0 μg/ml) and (B) extracted plasma sample from a volunteer receiving an oral dose (1000 mg) of CH; concentrations found: MTCA, 25.5 μg/ml; TCE, 9.4 μg/ml; internal standard, 5.0 μg/ml. Peaks: CH = chloral hydrate; MTCA = methylated trichloroacetic acid; IS = internal standard, 1,2,3-trichloropropane; TCE = trichloroethanol.

Fig. 4 shows the plasma concentration–time profile for this patient. A gradual increase in TCA is also seen in the neonate. Unfortunately TCA concentrations are still increasing at the 165-h collection period. Elevated levels of this acidic metabolite may not be desirable as it is known to be highly protein-bound and evidence suggests it may be cardiotoxic [9]. Further studies are in progress. TCE concentrations remained within the therapeutic range, reported to be between 10 and 100 μg/ml [19,20]. TCE-G elimination appears to be

TABLE I

RECOVERY OF CH, TCA AND TCE FROM SPIKED PLASMA AND URINE SAMPLES

Drug/ metabolite	Concentration added (μg/ml)	Mean concentration recovered ($n=3$) (μg/ml)	Recovery (mean ± S.D.) (%)	Coefficient of variation (%)
Plasma				
CH	5.0	5.04	100.70 ± 1.20	1.19
TCA	10.0	10.13	101.26 ± 0.83	0.82
TCE	20.0	20.33	101.52 ± 0.80	0.78
TCA-TCE	12 5 (TCA)	12.92	103.33 ± 1.36	1.31
	8.0 (TCE)	7.83	97.80 ± 0.82	0.84
CH-TCA-TCE	5.0 (CH)	4.75	95.0 ± 3.34	3.50
	5.0 (TCA)	4.76	95.26 ± 0.85	0.90
	5.0 (TCE)	4.92	98.39 ± 1.11	1.12
Urine				
TCA-TCE	10.0 (TCA)	10.16	101.57 ± 0.48	0.47
	5.0 (TCE)	4.96	99.13 ± 0.64	0.64

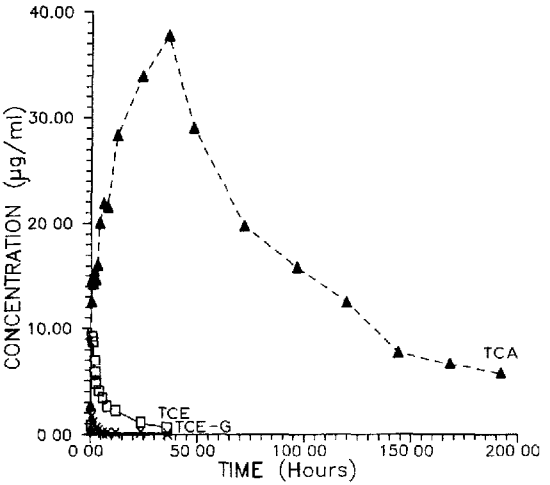

Fig. 2. Mean plasma concentration-time profile of TCE, TCE-G (glucuronide) and TCA after oral administration of a single 1-g dose of CH to a healthy male volunteer.

slower in the neonate. This may be partially explained by the fact that renal excretion of drugs, in the neonate, is reduced as a result of a lower glomerular filtration and tubular secretion rate [21,22].

The differences between the neonate and the adult with respect to TCA metabolism are presently being investigated in our laboratory. In addition,

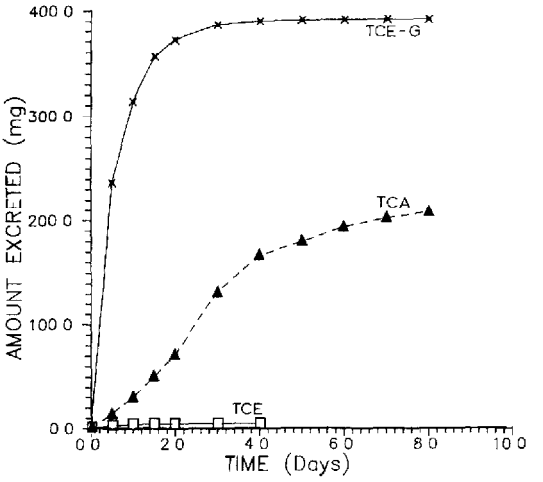

Fig. 3. Cumulative urinary excretion–time profile of TCE-G, TCA and TCE after oral administration of 1 g of CH to a healthy male volunteer.

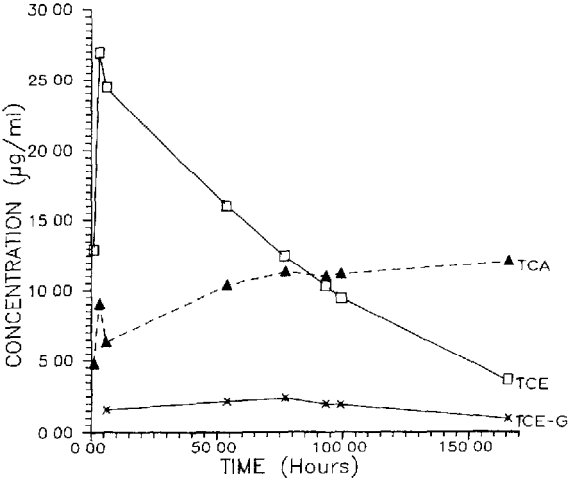

Fig. 4. Mean plasma concentration–time profile of TCE, TCE-G and TCA after oral administration of 80 mg to a 1830-g male infant

multiple dosing is common with CH. Whether this affects the metabolic profile in the neonate will also be determined using this analytical procedure.

CONCLUSION

A simple, rapid and sensitive method has been developed which allows for the simultaneous determination of CH and its metabolites in biological fluids. The described method is applicable to single-dose pharmacokinetic studies of

CH in adults. The method also meets the important requirement of using very small sample volumes and is sufficiently sensitive and reliable to undertake disposition studies in the neonate.

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