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Short communication

Determination of paraldehyde by gas chromatography in whole blood from children

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Abstract

A rapid, sensitive and selective gas chromatographic method with flame ionization detection was developed for the determination of paraldehyde in small blood samples taken from children. Whole blood samples (300 μl) collected in a 3 ml Wheaton® glass sample vial were spiked with acetone (internal standard: 15 ng) followed by addition of concentrated hydrochloric acid. The mixture was heated in the sealed airtight sample vial in a water bath (96 °C; 5 min) to depolymerize paraldehyde to acetaldehyde. A 2 ml aliquot of the headspace was analyzed by gas chromatography with flame ionization detector using a stainless steel column (3 m × 4 mm i.d.) packed with 10% Carbowax® 20M/2% KOH on 80/100 Chromosorb® WAW. Calibration curves were linear from 1.0–20 μg ($r^2 > 0.99$). The limit of detection was 1.5 μg/ml, while relative mean recoveries at 2 and 18 μg were 105.6 ± 8.4 and 101.2 ± 5.9%, respectively ($n = 10$ for each level). Intra- and inter-assay relative standard deviations at 2, 10 and 18 μg were <15%. There was no interference from other drugs concurrently used in children with severe malaria, such as anticonvulsants (diazepam, phenytoin, phenobarbitone), antipyretics/analgesics (paracetamol and salicylate), antibiotics (gentamicin, chloramphenicol, benzyl penicillin) and antimalarials (chloroquine, quinine, proguanil, cycloguanil, pyrimethamine and sulfadoxine). The method was successfully applied for pharmacokinetic studies of paraldehyde in children with convulsions associated with severe malaria.

Keywords: Paraldehyde

1. Introduction

Severe falciparum malaria is a major cause of childhood deaths in sub-Saharan Africa [1,2]. Convulsions are a common complication of severe disease, and occur in 30% of all children admitted with malaria [3,4]. Protracted and repetitive convulsions are often refractory to treatment, and are associated with an increased risk of death and neurological sequeale in children with severe malaria [5,6]. Thus, rapid termination and prevention of convulsions are a priority and may improve the outcome in children with severe malaria.

Paraldehyde is still used routinely in some settings, including ours, for treatment of convulsions. It is a drug that has been used in the treatment of convulsions for more than a century, although its pharmacokinetics and clinical effects have not been fully elucidated in children. It is useful in resource poor countries since it is cheap and can be administered by intramuscular or rectal routes that do not require a lot of expertise, particularly in peripheral health facilities. Varying doses (0.1–0.3 mg/kg) have been recommended [7,8], but these doses have not been subjected to pharmacokinetic analysis or to rigorous evaluation for clinical effectiveness. There have been no studies on paraldehyde in

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children in resource-poor countries. Part of the reason may be difficulties associated with the assay of paraldehyde using some of the currently available methods. The other reason is that paraldehyde is no longer widely used in the west. We routinely use paraldehyde as one of the first line treatment for convulsions associated with severe malaria in children. For pharmacokinetic studies in such children, the ideal analytical method for paraldehyde should be one that is sensitive enough to allow use of small quantities of biological fluid (since the children often have severe anaemia), and selective to avoid interference from concurrently administered drugs (antimalarials, other anticonvulsants, antibiotics, antipyretics/analgesics).

Several analytical techniques for quantifying paraldehyde in biological fluids have been reported, including chemical and gas chromatographic methods. Most of these assay procedures are based on depolymerization of paraldehyde by acid and heat to its monomer, acetaldehyde. Acetaldehyde is then measured by oxidimetric titration [9], colorimetry [10], UV spectrophotometry [11], coupled enzymatic reduction [12], or gas chromatography with flame ionization detection [13–15]. Gas chromatography has also been used to quantify intact paraldehyde in biological fluids by direct injection [16,17] and deproteinization of serum using ultrafiltration followed by wide-bore capillary gas chromatography [18]. However, these techniques have some limitations, including lack of selectivity [10] or adequate sensitivity [18], laborious sample clean-up procedures [11,16] and use of expensive disposable ultrafiltration units [18], which may be unaffordable for routine use in many resource-poor countries (where paraldehyde is routinely used).

We report a simple, sensitive and selective gas chromatographic method with flame ionization detection that requires only 300 μl of whole blood. We have used this method to monitor paraldehyde whole blood concentrations in a group of children with severe malaria who had been administered paraldehyde to control associated convulsions.

2. Experimental

2.1. Chemicals and reagents

Paraldehyde (99.5% (w/v); Acros Organics, New Jersey, USA) was used as the analytical standard. HPLC-grade acetone (the internal standard) and concentrated hydrochloric acid (AnalaR® grade; specific gravity 1.18) were obtained from BDH (Dorset, UK). Distilled water was prepared as required using a Manesty Type S 75 water distiller (Manesty Machines Ltd., Liverpool, UK) while deionized water was prepared in-house using Elgastat C–114 water deionizer cartridges (Elga Ltd., High Wycombe Bucks, England). All other reagents were of analytical grade or better. White spot nitrogen, hydrogen and compressed air were purchased from BOC Ltd. (Nairobi, Kenya).

2.2. Preparation of standard solutions

A stock solution (10 mg/ml) of paraldehyde was prepared by diluting 10 μl (density: 0.999 g/ml; about 10 mg paraldehyde) of the commercial preparation of paraldehyde to 1 ml with distilled water. Working standard solutions (100, 10 and 1 μg/ml) of paraldehyde were prepared from the stock solution by making appropriate serial dilutions in distilled water. A stock solution of acetone (7.5 μg/ml) was prepared by diluting 20 μl (density: 0.79 g/ml, purity: 100%; 15.8 mg) of the HPLC grade acetone to 2 ml with distilled water. A working solution (0.75 μg/ml) was prepared by making a 1:10 dilution of the stock solution in water.

2.3. Gas chromatographic conditions

The GC system comprised a Trace GC 2000 gas chromatograph (Model no. K07332000000000; ThermoQuest™ Italia S.p.A., Milan, Italy) equipped with a flame ionization detector (ThermoQuest™ Italia S.p.A). A stainless steel column (3 m × 4 mm i.d.) packed with 10% Carbowax® 20M/2% KOH on 80/100 Chromosorb® WAW (Supelco, Bellefonte, PA, USA) was used. Analysis of paraldehyde was carried out under the following conditions: column oven conditioning and operating temperatures of 200 °C and 120 °C, respectively; oven temperature, 100 °C; detector temperature, 160 °C; carrier gas (white spot nitrogen), at a flow rate 50 ml/min with gas saver off; hydrogen flow, 50 ml/min and air flow rate, 400 ml/min. The nitrogen gas was further purified by passing it through a moisture trap (molecular sieve 5 Å; 200 cm^3; 61 cm × 2.5 cm), an oxygen trap (Supelpure-O trap, 120 cm^3) and a hydrocarbon trap (Supelcarb HC, 120 cm^3) [Supelco, Bellefonte, PA, USA] connected in series. The chromatographic peaks were recorded on a flat bed chart recorder (Servogor 124; BBC Goerz Metrawatt, Belmont Instruments, Glasgow, UK) set at a chart speed of 30 cm/h.

2.4. Sample preparation

Drug-free heparinized whole blood samples (0.3 ml) from healthy volunteers were aliquoted into 3 ml Wheaton® sample vials (Millville, NJ, USA) with an air airtight Teflon-lined screw cap, reinforced with aluminium foil. The same procedure was followed for blood samples from patients. In the latter case, the sample bottles were stored at −20 °C until analysis of the blood for paraldehyde. Acetone (20 μl of the working solution; 15 ng, internal standard) was added (after thawing of the blood in the case of stored samples) and the mixture vortexed for 15 s. Concentrated hydrochloric acid (20 μl; 11.8 M) was then added and the vial immediately sealed, and contents mixed by vortexing for 30 s. The mixture was heated in a boiling water bath (96 °C; 5 min) and an aliquot (2.0 ml) of the hot headspace was removed with a gas-tight syringe (Gastight® #1002;

Hamilton Co., Nevada, USA) and injected onto the gas chromatograph.

2.5. Preparation of calibration curves

Calibration curve standards were prepared by adding known quantities (1.0–20.0 μg) of paraldehyde to aliquots (300 μl) of drug-free whole blood, and 20 μl (15 ng) of the internal standard working solution. The samples were assayed as described in the extraction procedure. Calibration curves were constructed by plotting peak height ratios of drug to that of the internal standard against known amounts of the drug. The results were analyzed by least-squares linear regression to estimate the slope, intercept and correlation coefficient (r^2).

2.6. Selectivity

Various anticonvulsants (diazepam, phenobarbitone, phenytoin, fosphenytoin), antibiotics (gentamicin, chloramphenicol succinate and chloramphenicol base and benzyl penicillin), antipyretics/analgesics (paracetamol and salicylate) and antimalarials (chloroquine, quinine, proguanil, cycloguanil, pyrimethamine and sulfadoxine) commonly used in the management of severe malaria and associated complications were evaluated for interference with the assay for paraldehyde. Drug-free whole blood was spiked with therapeutic concentrations of these drugs followed by extraction and analysis as described above.

2.7. Recovery and reproducibility

The analytical recovery of the extraction procedure for paraldehyde was assessed by spiking 300 μl aliquots of drug free whole blood with 2.0 and 15 μg ($n = 10$ for each level) of paraldehyde and 15 ng of the internal standard. In another set of tubes, equivalent amounts of paraldehyde and internal standard were added to distilled water (300 μl). The samples were processed as described in the extraction procedure above, and 2 ml aliquots of the headspace injected onto the chromatograph. Relative recovery was assessed by comparing peak height ratios of paraldehyde to internal standard for the extracted whole blood samples, with those from equivalent amounts extracted from distilled water.

Quality control (QC) samples were prepared by spiking drug-free whole blood (5 ml) with various quantities of paraldehyde, corresponding to the low (LQC), medium (MQC) and high (HQC) levels of the calibration curves. The nominal concentrations for the LQC, MQC and HQC levels were 6.67, 33.4 and 60.0 μg/ml (equivalent to 2, 10, and 18 μg per 300 μl), respectively. Within-day precision and accuracy were determined by analyzing on the same day 300 μl aliquots of each of the QC samples ($n = 5$ for each level). Between-day precision and accuracy were assessed by analyzing duplicates of each of the QC samples for 5 days. Relative standard deviations (R.S.D.) values were used as an index of precision. Accuracy was calculated by comparing the mean experimental amounts of assayed QC standards with their nominal values.

2.8. Validation study

In a separate study investigating the clinical pharmacology of paraldehyde, children with severe malaria and convulsions were recruited (after obtaining informed consent from the parents/guardians and ethical clearance from the KEMRI/National Ethical Review Committee) and administered paraldehyde intramuscularly (IM; 0.2 ml/kg of undiluted paraldehyde) or rectally (PR; 0.2 ml/kg of a 10% (v/v) solution in olive oil) [19]. Blood samples (0.3 ml) were collected pre-dose and at 5, 10, 15, 20, 25, 30, 40, 60 min, and 2, 4, 6, 8, 12, 24, 36 and 48 h after paraldehyde administration. The blood was collected into heparinized 3 ml Wheaton® sample vials (Millville, NJ, USA) with an air airtight Teflon-lined screw cap, reinforced with aluminium foil, and tightly capped. The samples were stored frozen at −20 °C until analysis for paraldehyde as described above. Concentration–time profiles for two of the patients (one from the IM paraldehyde and one from the PR paraldehyde group) are reported.

3. Results and discussion

3.1. Chromatography

Chromatograms of acetaldehyde (paraldehyde monomer) and acetone are shown in Fig. 1. Under these chromatographic conditions, acetaldehyde and acetone (internal standard) had retention times of 2.0 and 2.5 min, respectively, and were resolved to baseline throughout the calibration curve range of 1.0–20 μg. There was no interference from endogenous compounds or any of the drugs screened. Acetone was not detectable either in any of the drug-free whole blood samples from healthy volunteers, or in pre-dose whole blood samples from children with severe malaria and convulsions. The limit of detection (lowest concentration yielding a signal-to-noise ratio >3) was 1.5 μg/ml.

3.2. Recovery, calibration curves and reproducibility

The mean (±S.D.) percentage relative recoveries at 2.0 and 15 μg were 105.6 ± 8.4% ($n = 10$) and 101.2 ± 5.9% ($n = 10$), respectively. Calibration curves for paraldehyde in whole blood over the range 1.0–20 μg (3.3–66.6 μg/ml) were linear ($r^2 > 0.99$). The intra-assay R.S.D.s at 2.0, 10.0 and 18.0 μg of paraldehyde were 3.5% ($n = 5$), 7.7% ($n = 5$) and 4.6% ($n = 4$), respectively. The inter-assay R.S.D.s at the above concentrations were 5.3% ($n = 10$), 12.8% ($n = 10$) and 5.4% ($n = 9$), respectively.

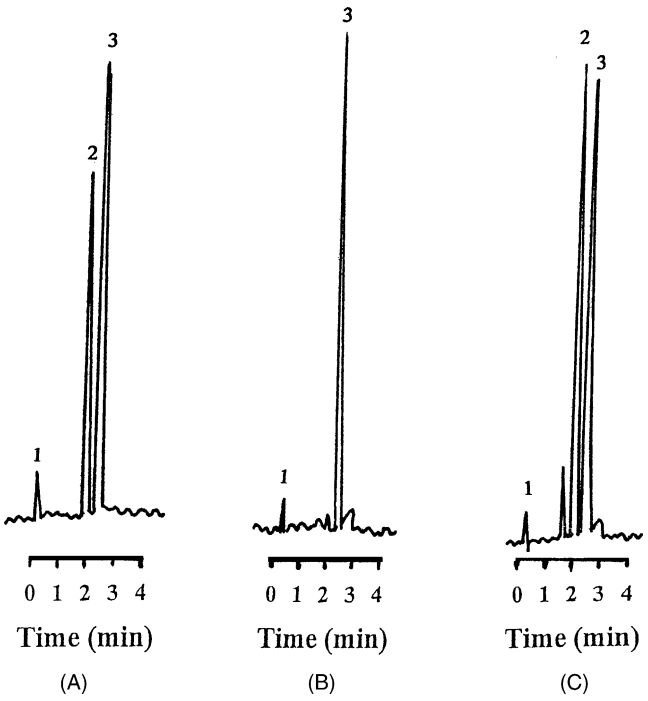

Fig. 1. Gas chromatograms of: (A) extracted spiked whole blood (300 μl) containing 15 μg (50 μg/ml) of paraldehyde [2] and 15 ng of the internal standard (acetone) [3]; (B) extracted pre-dose whole blood sample (300 μl) spiked with 15 ng of the internal standard [3]; (C) extracted whole blood sample obtained after 2 h following intramuscular administration of 0.2 ml/kg (100% (w/v)) paraldehyde to a child with severe malaria and convulsions. The estimated paraldehyde concentration was 52 μg/ml. Peaks: 1, injection event mark; 2, acetaldehyde (paraldehyde monomer); 3, internal standard.

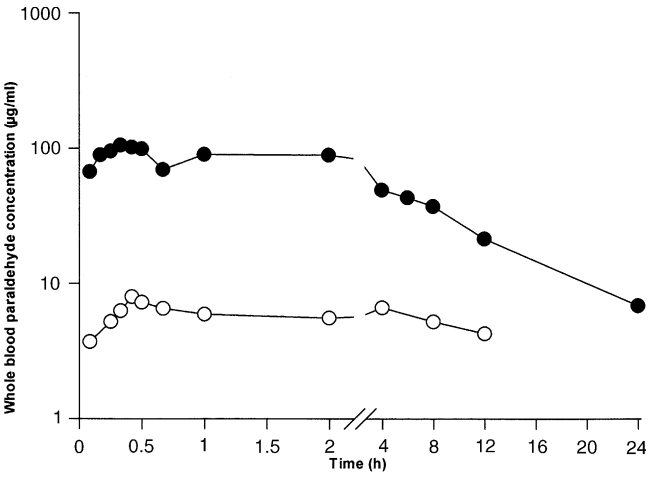

Fig. 2. Whole blood paraldehyde concentration versus time profiles following administration of a single dose of paraldehyde either intramuscularly (0.2 ml/kg undiluted paraldehyde; closed circles) or rectally (0.2 ml/kg of a 10% (v/v) solution in olive oil; open circles) in two children.

3.3. Validation study

This assay method was successfully used to quantitatively measure the concentrations of paraldehyde in whole blood samples obtained from pediatric patients who were administered paraldehyde either intramuscularly (0.2 ml/kg) or rectally (0.2 ml/kg of 10% (v/v) paraldehyde). Fig. 2 shows concentration versus time profiles from two of the patients.

3.4. Discussion

Paraldehyde has been used in the treatment of convulsions but there are no studies relating plasma levels and clinical effects. In this study, we report a simple, selective and rapid method for assay of paraldehyde (as its monomer acetaldehyde) in small volumes of whole blood. Paraldehyde reacts with plastic and rubber, and sample collection and handling during analysis may present a major challenge. We have adapted a pragmatic method for sample handling that avoids contact with plastic or rubber, by covering the screw caps with aluminium foil. This ensures that there is no direct contact of the paraldehyde with the rubber or Teflon lined screw caps during storage or on heating prior to assay.

Most of the previously reported assay procedures are based on depolymerization of paraldehyde by acid and heat to its monomer, acetaldehyde, which is then measured by gas chromatography with flame ionization detection [13–15]. Lampkin [13] reported a GLC method for determination of paraldehyde in pharmaceutical preparations. Although the method only involves dilution of the paraldehyde sample prior to analysis of its paraldehyde content, this assay may be unsuitable for analyzing biological samples, which may require clean up before injection onto the chromatograph. Koren et al. used gas chromatography to quantify intact paraldehyde in 2 μl serum samples by direct injection [17]. However, the infants in this study were administered paraldehyde either as a 0.2 mg/kg intravenous bolus followed by an infusion (16 mg/(kg h)), or a 400 mg/kg bolus, and thus higher serum concentrations (>100 μg/ml) were achieved compared to our study where paraldehyde was administered IM (0.2 mg/kg) or PR (0.2 mg/kg of 10% (v/v) solution). Using gas chromatography, paraldehyde was determined by direct injection of aliquots of blood, urine, reference solutions and gastric contents [16]. The authors reported that blood paraldehyde concentrations ranging from 30–150 μg/ml were achieved following therapeutic use (dose not stated) of paraldehyde, which is consistent with our findings. Hessel employed serum deproteinization using ultrafiltration followed by wide-bore capillary gas chromatography to quantify intact paraldehyde [18]. However, this technique has some limitations, including lack of adequate sensitivity and use of expensive disposable ultrafiltration units, which may be unaffordable for routine use in many resource-poor countries, where paraldehyde is routinely used.

Most of the doses currently recommended for control of convulsions are empirical and not based on previous pharmacokinetic studies. We evaluated the doses previously used for treatment of convulsions in our unit to correlate the blood concentrations and clinical effects. The results show that paraldehyde is well absorbed after both IM and

PR administration in children with severe malaria. Maximum paraldehyde concentrations of about 105 and 8 μg/ml were achieved within 20 min following IM and PR administration of paraldehyde, respectively (Fig. 2). However, the concentrations achieved following IM and PR administration (0.2 ml/kg in both cases) were insufficient to terminate convulsions in 84% of the children. A concentration of >100 μg/ml has been proposed to be required to terminate convulsions in neonates, while concentrations between 150–200 μg/ml have been suggested to be effective in all ages [17]. Previous reports on efficacy of paraldehyde were based on studies following IV infusion. A 90% efficacy was reported in a study in neonates in which multiple IM dose had been administered, although most of the neonates had also received either phenobarbitone or phenytoin concurrently [20].

In conclusion, the gas chromatographic method for the determination of paraldehyde described here is simple, sensitive and selective. The method requires only 300 μl of whole blood. Both the sample collection and preparation procedures are simple, and can be used in resource-poor facilities. We have successfully used this assay method to study the pharmacokinetics of IM and PR paraldehyde in children with convulsions associated with severe malaria.

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